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HOW NATURE SHAPED ECHOLOCATION IN ANIMALS

Topic Editors
Mariana L. Melcón and Cynthia F. Moss

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HOW NATURE SHAPED ECHOLOCATION IN ANIMALS

Topic Editors:
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On the top: fish-eating bat (Myotis vivesi) looking for prey (courtesy of Marco Tschapka). On the bottom: Commerson's dolphins (Cephalorhynchus commersonii) swimming in a bay (courtesy of Fundación Cethus)

Echolocation has evolved in different groups of animals, from bats and cetaceans to birds and humans, and enables localization and tracking of objects in a dynamic environment, where light levels may be very low or absent. Nature has shaped echolocation, an active sense that engages audiomotor feedback systems, which operates in diverse environments and situations. Echolocation production and perception vary across species, and signals are often adapted to the environment and task.

In the last several decades, researchers have been studying the echolocation behavior of animals, both in the air and underwater, using different methodologies and perspectives. The result of these studies has led to rich knowledge on sound production mechanisms, directionality of the sound beam, signal design, echo reception and perception. Active control over echolocation signal production and the mechanisms for echo processing ultimately provide animals with an echoic scene or image of their surroundings. Sonar signal features directly influence the information available for the echolocating animal to perceive images of its environment. In many echolocating animals, the information processed through echoes elicits a reaction in motor systems, including adjustments in subsequent echolocation signals. We are interested in understanding how echolocating animals deal with different environments (e.g. clutter, light levels), tasks, distance to targets or objects, different prey types or other food sources, presence of conspecifics or certain predators, ambient and anthropogenic noise. In recent years, some researchers have presented new data on the origins of echolocation, which can provide a hint of its evolution. Theoreticians have addressed several issues that bear on echolocation systems, such as frequency or time resolution, target localization and beam-forming mechanisms.

In this Research Topic we compiled recent work that elucidates how echolocation – from sound production, through echolocation signals to perception- has been shaped by nature functioning in different environments and situations. We strongly encouraged comparative approaches that would deepen our understanding of the processes comprising this active sense.

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Introduction to special issue, "How nature shaped echolocation in animals"

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This special issue, "How nature shaped echolocation in animals," is dedicated to Elisabeth Kalko and Björn Siemers, two extraordinarily creative, passionate, and important researchers in the field of echolocation. Both Eli and Björn passed away suddenly and at young ages, leaving a gaping hole in our research community. Eli, with enormous talents as a naturalist and a contagious enthusiasm to understand the lives of animals in the field, broke new ground in her discoveries of the diversity and richness of bat behaviors. Björn combined exceptional imagination and scientific rigor to make keen observations on bat echolocation and to launch a world class program combining laboratory and field studies.

For those who did not have the chance to meet Elisabeth Kalko (see **Figure 1**) and Björn Siemers (see **Figure 2**), we hope that the obituaries below will give you some understanding of their contribution and personalities. And for those who had the pleasure of knowing them, this is just another way to remember them.

Professor Dr. Elisabeth Kalko, Director of the Institute of Experimental Ecology at the University of Ulm, died most unexpectedly on September 26, 2011, at the age of 49. She died in her sleep during a research trip to Mount Kilimandjaro in Tanzania. As the website of the Smithsonian's Tropical Research Center in Panama City put it, she passed away while doing what she loved most: research with bats.

Elisabeth Kalko was born in Berlin on April 10, 1962. After receiving her "Abitur" [German higher education entrance certificate; preparatory school diploma] from the Justinus-Kerner Gymansium in Heilbronn she began her studies in biology at the University of Tübingen in the fall of 1981 and completed a Master's degree in 1987. From 1984 until completing her PhD, she held a scholarship from the German National Academic Foundation.

With her MS project in animal physiology in Tübingen, "Hunting and Echolocation Behavior of the Daubenton's bat, *Myotis daubentonii*, in the wild," Kalko had already discovered the research area that would be the focus of her future career.

In 1991, Kalko completed her doctoral work on "The echolocation and hunting behavior of three European species of common pipistrelles, *Pipistrellus pipistrellus*, *P. nathusii*, *P. kuhlii*, in the wild" and graduated summa cum laude in Tübingen. Her work was honored with the Fritz Lang Prize of the German Society for Mammalian Biology (DGS).

The publications resulting from Kalko's graduate research set new standards for conducting field work on bat echolocation and are frequently cited to this day. Because of the high quality of this work, Kalko quickly became internationally renowned. Everyone spoke highly of her unique ability to capture bat behavior with tireless energy, great patience, an intuitive understanding of animal behavior, and an extraordinary empathy for nature. This quickly made her an important figure in bat research. Kalko's extraordinary impact is reflected in a statement by Donald Griffin, the discoverer of bat echolocation: After she enthusiastically showed him her field data, he spontaneously called her the "Jane Goodall of bats." Coming from Griffin, a critical scientist who was known to express praise sparingly, this was especially noteworthy recognition.

After she received her doctoral degree and during the postdoctoral phase of her career, Kalko was able to pursue her research tirelessly and with great success through two DFG-[German Research Foundation]-funded projects, "Diversity in Tropical Bats: Resource Utilization, Habitat Selection, and Niche Specializations of a Tropical Species Community" and "Comparative Studies on the Organization, Structure, and Dynamics of Neotropical Bat Communities in Disturbed and Undisturbed Forest Systems." These research topics also formed the basis for her Heisenberg-funded habilitation work, which she completed at the University of Tübingen in 1999 with a thesis on "Diversity, Structure and Dynamics of Neotropical Bat Communities."

Even before she completed her habilitation, Kalko was awarded a professorship in Experimental Ecology at the University of Ulm. At the same time she was promoted to staff scientist at the STRI in Panama. The good working conditions in Ulm and


Foto Heike Wägele

FIGURE 1 | Elisabeth Kalko. April 10, 1962 to September 26, 2011.

Panama enabled Kalko to build a highly productive research group and to considerably broaden her field of interest and research, as described in an excerpt on her University of Ulm website:

> "My research focuses on community ecology, sensory ecology, behavioral ecology, ecophysiology and diversity patterns of vertebrate assemblages, particularly in the species-rich tropics with a focus on bats (Chiroptera). I am particularly interested in functional diversity and the effects of changes in land use and climate change on biodiversity patterns and ecosystem services with the ultimate goal to feed the results of my studies into applied sciences, particularly into conservation biology and into the emerging field of zoonotic diseases with regard to wildlife and human health. Another focus of my research deals with sensory systems of bats with special emphasis on ecological and evolutionary aspects of their echolocation system and foraging strategies integrating multiple sensory cues, i.e., olfaction and vision. As a third cornerstone of my research I am concentrating on bat-plant interactions, particularly frugivory and the adaptations between consumers/dispersers and plant traits. My study areas cover temperate zones, particularly Europe, and the tropics, mainly Central- and South America (Panama, Costa Rica, Mexico, Venezuela, Bolivia, Peru) and Africa (Tanzania, Benin, Ivory Coast, Ghana)."

The great success of Kalko's work is evident in the many academic honors she received, such as her election to the National Committee of Global Change in Germany in 2002 and to the Heidelberg Academy of Sciences [HAW] in 2004. In 2005, she became vice president of the Society for Tropical Ecology [GTÖ], followed in 2008 by her election as a member of the Senate Commission on Biodiversity Research of the German Research Foundation [DFG], and as chair of DIVERSITAS Germany. In 2011, shortly before her death, she became a member of the University Council at the University of Ulm.

Kalko was a talented teacher and received the State Teaching Award of Baden-Wüttemberg. She captured the attention of her audience with her great expertise in behavioral ecology and her enthusiasm for nature, making a lasting impression on the listener. This ability was well-known at the STRI in Panama. Whenever important politicians or VIPs arrived from Washington, Kalko was asked to guide them through the forest and discuss her research, which inevitably had favorable effects on future research funding. Everyone who experienced Kalko in the field will certainly agree that no one could convey biological knowledge, love for nature, and amazement about the natural world better than she did.

Elisabeth Kalko had great fervor for her work, was passionately devoted to science, and viewed her research as both a job and a calling. She launched many projects, positively influenced scientists and students, and touched them with her engaging personality. German Zoology has experienced a great loss with her passing. We deeply miss her.

—Prof. Dr. H.-U. Schnitzler and Dr. A. Denzinger

On May 23, 2012, Assistant Professor Dr. Björn Martin Siemers died as a result of an infection, within only a few hours, and just two days before his 40th birthday. His death came as a great shock to his wife and two children, his family, his workgroup at the Max Planck Institute for Ornithology in Seewiesen, and to his many students, colleagues, and friends.

Björn Siemers was born in Stuttgart on May 25, 1972. Early on in his life it was already clear that he would become a natural scientist, as there was nothing he enjoyed more than imaginary research expeditions with his brother. After his "Abitur" [German higher education entrance certificate; preparatory school diploma] he studied animal physiology, zoology, and genetics, combined with a minor in law at the University of Tübingen. In 1994–1995 he was a visiting student at the University of Sao Paulo in Brazil for study abroad, funded by the DAAD [German Academic Exchange Service], where he studied primatology, entomology, and neurobiology. Despite his enthusiasm for primates and his decision to become a primatologist, when he returned to the University of Tübingen he began working with bats as part of his major practical lab course ("Grosspraktikum") in animal physiology. Bat research continued to impact his career development and resulted in important discoveries during his master's and doctoral work and in many subsequent investigations. However, his research profile and his scientific collaborations over the past few years show that he never fully abandoned primatology.

Siemers's graduate work laid the foundations for his primary area of research: sensory ecology. In his master's project he investigated the hunting and echolocating behavior of the Natterer's bat and showed for the first time that these bats are able to locate their prey even if the prey is positioned very close to background clutter. For his doctoral work he expanded his research by conducting comparative studies of prey perception in different species of *Myotis*, completing his research in 2000. He demonstrated that the echolocation calls of the different *Myotis* species, which actively find their prey using echolocation, are characterized by species-specific differences, particularly in their call bandwidth. This can be interpreted as an adaptation to habitat-specific echolocation tasks. The wider the species-specific signal

FIGURE 2 | Björn Siemers (May 25, 1972 to May 23, 2012) in front of the "autograph wall" in the former Von-Holst-House in Seewiesen. In the background is a bat drawn by Donald Griffin in 1961.

bandwidth, the smaller the separation a bat could detect between prey and background. These experimental studies were the first to confirm a previously untested hypothesis derived from sonar theory. Subsequent publication of the results in *Nature* earned Siemers international acclaim. In the 5½years following his doctoral degree he worked as Assistant Professor in animal physiology and further qualified himself with additional studies on the sensory ecology of prey perception in bats and primates, work which cumulatively led to his habilitation at the University of Tübingen in 2006. Due to his extraordinary scientific achievements and approaches, immediately after his habilitation he was selected for a position as "Independent Young Scientist" at the Max Planck Institute, after a highly competitive selection process. This is a particularly great achievement, since only 2% of the applicants were selected. With his usual dynamism and great enthusiasm he began building a "sensory ecology" research group at the Max Planck Institute for Ornithology in Seewiesen in the summer of 2006. This research group focused on comparative studies of sensory and cognitive specializations for foraging in animals, and the resulting niche differentiation.

At the time of his death, Siemers's group consisted of 15 members, with whom he conducted research both nationally and internationally. Siemers's great productivity resulted in many high quality publications in top tier journals, such as *PNAS* and *Nature*. Also impressive was his ability to convey his broad knowledge of bats beyond the halls of academia. The books he published in collaboration with the well-known animal photographer Dietmar Nill, *Fledermäuse—Das Praxisbuch* [*Bats—A Practical Guide*] and *Fledermäuse. Eine Bilderreise in die Nacht* [*Bats. A Photographic Journey into the Night*], are an excellent introduction to the lives of these animals. All aspects of a bat's life are made comprehensible, with exciting narratives and surprising facts, and important species are introduced in brief portraits. With a strong media presence, Siemers impressed his audience with extraordinary stories and excellent photographic and video material.

In 2009, Siemers and colleagues from Israel and the LMU in Munich received $900,000 in funding from the Human Frontiers Science Program for a project entitled "Listening through the Looking Glass: Perception and Neural Encoding of Mirror Images of Biosonar." In 2011, Siemers was awarded the prestigious "European Starting Grant" in the amount of 1.5 Million Euros from the European Research Council on the topic, "Sensory and Cognitive Ecology of Interspecific Interactions in Bat Communities." Considering these many successes, a full professorship seemed only a question of time.

With the untimely death of Siemers, German Zoology loses not only a promising scientist but also an exceptional and kind human being. Siemers's curiosity, eagerness to engage in discussions, willingness to help others, and cheerfulness were contagious and inspired everyone. Anyone greeted by Siemers in the morning with his cheery "Good Morning" would be hard pressed to start the day in a bad mood. His optimism and positive attitude were astounding. Though his disability might have given him reason to bear a grudge against fate, he radiated vital energy and optimism and took delight in his work and life. He accepted life in its entirety. He believed in the good in every person and also in institutions, and made a positive impact with this attitude. These qualities made Siemers a popular teacher as well. He always listened to his students with a sympathetic ear. They cherished him for it and felt accepted. His humor, too, was quite endearing, and like almost nobody else he was able to laugh at himself—even when we were amused by his often [overly] professorial statements.

Björn Siemers focused his life's priorities not only on his career and work, but also—and always—on his family and children. Siemers will live on in our thoughts and be with us forever.

—Dr. A. Denzinger and Prof. Dr. H.-U. Schnitzler

Both obituaries translated by Dr. S. Blumenrath and reprinted with permission of the authors. Original published in ZOOLOGIE 2012, Mitteilungen d.Dtsch.Zool.Ges.

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Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats

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Throughout evolution the foraging and echolocation behaviors as well as the motor systems of bats have been adapted to the tasks they have to perform while searching and acquiring food. When bats exploit the same class of environmental resources in a similar way, they perform comparable tasks and thus share similar adaptations independent of their phylogeny. Species with similar adaptations are assigned to guilds or functional groups. Habitat type and foraging mode mainly determine the foraging tasks and thus the adaptations of bats. Therefore, we use habitat type and foraging mode to define seven guilds. The habitat types open, edge and narrow space are defined according to the bats' echolocation behavior in relation to the distance between bat and background or food item and background. Bats foraging in the aerial, trawling, flutter detecting, or active gleaning mode use only echolocation to acquire their food. When foraging in the passive gleaning mode bats do not use echolocation but rely on sensory cues from the food item to find it. Bat communities often comprise large numbers of species with a high diversity in foraging areas, foraging modes, and diets. The assignment of species living under similar constraints into guilds identifies patterns of community structure and helps to understand the factors that underlie the organization of highly diverse bat communities. Bat species from different guilds do not compete for food as they differ in their foraging behavior and in the environmental resources they use. However, sympatric living species belonging to the same guild often exploit the same class of resources. To avoid competition they should differ in their niche dimensions. The fine grain structure of bat communities below the rather coarse classification into guilds is determined by mechanisms that result in niche partitioning.

Keywords: bat, echolocation, guild, community structure, habitat, foraging behavior

DIVERSITY IN BATS

The order Chiroptera consists of 19 families including the Pteropodidae. The key character that distinguishes bats from all other mammals is the capacity of powered flight and in microchiropteran bats the use of a tonal echolocation system (Denzinger et al., 2004; Schnitzler et al., 2004; Jones and Teeling, 2006). Microchiropteran bats comprise about 1000 species and are one of the most diverse groups within terrestrial mammals. In the course of evolution, numerous adaptations in behavior and in sensory and motor systems allowed bats to radiate into a multitude of niches at night which are occupied by other animals during the day. Bats exploit a great variety of food sources including insects and other arthropods such as scorpions and spiders, fish, small vertebrates, fruit, nectar and pollen, and even blood. They forage for airborne prey, glean food items from the ground or from vegetation, or forage above water surfaces for insects or fish. Bats occupy all terrestrial areas with the exception of the polar region and high mountain ranges and even use extreme habitats, i.e., *Otonycteris hembrichii* feeding in the desert on scorpions, or *Myotis vivesi* living on small isolated islands and hunting for fish in the ocean.

AIMS OF THIS STUDY

To understand the factors which underlie the radiation of bats into so many different directions, we have to identify the mechanisms that structure the high diversity in bats. There have been many approaches to classify bats into groups that face similar constraints (for review see: Fenton, 1990; Kalko et al., 1996; Kalko, 1997; Schnitzler et al., 2003). Food and feeding mode was often used as a basis for categorization leading to feeding associations like aerial insectivory, foliage-gleaning insectivory, piscivory, sanguinivory, nectarivory, frugivory, omnivory, and carnivory (McNab, 1971; Hill and Smith, 1984). Wing morphology and diet have been also used to separate bats into groups like: fast hawking, slow hawking, trawling, gleaning and hovering, fly-catching and perch hunting (Norberg and Rayner, 1987). Patterns of habitat use and variations of this approach have been used to identify groups of bats with similar foraging behaviors (Aldridge and Rautenbach, 1987; Crome and Richards, 1988; Neuweiler, 1989; Fenton, 1990). Elisabeth Kalko, who is honored with this edition of Frontiers in Integrative Physiology developed—together with others—this habitat oriented approach further and arranged bats that live under similar ecological conditions and perform similar echolocation tasks into guilds or functional groups (Kalko et al., 1996; Schnitzler and Kalko, 1998, 2001; Schnitzler et al., 2003; Denzinger and Schnitzler, 2004). The aims of this paper are to critically discuss the studies which have used the guild concept for classification of microchiropteran bats, and to further refine this approach. We will examine whether the arrangement of bats in functional groups is suited to identify the driving forces which determine the organization of bat communities. With our work we also want to honor Björn Siemers, to whom this edition of Frontiers in Integrative Physiology is also dedicated. In his scientific work Björn Siemers investigated the role of sensory and cognitive abilities of bats for defining a species' niche. Here we will discuss his approach on niche partitioning in bats within the guild concept.

THE GUILD CONCEPT

Root (1967) defined a guild as "a group of species that exploit the same class of environmental resources in a similar way." Bats belonging to different guilds should therefore differ in the environmental resources they exploit and/or in the way how they do this. The basic idea behind the guild concept is that bats performing the same tasks share similar adaptations. We will outline that the attribution of bats into functional groups or guilds helps us to understand the organization of the highly diverse microchiropteran bat communities.

BASIC ECHOLOCATION TASKS OF FORAGING BATS

Foraging bats continuously emit echolocation signals and analyze the sound complex consisting of the emitted signal and the returning echoes in their auditory system to perform the basic echolocation tasks: detection, localization and classification. For detection, bats have to decide whether they perceive echoes form their own emitted signals or not. For localization bats determine the target distance by measuring the time delay between the emitted signal and the echo, and the target direction by using binaural and monaural echo cues. For classification bats use echo features such as spectrum and modulation patterns which encode the nature of the reflecting target (Schnitzler and Kalko, 1998, 2001; Schnitzler et al., 2003).

All bats have to perform several tasks in parallel when searching for food:

SPATIAL ORIENTATION

Bats need to know their own position in relation to the world around them. This self-positioning has two aspects: navigation and obstacle avoidance. Bats navigate from their roosts to their hunting grounds and back. Thus, they have the ability to find, learn and return to specific places (Trullier, 1997; Schnitzler et al., 2003; Thiele and Winter, 2005). Each identified target can serve as a potential landmark for orientation in space. Landmarks within the perceptual range of a bat are used for route planning and route following. For long-range navigation, however, other senses like vision and the magnetic sense must be used (Schnitzler et al., 2003; Holland et al., 2006, 2008; Wang et al., 2007). Background objects are physical structures which may influence the flight behavior of bats. The closer a bat forages to the background, the smaller the available space for food acquisition maneuvers, and the higher the collision risk. The sensory and motor problems of foraging under these restricted conditions are reflected in specific sensory and motor adaptations. Distance dependent changes in echolocation behavior in the vicinity of background targets suggest that bats collect information needed for flight path planning and for collision avoidance. Adaptations in wing morphology that increase maneuverability of the bats also help them to forage successfully in restricted spaces (Aldridge and Rautenbach, 1987; Norberg and Rayner, 1987; Fenton, 1990; Norberg, 1994).

BIOTOPE RECOGNITION

The properties and the composition of the environment are important information for bats. Typical foraging grounds like forest edges, trees and bushes, meadows, and water surfaces are indicators for specific prey. In other words, they are biotopes which provide specific resources. Therefore, biotope recognition is fundamental for bats. Bats can use statistical properties of echoes from vegetation for the classification of typical biotope elements such as trees and bushes (Yovel et al., 2009, 2011).

FOOD FINDING

Foraging bats have to find food. The ability to detect, classify and localize a food item strongly depends on where the food item is positioned. An insect flying far from the bat in open air constitutes a different foraging task from an insect sitting on a leaf. For many bats species, echolocation delivers all information necessary to find the food. If echolocation is not sufficient sensory cues such as odor or prey-generated sounds are used to find food.

The three tasks—spatial orientation, biotope recognition and food finding—often have to be performed in parallel. For example, an oak tree may be an important landmark along the foraging route and at the same time may also be an obstacle which needs to be avoided. Additionally, it may be an indicator for specific prey which has to be identified.

The psychophysics of hearing limits the processing of echo information. The emitted signal produces a forward-masking effect if it overlaps with or is close in time to the food echo. The echoes from background targets or clutter echoes produce a backward-masking effect if they overlap with or are close to the food echo. These masking effects prevent or reduce the chance of finding food. Comparative studies in the field and in the laboratory revealed that bats tend to avoid overlap of the target echo with the emitted signal as well as with clutter echoes from background targets (Kalko and Schnitzler, 1989, 1993). An exception are bats that use CF-FM signals consisting of a long component of constant frequency (CF) followed by a shorter downward frequency modulated terminal component (FM). These bats tend to avoid an overlap of the FM component.

Due to the masking effects of the emitted signal and of the clutter echoes bats can only find food items without interferences if their echoes are positioned in the overlap-free window. This window is defined as the area between signal overlap zone where the emitted signal overlaps with the food echo and the clutter overlap zone where the food echo overlaps with clutter echoes from the background (**Figure 1**) (Kalko and Schnitzler, 1993; Schnitzler

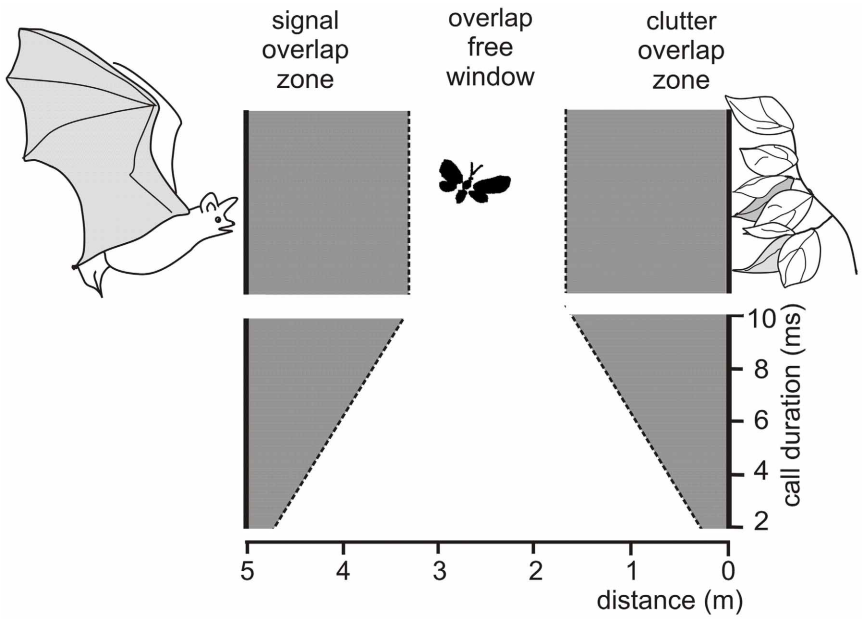

FIGURE 1 | Schematic drawing illustrating the conditions for overlap between emitted signal, prey echo and background echoes a bat encounters when foraging at a distance of 5 m to vegetation. The prey echo overlaps with the emitted signal when an insect flies in the signal overlap zone and with the clutter echoes from the background when it flies in the clutter overlap zone. In the overlap-free window no overlap occurs. The width of the overlap zones depend on signal duration. At durations between 10 and 2 ms, the overlap zones range between 1.70 and 0.34 m, if a sound speed of 340 m/s is assumed. A reduction of signal duration by 1 ms reduces the width of each overlap zone by 0.17 m and thus increases the width of the overlap-free window by 0.34 m.

and Kalko, 1998, 2001; Schnitzler et al., 2003). The width of the signal and the clutter overlap zone depends on signal duration. For example, at an assumed sound speed of 340 m/s a signal duration of 10 ms produces an overlap zone which is 1.7 m wide. If undisturbed detection of a food item is only possible beyond the signal overlap zone, signal duration can be used as a rough measure for the minimal detection distance. Each increase of sound duration by 1 ms increases the width of the signal overlap zone and with it the minimal detection distance by 0.17 m. Sound duration also controls the width of the overlap-free window. A reduction of 1 ms widens the window by 0.34 m as it reduces each of the overlap zones by 0.17 m.

The degree of masking also depends on the frequency structure and on the SPL of the interfering signals and decreases with increasing steepness of a signal (Schnitzler et al., 2003). Thus, the masking zone can be smaller than the overlap zone calculated from signal duration if bats use signals which are more masking-tolerant. For example, *Myotis nattereri* use steeply modulated signals of large bandwidth which tolerate some overlap between prey and clutter echoes (Siemers and Schnitzler, 2000) (**Figure 6**). All bats using long CF-FM signals have solved the masking problem in a different way: They compensate for Doppler shifts and keep the target echo of the CF component in the extremely sharply tuned neurons of their auditory fovea whereas the CF component of the emitted signal has a lower frequency and falls in an area where the auditory threshold is high (Schnitzler and Denzinger, 2011). Therefore, masking of the CF component is prevented.

FORAGING HABITATS AND FORAGING MODES

Comparative studies showed that the distance between bat and background or food and background is the most relevant ecological condition for foraging bats. According to these conditions, foraging areas of bats or habitats have been defined (Aldridge and Rautenbach, 1987; Neuweiler, 1989; Fenton, 1990; Schnitzler and Kalko, 1998, 2001; Schnitzler et al., 2003; Denzinger and Schnitzler, 2004). The definitions differ partially but all approaches have in common that they separate three main types of foraging areas which Fenton (1990) named open, edge and closed habitats (for review see Schnitzler et al., 2003). We will use the terms open, edge and narrow space as first proposed by Schnitzler et al. (2003).

In our definition habitat is not just the place where an animal lives. We follow Krausman's review (1999) and define that a foraging habitat is determined by the resources and conditions which a species encounters when searching for food. This functional definition implies that species forage in the same habitat as long as they have to perform similar tasks to exploit similar resources under similar conditions. The spatial extend of such a functionally defined habitat is species-specific.

Our habitat definition is based solely on the sensory abilities of bats to perform habitat-specific tasks. Habitats differ according to the spatial relations between bat and background or food and background. The proximity of a bat to the food items and to background objects poses also a motor task (Fenton, 1990). Bats foraging in the open fly long distances with high speed and gleaning bats maneuver close to the background to get the food while also avoiding collisions. Therefore, not only the sensory system has been adapted to habitat specific tasks but also the motor system (Aldridge and Rautenbach, 1987; Norberg and Rayner, 1987; Fenton, 1990; Norberg, 1994).

According to clutter conditions we define three types of foraging habitats which are developed from former definitions of Aldridge and Rautenbach (1987); Neuweiler (1989); Fenton (1990); Schnitzler and Kalko (1998, 2001); Schnitzler et al. (2003) and Denzinger and Schnitzler (2004) (**Figure 2**).

OPEN SPACE

Bats foraging in "open space" exploit the resource of airborne insects flying far from background targets and catch their prey in the "*aerial*" mode (**Figure 2**). Under these conditions echoes from the background reach the bat considerably later than the echoes from the prey and do not disturb their detection. In open space bats do not react to the background in their echolocation behavior.

EDGE SPACE

Bats foraging in "edge space" exploit the resource of airborne prey found near the edges of buildings and vegetation, in gaps, or above the ground and water surfaces, and catch their prey in the "*aerial*" mode (**Figure 2**). Under these conditions the pairs of emitted signal and prey echo are followed by background echoes. As long as the background echoes do not overlap with the prey echoes, no masking of the prey echo occurs. In edge space bats react to the background in their echolocation behavior.

A special edge space condition is used by bats that exploit the resource of prey which is found on or just above calm water surfaces. Foraging bats fly low over water and emit their signals in forward direction. Their sound waves propagate in the air above water and partly come back as direct echoes if they hit prey or a background target. However, most of the emitted waves and of the returning echoes hit the mirror-like water surface. These waves are reflected away. Only the waves which hit the water perpendicularly, direct below the bat, produce a strong echo. The two-way travel time of this echo from below encodes the flight height of the bat and indicates water (Greif and Siemers, 2010). As trawling foragers fly low over water, the surface echo appears first and often overlaps with the emitted signal. Echoes from prey ahead of the bat appear later. Echoes from surface prey always contain a direct and a reflected component. The overall amplitude of this combined echo is larger than the direct echo produced by the same target in air due to the additional mirrored echo (Siemers et al., 2001, 2005). Background targets such as the shore produce

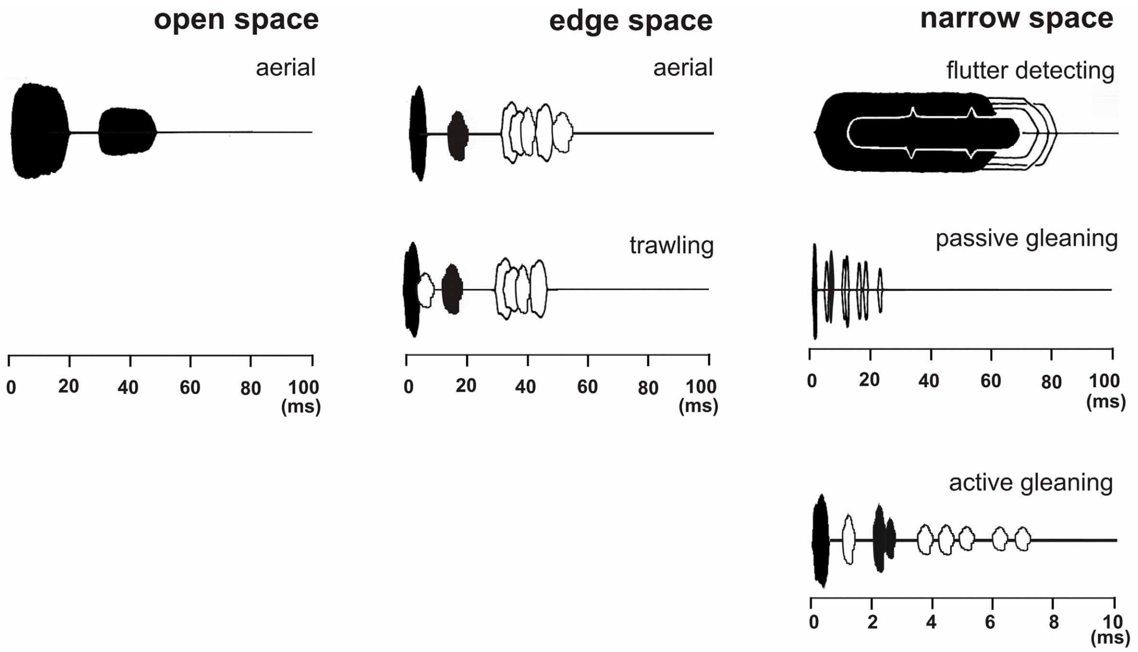

FIGURE 2 | Echolocation scenes of bats that search for prey in three different foraging habitats with typical foraging modes. The emitted signal (black) and the returning echoes from prey (black) are displayed together with echo trains from background targets (white). In the depicted echolocation scene which covers a time range of 100 ms a bat foraging in open space in the aerial mode perceives a pulse-echo pair consisting only of the emitted signal and the returning echo (both in black) as long as the background is further away than 17 m. Bats foraging in edge space in the aerial mode perceive a pulse-echo pair that is followed by clutter echoes from the background (in white). When foraging in the trawling mode above the water an additional surface echo returns from below immediately after signal emission (in white). In narrow space the target echo is positioned in the clutter overlap zone. Here three different foraging modes are employed. In flutter detecting foragers the echoes of the long CF-FM signals are modulated in the rhythm of the insect's wing beat and can therefore be discriminated from unmodulated background echoes. Passive gleaning foragers use very short signals. They have no chance to find the food echo (black) between the clutter echoes (white) and they rely on other senses for the detection and localization of the food item. Active gleaners exploit favorable short range favorable echolocation situations where the food echo is isolated enough or is so conspicuous that it can be found between clutter echoes.

an additional echo complex after the prey echo so that the auditory scene is similar to the situation in edge space. If background targets are far away, e.g., if the bat flies in the middle of a lake, even an open space-like auditory scene may occur, but with the important difference that the emitted signal is always followed by the surface echo from below (**Figure 2**).

NARROW SPACE

Bats foraging in "narrow space" exploit either animal prey which is positioned on or near background objects like vegetation or the ground, or they forage for fruits and flowers which are part of the background. Food echoes from animals either overlap with or are so close to background echoes that they are masked. Food echoes from plants must be discriminated from other background echoes. In narrow space bats have difficulties to find food echoes between clutter echoes only by echolocation. Three different foraging strategies have been evolved to cope with this problem.

Flutter detecting mode

Some bats specialize in finding their food using the "flutter detecting" foraging mode. They recognize insect echoes from their long CF-FM signals, which are modulated in the rhythm of the beating wings, and discriminate them from unmodulated clutter echoes (**Figure 2**).

Passive gleaning mode

Other bats have no chance to find the food echo in the dense clutter echoes from the background. They have to rely on other senses and use food generated cues to find it. They operate in the "passive gleaning" mode (**Figure 2**).

Active gleaning mode

Some bats are still able to find food, which is either part of the substrate or positioned on substrate, only by echolocation even under challenging clutter conditions. They forage in the "active gleaning" mode. Active gleaners use their echolocation system to exploit on short range favorable echolocation situations. Either they profit from food echoes that are isolated enough in time such that they can be identified between the clutter echoes (**Figure 2**), or they search for conspicuous food echoes, e.g., from flowers and fruits that can be discriminated from clutter echoes.

BORDERS BETWEEN FORAGING HABITATS

So far we have defined three foraging habitats where bats exploit different resources and perform different echolocation tasks. However, we have not yet defined the borders between them. The distances between bat and background and between food item and background have been identified as the most relevant ecological constraint which have shaped the foraging and echolocation behavior of bats. These distance-dependent effects have been used to define the borders between habitats (Schnitzler and Kalko, 1998, 2001; Schnitzler et al., 2003; Denzinger and Schnitzler, 2004).

The border between open and edge space is indicated by the bats' echolocation behavior (**Figure 4**). In open space bats do not react to the background, whereas in edge space they do. We hypothesize that in edge space bats react in their echolocation behavior to collect information necessary to maneuver in relation to background objects and to avoid collisions. The border between open and edge space is species-specific. *Vespertilio murinus* varied signal structure systematically in relation to the background. Above 6 m in horizontal direction and 5 m in vertical direction from the background, bats did no longer change their signal structure. According to our definition, this switch indicates the border between open and edge space (Schaub and Schnitzler, 2007) (**Figure 3**). Data from other species also show such a border. In *Pipistrellus kuhlii* the border was found at a height of about 5 m, in *Pipistrellus pygmaeus* at 3 m, and in *Eptesicus serotinus* and *Eptesicus nilssonii* at about 8–10 m (Kalko and Schnitzler, 1993; Rydell, 1993; Jensen and Miller, 1999). The species-specific spatial extend of the edge space may reflect the ability of the different species to maneuver near background objects. Fast flying bats with a lower maneuverability need more space for collision avoidance than bats which fly slower and have broader wings that equip them better for obstacle avoidance.

The border between edge and narrow space has been defined by the relation between food echo and clutter echoes from the background (Schnitzler and Kalko, 1998, 2001; Schnitzler et al., 2003; Denzinger and Schnitzler, 2004). This definition implies that a bat is in narrow space if the food item is positioned in the clutter-overlap zone where background echoes overlap with the food echo. A better definition for narrow space would be if the food echo is masked by the clutter echoes. For example, shallow modulated narrowband signals have a stronger masking effect and a wider masking zone than steeply modulated broadband signals of the same duration. However, it is very difficult to determine the exact extension of the masking zone. For practical reasons, we therefore define that the narrow space begins with the clutter overlap zone (**Figure 4**).

BAT GUILDS

The guild concept opened the way to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats by attributing species which perform similar tasks and share similar adaptations to guilds. These guilds were first defined by habitat type (uncluttered, background-cluttered, and highly-cluttered space), foraging mode (aerial-hawking, trawling and gleaning) and diet (insectivore, piscivore, carnivore, sanguivore, frugivore, nectarivore, omnivore) (Schnitzler and Kalko, 1998, 2001). In a second attempt the terms for the three habitat types were changed to the more neutral terms open, edge and narrow space to avoid misinterpretations concerning the role of background echoes in the echolocation process. Background echoes are not only disturbing clutter, but they also carry relevant information which is used for biotope and landmark recognition, and collision-free maneuvering. Additionally, diet was no longer used to classify guilds because echolocation and foraging behavior are mainly influenced by habitat type and foraging mode but not by the prey type. However, by that time it was not yet known that there are bats which find their food in narrow space by using the active gleaning mode so that only 5 guilds were defined

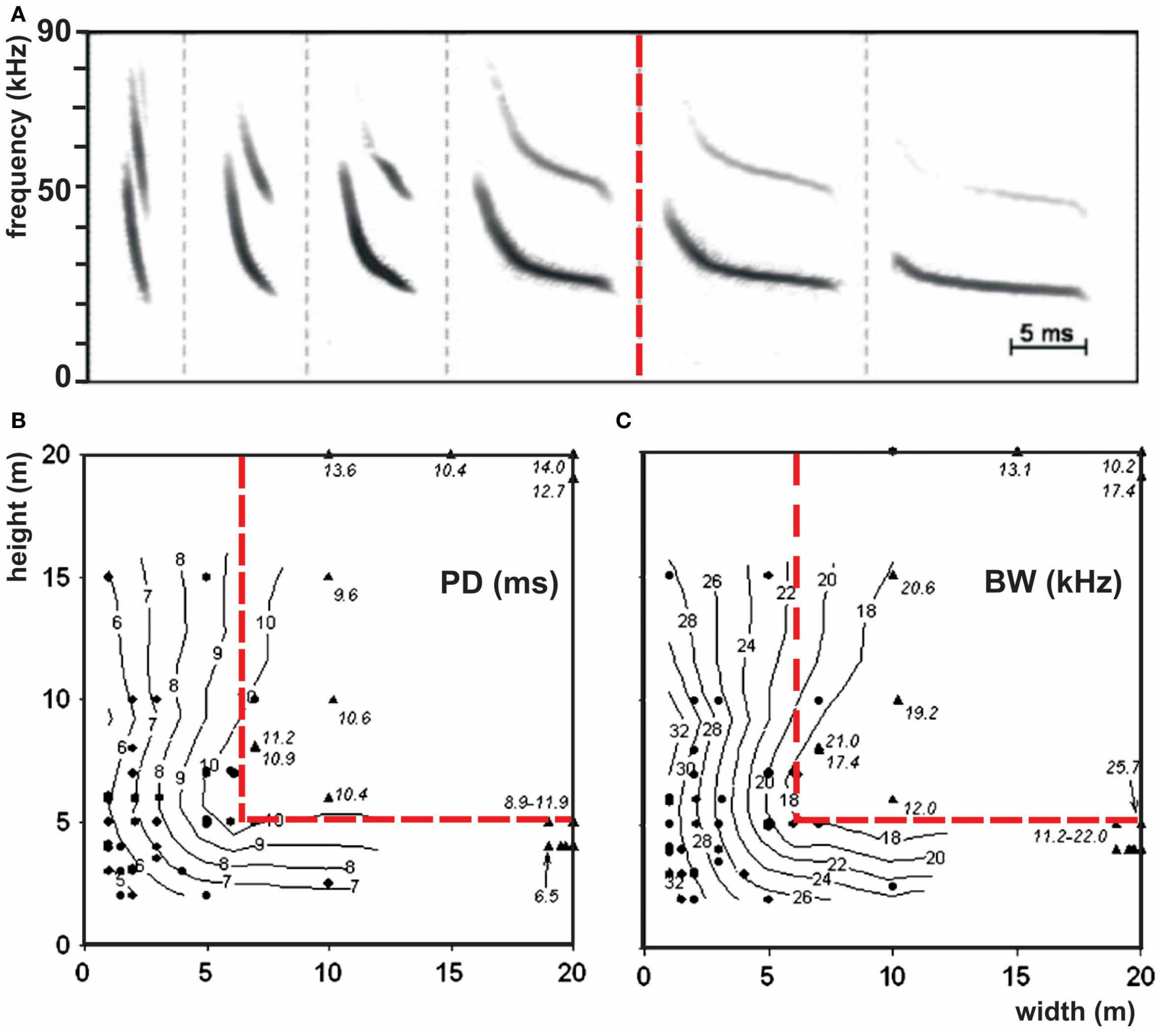

FIGURE 3 | Border between open and edge space. (A) Repertoire of the echolocation signals of *Vespertilio murinus* while foraging in open (right to the red line) and in edge space (left to the red line) and **(B)** isocontour plots of the signal parameters pulse duration and **(C)** bandwidth as a function of the horizontal and vertical distances to the background. Each dot represents the mean value of a sequence which was emitted at the indicated position. The red line separates open space from edge space according to our definition that bats react to the background in edge space by changing signal structure but not in open space [adapted from Schaub and Schnitzler (2007)].

(Schnitzler et al., 2003). Later an additional guild was added taking into consideration that some bats operate in narrow space in the active gleaning mode (Denzinger and Schnitzler, 2004). Here we will introduce a further guild that comprises all nectar, pollen and fruit eaters because these bats use the passive and the active mode to find their prey. Thus, we propose that 7 guilds are sufficient to structure even the most diverse bat communities.

OPEN SPACE AERIAL FORAGERS

Bats that hunt for airborne prey in open space face the problem that their prey is often distributed over large spaces and is therefore difficult to find. Bats that have to cope with this echolocation task are assigned to the guild of "open space aerial foragers." They have evolved echolocation systems for long range detection of prey. They use narrowband and shallowly modulated search signals with rather long call durations of about 8 ms to 25 ms. The long and narrowband signals increase the probability to detect an insect echo, as the signal energy of the echo is concentrated for a substantial time in the corresponding neuronal filters within the auditory system. Additionally, long signals increase the chance to perceive glints in insect echoes, which are short amplitude peaks generated by the fluttering wings in the instant when the wing is perpendicular to the impinging sound waves (Schnitzler, 1987). The frequency of the relevant harmonic of the narrowband echolocation calls is generally below 30 kHz and the calls are often emitted only every second or third wing beat resulting in long pulse intervals (**Figure 5**). The average source levels range between 104 and 111 dB SPL calculated for 1 m in front of the bat's mouth (re 1 m) (Holderied and von Helversen, 2003). The low frequencies and high source levels guarantee large detection ranges. For example, estimations of maximum detection distances for *Nyctalus noctula*, a typical open space bat from Europe, range from 10 to 3.5 m for insects with target strengths between −40 and −65 dB (Stilz and Schnitzler, 2012). In open space foragers maximum detection distances for flying insects beyond 20 m to 25 m are very unlikely even under the most favorable conditions with low signal frequency, high emission SPL, optimal beam width, high target strength and optimal temperature and humidity (Holderied and von Helversen, 2003; Jung et al., 2007; Stilz and Schnitzler, 2012; Jakobsen et al., 2013).

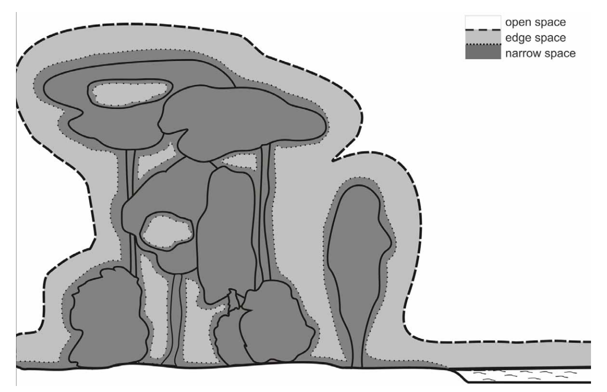

FIGURE 4 | Foraging habitats of bats. The borders between open and edge space is determined by the echolocation behavior of the bats. Bats react to background targets in edge space but not in open space. The border is species specific. The narrow space begins with the clutter overlap zone which depends on signal duration.

The echoes of the long distance echolocation signals of open space bats also deliver information that can be used for navigation and for biotope recognition. According to Stilz and Schnitzler (2012), *N. noctula* is able to perceive echoes from a forest edge up to a maximal distance of 37 m and from a water surface up to 54 m. Open space bats do not react to the background in their echolocation behavior. This may indicate that they do not need to adjust their flight maneuvers in relation to the background.

After the detection of prey all open space foragers start with an approach sequence where pulse interval and pulse duration are reduced and signal bandwidth is increased with decreasing distance to prey. The approach sequence always ends with a distinct terminal group consisting of buzz I and buzz II. In buzz I pulse interval is further reduced, buzz II is characterized by a minimal and constant pulse interval of approximately 6 ms, and in some species also by a lower signal frequency (**Figure 5**). Open space aerial foragers are mainly found in the

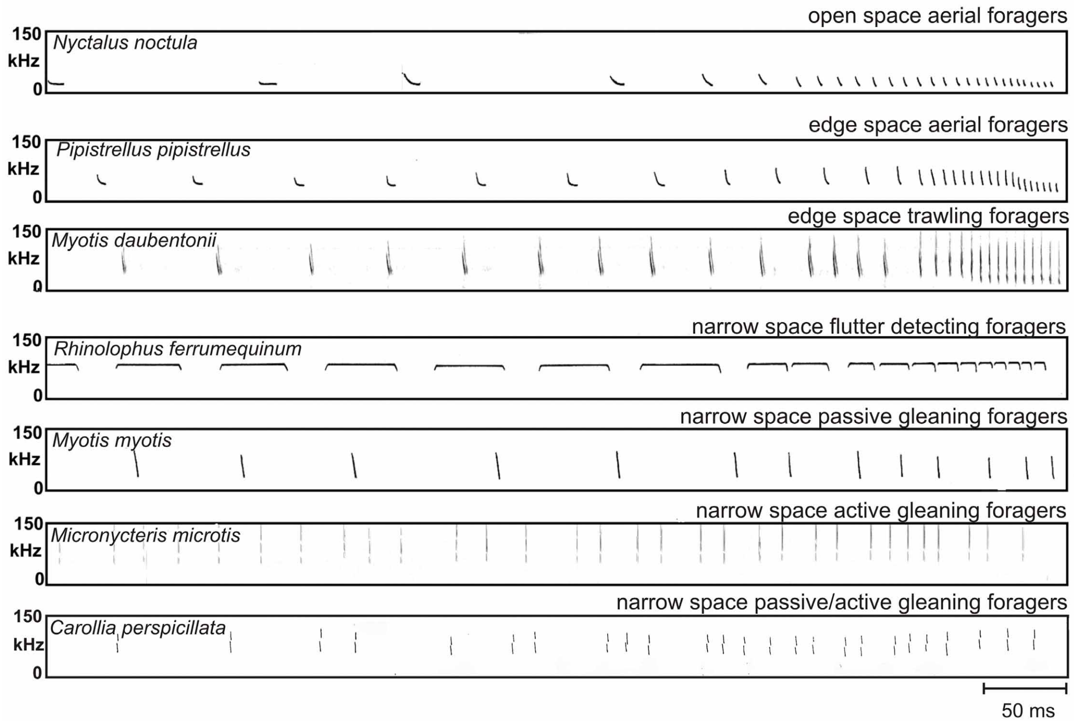

FIGURE 5 | Search and approach signals of a representative species from each guild. The approach sequences of open space and edge space foragers end with a terminal group consisting of buzz I and buzz II. Narrow space flutter detecting foragers maintain the CF-component of the calls even in the shortest signals of the terminal group. The approach sequences of all other narrow space gleaning foragers lack a distinct terminal group. The approach signals of narrow space passive gleaners are often arranged in groups, but grouping is less distinct and pulse intervals are larger than in active and passive/active gleaning foragers. Echolocation is exclusively used for landing control. The approach signals of narrow space active and passive/active gleaning foragers are clearly arranged in groups of two to five. Repetition rate is higher than in passive gleaning foragers. Echolocation is used to approach a stationary identified food item and to evaluate the orientation of the prey in order to grab it.

families of Rhinopomatidae, Emballonuridae, Vespertilionidae and Molossidae.

EDGE SPACE AERIAL FORAGERS

Bats that hunt for airborne prey in edge space have to find their food in the vicinity of background targets. In parallel they have to determine their own position, adjust their flight path and flight maneuvers in relation to the background, and avoid collisions. Additionally, they have to collect the information necessary for biotope recognition. These bats are assigned to the guild of "edge space aerial foragers." To perform these echolocation tasks, edge space foragers use mixed search signals containing a shallowly modulated narrowband component preceded and/or followed by a broadband, steeply downward frequency-modulated component. The signals have an intermediate duration of about 3–10 ms and are emitted every wing beat or, if bats fly close to the background, in groups of two signals. The frequency of the shallowly modulated component is species-specific and mostly between 30 and 60 kHz in the relevant harmonic. The shallowly modulated part is suited for the detection of insects at intermediate distances, i.e., between 1.5 and 7 m (Stilz and Schnitzler, 2012). The broadband and steeply modulated signal component is suited to localize and classify background targets. Thus, it is most likely used to control maneuvers in the vicinity of background objects, including obstacle avoidance. The source levels of edge space aerial foragers are somewhat lower than those of bats that forage in open space and range from 101 to 107 dB SPL re 1 m (Holderied and von Helversen, 2003; Surlykke and Kalko, 2008).

Bats change the structure of their signals when they come closer to the background (Schaub and Schnitzler, 2007). Bandwidth is increased, duration is reduced (**Figure 3**) and often two signals per wing beat are emitted to increase the update rate. The reduction of signal duration keeps the overlap-free window open (Kalko and Schnitzler, 1993) and a higher sweep rate resulting from a shortening of the signal duration and an increase in bandwidth additionally increases the localization accuracy. At least for some species it has been shown that they also reduce the emission SPL when they approach the background (Surlykke and Kalko, 2008; Brinkløv et al., 2010). The approach sequences of edge space bats also end with a terminal group consisting of buzz I and buzz II (Schnitzler et al., 1987; Denzinger et al., 2001; Ratcliffe et al., 2011). Edge space aerial foragers are mainly found in the families of Emballonuridae, Mormoopidae, Vespertilionidae, and Mollossidae.

EDGE SPACE TRAWLING FORAGERS

Bats belonging to the guild of "edge space trawling foragers" are found in at least three bat families: Vespertilionidae [*Myotis adversus* (Thompson and Fenton, 1982), *Myotis albescens* (Kalko et al., 1996), *Myotis daubentonii* (Kalko and Schnitzler, 1989), *Myotis dasycneme* (Britton et al., 1997), *Myotis capaccinii* (Kalko, 1990), *Myotis vivesi* (Blood and Clark, 1998), *Myotis ricketti* (Ma et al., 2007)], Noctilionidae [*Noctilio leporinus* (Schnitzler et al., 1994), *Noctilio albiventris* (Kalko et al., 1998)] and Phyllostomidae [*Macrophyllum macrophyllum* (Weinbeer and Kalko, 2007)]. Trawling foragers fly at low height above water. They either hunt for insects drifting on or flying just above calm water surfaces or for fish. Fish is detected either directly when it jumps out of the water or by the water drops arising when the fish breaks through the water surface. The sound waves that hit the water are reflected away from the bat except for those that hit the water surface in a perpendicular way, right below the bat. This echo encodes the flight height. When trawling bats hunt for prey in the vicinity of the shore they encounter similar echolocation scenes as edge space aerial foragers. On clean water surfaces the isolated prey echo is followed by the background echoes from the shore. Edge space trawling foragers have difficulties to detect prey if the water is turbulent or covered with ripples (Frenckell and Barclay, 1987; Rydell et al., 1999; Warren et al., 2000) or if plants or debris is floating on the water surface (Boonman et al., 1998). In this case the prey echo is hidden in clutter echoes (Siemers et al., 2001). If trawling bats search for prey far away from the shore, e.g., on a lake, the echolocation scene may even be similar to that of open space bats.

In search flight *Myotis* species emit mixed signals which contain steeply modulated components with a more shallowly modulated component in between. The species-specific peak frequencies of the shallowly modulated components are between 30 and 60 kHz. The signals have an intermediate duration of 3–7 ms and either one or two signals per wing beat are emitted (Kalko and Schnitzler, 1989; Jones and Rayner, 1991; Britton et al., 1997) (**Figure 5**). *M. macrophyllum* emits multiharmonic signals. The main energy is in the second or third harmonic with frequencies above 50 kHz. Signals have an intermediate duration of 2–4 ms (Brinkløv et al., 2010). *N. leporinus* and *N. albiventris* use a combination of pure CF-signals and mixed signals with a CF-component that is followed by a frequency modulated component. The species-specific constant frequencies are 55 and 70 kHz, respectively. The signals are usually emitted in groups. When flying low over water, the signal duration is around 6 ms but can reach up to 21 ms in *N. albiventris* when flying in high search flight (Schnitzler et al., 1994; Kalko et al., 1998). The source levels of edge space trawling foragers recorded in the field vary somewhat between species. In *M. daubentonii* the mean source level was about 100 dB SPL re 1 m (Surlykke et al., 2009) whereas *N. leporinus* and *N. albiventris* cry out much louder and reach maximal mean source levels of around 116 dB SPL re 1 m (Surlykke and Kalko, 2008). In *M. macrophyllum* the mean source level depends on the distance to background and is 85 dB SPL re 1 m in a semi-cluttered condition and 91 dB SPL re 1 m in a more open situation (Brinkløv et al., 2010). The approach sequences of all trawling *Myotis* species end with a distinct terminal group consisting of buzz I and buzz II (**Figure 5**). In *M. macrophyllum* the pulse interval is continuously reduced down to 6 ms between the last calls (Weinbeer and Kalko, 2007), a typical value for buzz II in other species. In *Noctilio* the CF component is given up in the terminal group, which distinguishes the Noctilionids from Rhinolophids and Hipposiderids (Schnitzler et al., 1994; Kalko et al., 1998; Übernickel et al., 2013).

Edge space trawling foragers show several morphological adaptations to the trawling mode. The hind legs and interfemoral pouches are highly specialized to take prey from the water surface or out of the water. Piscivorous species have sharp claws.

NARROW SPACE FLUTTER DETECTING FORAGERS

All bats that search for prey in narrow space face the problem that the prey echoes are hidden in background echoes. Bats which belong to the guild of "narrow space flutter detecting foragers" have evolved specific adaptations to overcome this problem. They use echolocation to find their prey and evaluate flutter information in the echoes of their long CF-FM signals which is encoded in a pattern of distinct amplitude and frequency modulations produced by the moving wings of the prey. The modulations are analyzed in a highly specialized hearing system with an auditory fovea. Flutter detecting foragers compensate the Doppler shifts generated by their own flight speed to keep the echo frequency within the frequency range of the auditory fovea [reviewed in Schnitzler and Denzinger (2011)]. Flutter information not only facilitates the detection but also contains information about species, size, and aspect angle of the prey (von der Emde and Menne, 1989; von der Emde and Schnitzler, 1990; Roverud et al., 1991). The short terminal FM component of the CF-FM signals is well-suited to localize background targets and the CF additionally contains flow field information that bats might use to commute along landscape contours (Müller and Schnitzler, 1999; Schnitzler et al., 2003).

Flutter detection has been evolved at least twice, in Rhinolophids and Hipposiderids and in one species of Mormoopids, *Pteronotus parnellii*. Narrow space flutter detecting foragers either hunt on the wing or from perches in the flycatcher style. Fluttering prey flying close to vegetation or sitting on surfaces is either caught in the aerial mode or gleaned from surface. In search flight signal duration in Hipposiderids is around 5–20 ms, in *P. parnellii* around 15–35 ms and in Rhinolophids around 50–80 ms. Rhinolophids mostly emit one call per wing beat, whereas *P. parnellii* often emits groups of two and Hipposderids groups with more signals. The long signal duration accounts for the very high duty cycles in narrow space flutter detecting foragers. Therefore, these bats have also been classified as "high duty cycle bats" (Neuweiler and Fenton, 1988; Fenton, 1995). The CF frequency is species-specific and ranges from about 28 kHz in *Rhinolophus paradoxolophus* to 213 kHz in *Cleotis percivali*. The approach sequence ends with a distinct terminal group. All bats of this guild have in common that the CF component is always maintained even in the shortest signals of the terminal group (**Figure 5**).

NARROW SPACE ACTIVE GLEANING FORAGERS

Bats that search for food positioned on or near background objects (e.g., an insect) or which is part of the background (e.g., a fruit or a flower) face the problem that the food echoes are hidden in clutter echoes. If they use only echolocation to solve this problem, they are assigned to the guild of "narrow space active gleaning foragers." So far only one insectivorous bat species has been identified to be a strict active gleaner that finds the prey by echolocation alone. *Micronycteris microtis*, a phyllostomid bat, forages for stationary prey items like dragon flies that sit on large leaves (Geipel et al., 2013; own unpublished data). When searching for prey *M. microtis* explore one leaf after another by approaching them oblique from above. Within about one third of a second the bats decide whether a leaf is empty. From an empty leaf the bat receives an echo train with a clutter echo from the frontal part of the leaf and an echo train with many clutter echoes from the end of the leaf and from objects behind it. All sound waves hitting the flat surface of the leaf are reflected away from the bat. Echo trains from empty leaves therefore only contain the clutter pattern without an insect echo in between, whereas leaves with prey produce an isolated additional echo between the clutter echoes (**Figure 2**). Active gleaning from a flat surface thus somehow resembles the echolocation scene in the trawling mode but on a micro time scale. In both situations a flat surface reflects the sound waves away from the bat so that the echoes from prey sitting on this surface stick out if the echolocation signals are short enough. When the bat has detected a leaf with prey it hovers on the spot or backward before it makes the final approach flight. When searching for prey bats emit multi-harmonic, ultra-short (0.2 ms), broadband and high-frequency calls with low amplitude. The signals are arranged in groups. The terminal group just before the prey is grasped contains 3–5 signals. A distinct buzz is missing (**Figure 5**).

NARROW SPACE PASSIVE GLEANING FORAGERS

Bats that encounter echolocation scenes, where the echo train does not deliver enough information to distinguish between food and background echoes, rely on prey generated cues alone to find their food. These bats are assigned to the guild of "narrow space passive gleaning foragers." Animal eating passive gleaners feed on substrate bound prey such as insects, other arthropods, and small vertebrates and rely on prey generated sounds to localize the site with prey (Schmidt et al., 2000; Arlettaz et al., 2001; Goerlitz et al., 2008; Page and Ryan, 2008). Under favorable conditions vision may also play a role in prey detection (Bell, 1985; Eklöf and Jones, 2003).

After getting alerted bats approach the prey site which is indicated by prey generated cues with sufficient accuracy. Echolocation is only used to guide the approach to the site with prey. After landing on the prey bats use mainly tactile and olfactory cues to find the prey (Kolb, 1958). Under very favorable conditions passive gleaners are able to make the transition to active gleaning, e.g., if the prey is offered on a flat surface which produces no clutter echoes. So far, this transition has been demonstrated only in the laboratory (Marimuthu et al., 1995; Schmidt et al., 2000; Flick, 2008).

All animal eating narrow space passive gleaning foragers operate with short, broadband signals with low source levels. Often two to three signals are emitted within the rhythm of the wing beat. The signals are suited for spatial orientation including obstacle avoidance and biotope recognition. During the approach to the site with food, repetition rate is increased and signals are arranged in more or less distinct groups. The terminal group contains only a few signals. This echolocation pattern is typical for the approach to a landing site (**Figure 5**). Narrow space passive gleaning foragers are found in Phyllostomidae, Megadermatidae, Nycteridae, and Vespertilionidae.

NARROW SPACE PASSIVE/ACTIVE GLEANING FORAGERS

Frugivorous and nectarivorous bats feed on fruits and nectar of bat-pollinated flowers. These targets are part of the background and their echoes have to be found between the echoes of other background targets. Fruits and flowers advertise their nature and position by species-specific odor bouquets but also by a specific position in relation to the background. Often also specific reflection properties result in food-specific conspicuous echoes (von Helversen and von Helversen, 1999, 2003; von Helversen et al., 2003; Simon et al., 2006, 2011). There is evidence that fruit and nectar eating bats use odor for a rough localization of the food source in the passive mode and echolocation for the precise localization in the active mode. Therefore, we assign all frugivorous and nectarivorous bats to a new guild called "narrow space passive/active gleaning bats."

Field studies in frugivorous and nectarivorous bats clearly show that odor is the primary cue that attracts the bats (Rieger and Jakob, 1988; Laska, 1990; Hessel and Schmidt, 1994; Thies et al., 1998; von Helversen et al., 2000; Mikich et al., 2003; Korine and Kalko, 2005). Odor can be detected over long ranges, and guides the bats close to where the food is located. However, the localization accuracy for an odor source is not very high so that bats probably cannot home in on the food only by olfactory cues. Bats therefore have to switch from the odor-guided and rather imprecise passive mode to the echolocation-guided and far more precise active mode for food localization.

The precise localization of a food source by echolocation is facilitated by specific positions and properties of bat plants and flowers. For example, *Gurania spinulosa*, a flaggelichorous curcubit, exposes its cucumber shaped fruits on pendulous leafless branches in vegetation gaps. In a flight tent *Phyllostomus hastatus* not only approached the ripe fruits with the typical odor but also fruit models without odor if they were offered at the correct position. This approach was guided only by echolocation and would therefore fulfill the condition for active gleaning (Kalko and Condon, 1998). However, the experiments also revealed that the odor of ripe fruit in combination with the proper fruit position on pendulous branches is the most effective stimulus combination to evoke a response in bats. This suggests that odor also plays an important role under natural conditions. An odor- and echolocation-guided approach to food was also described for *Carollia* species approaching piper fruits (Thies et al., 1998) and for *Artibeus watsoni* and *Vampyressa pusilla* approaching figs (Korine and Kalko, 2005).

The precise localization of a food source by echolocation is also facilitated if the echo of a food item has characteristic echo properties and differs from other background echoes. Ensonification experiments have shown that a specific disc-shaped leaf or petal on the inflorescences of some bat-pollinated plants produced spatially invariant echoes with a characteristic spectral and amplitude pattern over a wide range of sound incidence angles. These conspicuous echoes are rather loud and stick out between spatially more variable background echoes (von Helversen and von Helversen, 1999; von Helversen et al., 2003; Simon et al., 2006, 2011). Behavioral studies have shown that bats use such echo beacons to localize flowers among other background echoes. The presence of a disk-shaped model leaf reduced the search time for an artificial feeder by 50% in *Glossophaga soricina* (Simon et al., 2011) and flowers were less visited if the echo producing structures were manipulated (von Helversen and von Helversen, 1999). However, in another approach von Helversen et al. (2000) showed that odor is a very important cue which attracts species of the genus *Glossophaga* to bat-pollinated flowers. They concluded for nectarivorous bats that the sense of smell plays an important role in searching for and localizing bat-pollinated flowers.

So far all studies with frugivorous and nectarivorous bats have shown that the passive and rather imprecise localization of food with odor as well as the active and precise localization of food with echolocation play a role in the foraging process. The degree of overlap between the two modes and their relative importance for the foraging process may differ between species. Our attribution does not exclude the possibility that under favorable conditions only odor or only echolocation can guide a species successfully to their food sources.

The echolocation signals of narrow space passive/active foragers are short, multi-harmonic and broadband. They have high frequencies and low source levels to reduce clutter echoes from the background. Signals are often arranged in groups and the approach sequences lack a typical buzz (**Figure 5**). The echolocation behavior is rather similar to that of the pure active gleaner *M. microtis* which may indicate that a stationary, rather large, identified food item is approached under the guidance of echolocation. Narrow space passive/active foragers are only found in the family of Phyllostomidae.

In theory, there might be animal eating bats that forage in the active mode and also use olfactory cues from prey to get close to the site with food. So far there are no hints that bats flying and searching for food in the active mode use olfactory cues to find their animal prey. If these bats would use olfactory cues they should be assigned to the guild of narrow space passive/active gleaning foragers.

ADAPTATIONS IN WING MORPHOLOGY

Bats are not only adapted in their echolocation systems to where and how they forage for prey but also in their morphology (Fenton, 1990). The most obvious ecomorphological adaptation is the shape of the wings, which reflects the demands on flight performance when foraging under particular ecological conditions. Meaningful parameters that describe the size and shape of wings are wing loading, aspect ratio and shape of the wing tip (Norberg and Rayner, 1987; Norberg, 1994). Typical open space foragers have small pointed wings with high aspect ratio which give high agility. Such a wing is adapted for a fast aerial hawking flight. Edge space foragers fly slower and are more maneuverable than open space foragers. Their wings have average aspect ratios and wing loadings and rounded tips. These wings are adapted for slow inexpensive flight in the vicinity of background objects. Edge space trawling foragers have long wings and a higher aspect ratio than most other bats but have only a medium wing loading. Such a wing is adapted for economic flight above water surfaces that allows also slow flight. All narrow space bats have short and broad wings with low aspect ratios, low wing loading and often very rounded wing tips which are adaptations for high maneuverability and slow flight in confined spaces (Norberg and Rayner, 1987; Norberg, 1994). The relation between habitat specific demands on flight performance and wing morphology is obvious. However, within guilds there are many fine grained differences in wing morphology that may reflect adaptations to different niches (Dietz et al., 2006).

ASSIGNING BAT SPECIES TO GUILDS

Bats can be highly flexible in their habitat use and also in their foraging modes (Fenton, 1990; Schnitzler and Kalko, 1998; Denzinger et al., 2004). Bats that mainly forage in the gleaning mode in narrow space can also fly in edge space and maybe forage there in the aerial mode, and edge space aerial foragers very often also search for prey in open space. When moving from one habitat to another and when changing the foraging mode bats also change their echolocation behavior and use the habitat- and mode-specific signal types and sound patterns. For example, aerial-hawking pipistrelles switch from more broadband mixed search signals in edge space to longer pure narrowband signals in open space (Kalko and Schnitzler, 1993). However, there are limits to the behavioral flexibility which are mainly determined by the motor capabilities of the bats (Schnitzler and Kalko, 1998). Typical open space foragers such as *Tadarida* species always forage in open space as their habitat-specific wing morphology is not suited for maneuvering near background targets. Most edge space aerial foragers do not have the motor abilities to maneuver in close vicinity to background objects necessary to exploit resources in narrow space. The access of a species to a more open habitat type is possible, but not the reverse (Fenton, 1990). Despite the behavioral flexibility found in some bats they can also be assigned to a specific guild according to their dominant foraging behavior for which their echolocation system and their wing morphology are best adapted.

The criterion for the attribution of bats to the guild of narrow space flutter detecting foragers is the use of long CF-FM echolocation signals for flutter detection and the compensation of Doppler shifts. All flutter detecting foragers maintain the CF component in all behavioral situations even in the shortest signals of the terminal group of the approach. *Noctilio* species and some smaller mormoopids sometimes also use CF-FM search signals. However, they switch to pure FM signals when approaching prey. Additionally, they do not have a sharply tuned auditory fovea and a sophisticated Doppler shift compensation system (Schnitzler and Denzinger, 2011). Therefore, we do not classify them as flutter detecting foragers.

Narrow space foragers are attributed to the guild of passive gleaning foragers if they find the preferred food source only based on passive cues. Bats that find their food relying only on echolocation are assigned to the guild of active gleaning foragers. In critical tests for the attribution to one of the guilds, passive gleaning foragers should approach a loudspeaker with playback signals from the prey, and active gleaning foragers should approach a stationary silent and non-smelling insect dummy on a leaf.

In this paper we propose a new guild of "narrow space passive/active foragers" that comprises all frugivorous and nectarivorous bats. Most bat fruits and flowers advertise their presence and position by species-specific odor bouquets as well as by specific reflection properties which produce a conspicuous echo. In their typical foraging pattern frugivorous and nectarivorous bats use both, odor and echolocation information, to find their food. We are aware that under favorable conditions odor alone or echolocation alone can guide bats to their food.

Some species are highly variable in their use of foraging modes and diets which makes it difficult to assign them to a specific guild. For example *Phyllostomus hastatus* "glean a wide variety of animal and vegetable food" (Kalko et al., 1996). They feed on insects and small vertebrates as well as on nectar, pollen, and fruit. Most likely, they use the passive/active mode for fruit and nectar acquisition, reason why we attribute *P. hastatus* to the guild of narrow space passive/active foragers.

With the guild concept we group together species that live under similar ecological conditions, perform similar tasks, and share similar sensory and motor adaptations. The foraging and echolocation behaviors of all members of a guild are so similar that the observed behavioral patterns of well-investigated species have a high predictive value for other less studied species of the same guild (**Figure 5**).

NICHE DIFFERENTIATION WITHIN GUILDS

Bat species from different guilds differ in their foraging behavior and in the environmental resources they use. Therefore, they do not compete for food even if they belong to the same genus. An example is *Myotis nattereri*, an edge space aerial-hawking forager, and *Myotis bechsteinii*, a narrow space passive gleaning forager. The diets of the two species differ significantly, reflecting the differences in the location where they search for prey and how they find it (Siemers and Swift, 2006).

In contrast, sympatric living species that belong to the same guild exploit similar resources and show rather similar foraging and echolocation behaviors. The members of a guild encounter the same possibly limited food resources and may face the problem of how to avoid competition. Sympatric living bats within a guild should therefore differ in at least one niche dimension. Niche differentiation can be achieved by several mechanisms such as differences in echolocation performance, sensory and cognitive abilities, maneuverability and other adaptations of the motor system, spatial segregation of foraging areas, and biogeography.

Differences in echolocation behavior especially in signal frequency but also in duration and bandwidth may account for niche partitioning within a guild (Denzinger et al., 2004; Siemers and Schnitzler, 2004). With decreasing frequency the maximum detection distance increases and directionality decreases (Stilz and Schnitzler, 2012). Thus, frequency has a huge effect on the search volume of bats which strongly increases with decreasing frequency. Signal frequency also determines the target strength of prey which depends on the relationship between the wavelength of the echolocation signal and target size. If the wing length of a prey insect is around and below the wavelength of the echolocation signal the target strength is reduced by Raleigh scattering (Houston et al., 2004). At a signal frequency of 10 kHz the critical Raleigh region is reached for wing lengths below 34 mm and at a frequency of 100 kHz for wing lengths below 3.4 mm. Bats operating with lower frequencies thus have a lower detection probability for small insects which may result in resource partitioning between sympatric species. Shi et al. (2009) present data which support this hypothesis. They compared two CF-bats with similar size but different CF-frequency and found that *Rhinolophus macrotis*, a low-frequency horseshoe bat with a CF-frequency of 57 kHz, fed in general on larger prey with wing lengths ranging from 5.2 to 37.1 mm than *R. lepidus* a high-frequency horseshoe bat with a CF-frequency of 91 kHz and wing lengths between 3.5 and 27.5 mm. Signal duration is another parameter which influences the detection probability for different sized insects. Long signals produce a wide signal overlap zone which hampers the detection of weak echoes from small insects at close range. Long signals with low frequency are mainly produced by open space foragers. Since long signals and also low frequencies reduce the probability for the detection of smaller insects in bats, Schnitzler and Kalko (1998) proposed the size filtering hypothesis. The lower the frequency and the longer the signals the larger is the just detectable prey. Bats with long signals and low frequencies are adapted for the long range detection of large insects but miss smaller ones whereas bats with shorter signals and higher frequencies have shorter detection ranges but, additionally, find insects which are smaller and fly closer to them. This general trend has been confirmed by a number of studies (e.g., Barclay, 1985, 1986; Kalko, 1995; Houston et al., 2004). The role of bandwidth in niche differentiation was demonstrated for some morphologically similar and sympatric edge space aerial/trawling species of the genus *Myotis* (Siemers and Schnitzler, 2004). The performance to detect prey in front of a clutter producing background depended on the bandwidth of the echolocation signals (**Figure 6**). The minimal detection distance decreased with increasing bandwidth thus indicating that differences in the echolocation system result in sensory based niche partitioning. Comparable studies with paleotropical species of the vespertilionid subfamilies Kerivoulinae and Murininae came to similar results (Schmieder et al., 2012).

There are, however, many other mechanisms besides echolocation that account for niche differentiation. Niche differentiation by spatial segregation in foraging areas has been shown for the passive gleaners *Myotis myotis* and *Myotis blythii*. While *M. blythii* depends on grassland habitats *M. myotis* selects foraging areas with access to ground-dwelling prey (Arlettaz, 1999). The spatial separation is also mirrored in the trophic niche separation of the two species (Arlettaz et al., 1997). The five species of European horseshoe bats constitute another interesting example for niche partitioning. They belong to the guild of flutter detecting foragers and have a rather similar echolocation behavior with only small differences in the species-specific frequency of the CF-FM signals. Nevertheless, they differ in foraging area, food preferences, and whether they search for fluttering prey from perches or on the wing (Dietz et al., 2007). Dietz et al. (2006) found differences in wing morphology between the species which may be just one among other mechanisms that account for the observed niche differentiation.

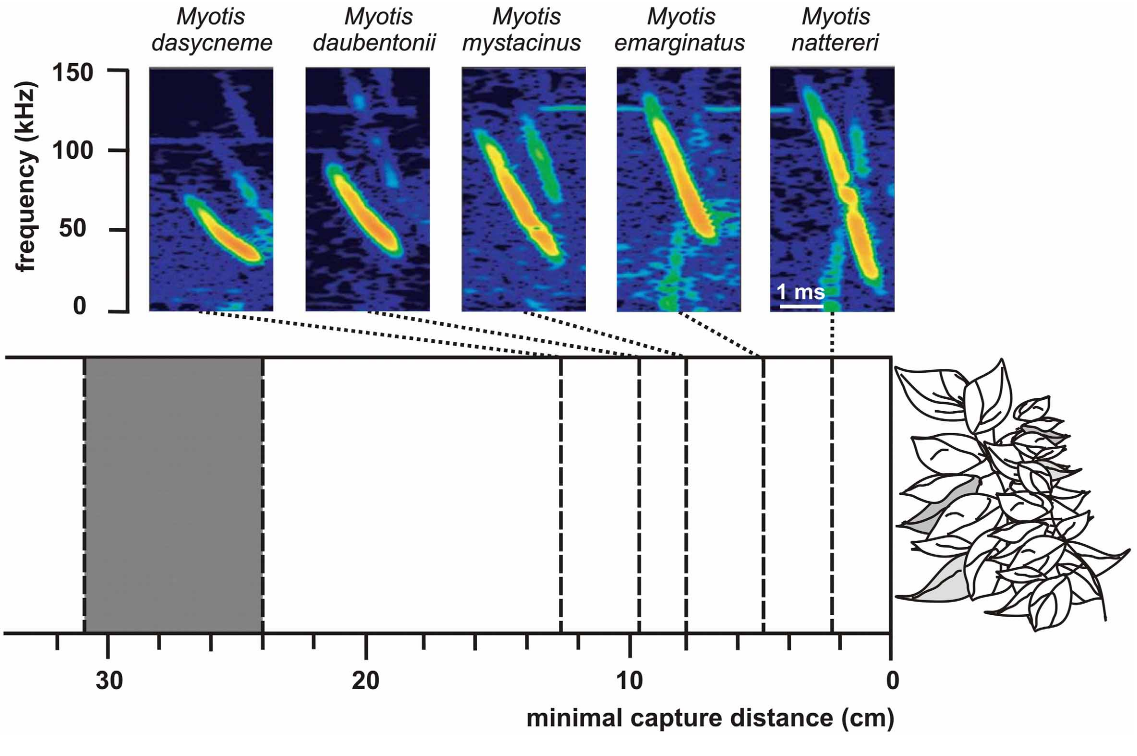

FIGURE 6 | Search call structure in relation to minimal capture distance (success rate 50%) in 5 sympatric *Myotis* species. The higher the signal bandwidth of a species the lower is the minimal capture distance for suspended mealworms. The gray block between 24 and 31 cm indicates the range of the outer borders of the clutter overlap zones of the five bats as calculated from the sound durations of the signals. Note that the performance which is an indicator for the masking effect of the clutter echoes strongly depends on signal structure [data from Siemers and Schnitzler (2004)].

CONCLUDING REMARKS

Many bat communities comprise a large number of species with a high diversity in foraging and echolocation behaviors. The assignment of species living under similar constraints and performing comparable tasks into functional groups or guilds identifies patterns of community structure and helps us to understand the factors that underlie the organization of the highly diverse bat communities. Bats within each guild forage under similar ecological conditions and share comparable sensory and motor adaptations. These task-specific adaptations have a high predictive value for the assignment of bats into a guild. Habitat and foraging mode predict the echolocation behavior of a species and vice versa echolocation behavior predicts to which guild a bat can be assigned. Bat species from different guilds do not compete for food as they differ in the environmental resources they use and in their foraging behavior. However, sympatric living species belonging to the same guild often exploit the same class of resources. To avoid competition they should differ in at least one niche dimension. The fine grain structure of bat communities below the rather coarse classification into guilds is determined by mechanisms that result in niche partitioning.

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Behavioral flexibility of the trawling long-legged bat, *Macrophyllum macrophyllum* (Phyllostomidae)

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We assessed the behavioral flexibility of the trawling long-legged bat, *Macrophyllum macrophyllum* (Phyllostomidae) in flight cage experiments by exposing it to prey suspended from nylon threads in the air and to food placed onto the water surface at varying distances to clutter-producing background (water plants). The bat revealed flexibility in foraging mode and caught prey in the air (aerial hawking) and from the water surface (trawling). *M. macrophyllum* was constrained in finding food very near to and within clutter. As echolocation was the prime sensory mode used by *M. macrophyllum* for detection and localization of food, the bat might have been unable to perceive sufficient information from prey near clutter as background echoes from the water plant increasingly overlapped with echoes from food. The importance of echolocation for foraging is reflected in a stereotypic call pattern of *M. macrophyllum* that resembles other aerial insectivorous and trawling bats with a pronounced terminal phase (buzz) prior to capture attempts. Our findings contrast studies of other phyllostomid bats that glean prey very near or from vegetation, often using additional sensory cues, such as prey-produced noise, to find food and that lack a terminal phase in echolocation behavior. In *M. macrophyllum*, acoustic characteristics of its foraging habitat have shaped its sonar system more than phylogeny.

Keywords: sensory ecology, aerial hawking, gleaning, bat echolocation, clutter, echo overlap

INTRODUCTION

Species with a flexible use of behavioral strategies while hunting are likely to have access to more resources and exploit habitats better than species which are restricted to a specific foraging mode and hence a specific type of prey (Neuweiler, 1989, 1990). Generally, flexibility in foraging behavior often requires specific sensory adaptations as the bats may face different perceptual challenges imposed by different foraging modes. In addition, characteristics of the foraging habitat, in particular the relative position of food to the background clutter, strongly affect how bats find food, and determine the role of echolocation while foraging (Neuweiler, 1990; Schnitzler et al., 2003b).

Trawling bats, which collect insects or small fish from the water surface, such as *Noctilio* sp. (Noctilionidae) and some *Myotis* sp. (Vespertilionidae), are also known to hawk aerial prey and thus exhibit high flexibility in foraging behavior. This allows them to also take advantage of the insect-rich space above water bodies (Jones and Rayner, 1988, 1991; Schnitzler et al., 1994; Britton et al., 1997; Kalko et al., 1998). While trawling, the smooth water surface reflects most of the call energy away from the low flying animals and thus, little or no clutter echoes interfere with prey perception (Boonman et al., 1998; Siemers et al., 2001b). This leads, in conjunction with rather high sound intensities (Surlykke and Kalko, 2008) and despite high calling frequencies, to increased prey detection distances (Siemers et al., 2005). Foraging over water thus poses a perceptual task that is similar to aerial hawking of insects in open space. In both cases, echolocation represents the prime cue for finding and locating food.

The situation of trawling bats hunting over smooth water surfaces however strongly contrasts with bats that collect stationary food (gleaning) in cluttered environments such as fruits or insects next to vegetation. Gleaning bats face the sensorial challenge that clutter echoes often overlap target echoes (clutter overlap zone, Denzinger and Schnitzler, 2013), and thus frequently use additional sensory cues, in particular vision, olfaction, or prey-generated acoustic cues for finding food (e.g., Fenton, 1990; Fuzessery et al., 1993; Arlettaz et al., 2001; Schnitzler and Kalko, 2001; Altringham and Fenton, 2004). Most New World leaf-nosed bats (Phyllostomidae) are classified as gleaners as they typically take food close to or from surfaces in narrow space habitats near or within vegetation (Schnitzler and Kalko, 2001). Echolocation in Phyllostomids is primarily used for orientation in space and supplemented with additional sensorial information for finding food. Probably as an adaptation to cluttered environments, Phyllostomid bats emit rather uniform, short, high-frequency multi-harmonic and steep frequency-modulated (FM) broadband echolocation calls, which are well suited for measuring distances in confined space and to assess surface structures (Kalko and Condon, 1998; Thies et al., 1998; Kalko, 2004; Geipel et al., 2013). Previously, Phyllostomid bats have been mostly regarded as "whispering" bats with low sound intensities, but recent studies point toward much higher sound intensities associated with high signal directionality (Brinkløv et al., 2009). During target approach, the echolocation behavior of foraging phyllostomid bats differs from aerial insectivores as they do not emit a characteristic terminal phase or buzz prior to prey capture (a series of very short calls emitted at a high repetition rate; Neuweiler, 1989; Schnitzler et al., 2003b). Terminal phases of aerial hawking bats are thought to increase the information flow of moving prey, while reducing the overlap between emitted signals and returning echoes (signal overlap zone, Denzinger and Schnitzler, 2013), and minimize doppler-dependent ranging errors for prey localization (Holderied et al., 2008).

Unique among phyllostomid bats, the long-legged bat, *Macrophyllum macrophyllum* hunts over water (Harrison, 1975). The acoustic characteristics of this habitat resemble more (semi)-open than cluttered space as most signal energy is reflected away from the smooth water surface. In contrast to all other phyllostomid bats studied so far, the call pattern of trawling *M. macrophyllum* resembles that of aerial insectivorous and other trawling bats of different families (Jones and Rayner, 1988, 1991; Schnitzler et al., 1994; Kalko et al., 1998; Schnitzler and Kalko, 2001; Siemers et al., 2001a; Weinbeer and Kalko, 2007). While trawling for prey above open water it comprises a distinct search, approach and terminal phase (Weinbeer and Kalko, 2007).

In nature *M. macrophyllum* exhibits high flexibility in its foraging behavior. It mostly trawls insects from smooth water surfaces (Weinbeer et al., 2006), but was also observed catching insects in the air, as well as foraging close to banks of protruding water plants, *Hydrilla verticillata* (Hydrocharitaceae; Meyer et al., 2005). Presence of clutter-producing objects on the water surface however may affect prey perception by echolocation and reduce capture success due to effects of echo overlap (Schnitzler and Kalko, 2001) and the lack of an echo-acoustic ground effect (Zsebok et al., 2013). This has been shown previously in the insectivorous trawling bat *Myotis daubentonii*. To avoid overlap effects between echoes of prey and clutter, *M. daubentonii* changes its foraging strategy from trawling to aerial hunting, when the amount of clutter producing duckweed floating on the water surface reached a certain threshold (Boonman et al., 1998).

Here we investigate how the Neotropical leaf-nosed bat *M. macrophyllum* adjusts its flight and echolocation behavior according to sensorial challenges while trawling or aerial hunting. In particular, we assessed if background clutter elicit a behavioral change in foraging strategy—as known for aerial insectivorous bats—or a switch to other sensorial cues for prey detection—as it has previously been documented for most Phyllostomids.

If *M. macrophyllum* behaves like other trawling bats and continues to use exclusively echolocation for finding prey close to clutter, we hypothesize that capture success should decrease with proximity to vegetation. Furthermore, echolocation behavior should remain highly structured including search and approach calls and a terminal phase prior to prey capture. However, if *M. macrophyllum* behaves similarly to other phyllostomid bats, it should rather use other sensory cues such as prey-generated noise, vision or scent in a clutter situation. In this case, we expected *M. macrophyllum* to forage successfully even with prey close to or within clutter while omitting a distinct terminal phase.

To test these propositions, we presented prey under controlled experimental conditions in a flight cage to individual *M. macrophyllum* and assessed how proximity of food to horizontal clutter on the water surface affects foraging and echolocation behavior. Prey was offered to the bats either suspended in the air or placed onto the water surface at varying distances to clutter producing water plants. Finally, we compared echolocation and foraging behavior of the bat during the different tasks and between flight cage and field conditions to assess the influence of confined space onto call structure.

MATERIALS AND METHODS

STUDY ANIMALS

Over a period of 6 months (January–June 2003) we studied foraging and echolocation behavior of *M. macrophyllum* by conducting behavioral experiments in the flight cage and additional observations of free flying individuals on Barro Colorado Island (BCI), a field station of the Smithsonian Tropical Research Institute in Panamá. For behavioral experiments we caught nine adult (four females, five males) *M. macrophyllum* at a known roost site (Meyer et al., 2005). They were subsequently transferred into a flight cage (4.5 m × 4.5 m × 2 m) located inside the forest of BCI and kept individually for four consecutive nights each. Temperature, humidity, and noise level in the flight cage were similar to ambient values. After the behavioral experiments, all individuals were released back into the colony. In addition to these experiments, we also studied flight and prey capture behavior of *M. macrophyllum* in the field, foraging for ordinary prey under unaffected, natural conditions close (within 50 m) to their colony in a small cove next to the field station (for details see Meyer et al., 2005).

EXPERIMENTAL SETUP IN THE FLIGHT CAGE

During behavioral experiments in the flight cage individual bats were exposed to prey (mealworms: larvae of *Tenebrio molitor*, Tenebrionidae) suspended in the air and on the water surface of a basin (3 m × 2 m) at varying distances to clutter-producing water plants. We chose mealworms as they come closest to one of the main foods of *M. macrophyllum* feeding mainly on small insects including water striders (pers. observations).

In the first set of experiments we tested the ability of *M. macrophyllum* to detect, classify and localize aerial prey using echolocation. We therefore suspended frozen (no movement, no scent) and live mealworms (wiggling, scent) on a thin (0.1 mm) nylon thread 20 cm above the water surface and recorded the bats' capture success. Experiments with mealworms suspended in air were arbitrarily interspersed by experiments with mealworms floating on the water surface (Weinbeer and Kalko, 2007) to impede accustoming of the bats to a particular situation.

To assess the influence of clutter overlap on prey detection ability of *M. macrophyllum*, we conducted a second set of experiments, in which we exposed foraging bats to various amounts of clutter. We positioned a mat of about 0.5 m × 1 m of *Hydrilla verticillata* (Hydrocharitaceae) on the surface of the water basin. *Hydrilla* is a common water plant that regularly occurs within the foraging habitat of *M. macrophyllum* in Panamá. We conducted six different trials, in which we either placed mealworms onto the water surface at 20 cm, 10 cm, and 0 cm distance to the clutter-producing plants, or presented mealworms 20 cm above the water surface either at 20 cm in front of the *H. verticillata* bank, at the edge, or 20 cm over the clutter mat (**Figure 1**).

The full set of experiments was conducted first in randomized order for each bat and was then repeated several times, also in randomized order. Capture attempts were defined as successful when the bats directed their flight toward the mealworm, touched it, and subsequently removed it from the water surface or from the thread. Behavioral sequences were defined as unsuccessful when bats searched for food emitting search calls only, but passed the mealworm three or more times without any obvious behavioral attempt to approach and remove it. All behavioral experiments were conducted under low intensity of infrared light conditions, which is beyond the spectral range of vision in Phyllostomids (Winter et al., 2003).

ANALYSIS OF FLIGHT AND ECHOLOCATION BEHAVIOR DURING BEHAVIORAL EXPERIMENTS AND IN THE FIELD

Flight behavior of bats during all experiments in the flight cage and all observations in the field was recorded with two CCD video cameras (Sanyo, VC 1950; resolution of half-frames: 20 ms) under infrared flash illumination. Simultaneously, echolocation calls of foraging *M. macrophyllum* were picked up by an ultrasound microphone, amplified and digitized (sampling rate: 312.5 kHz, 16 bit) with a custom-made system (Department of Animal Physiology, University of Tübingen, Germany). Calls were recorded at 1/15 of original speed onto a Sony Walkman professional (WM-DC6; Maxell XL-II 90 audiotape). Video sequences were synchronized with echolocation recordings (for details see Weinbeer and Kalko, 2007). For our analysis we randomly chose one video sequence per individual with a good signal-to-noise-ratio in the parallel acoustic recordings to avoid pseudoreplication. In total we thus analyzed nine video sequences, one of each bat hawking aerial prey in the flight cage, and eight sequences of bats in the field (originating from different individuals) with the program Simi Motion (Version 6.0, 2002, 85705 Unterschleißheim, Germany) for three-dimensional reconstruction of flight paths, speed, and bat-prey distance.

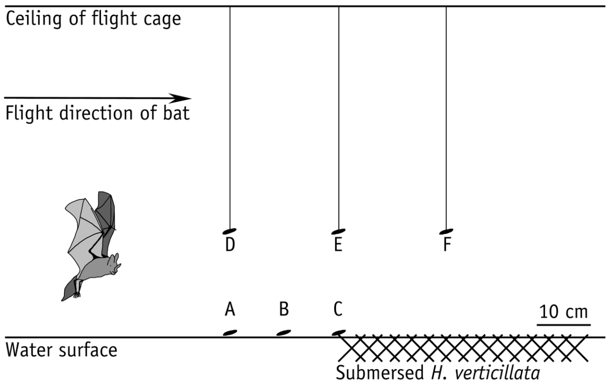

FIGURE 1 | Array of clutter experiments in the flight cage on BCI, Panamá, with *M. macrophyllum* searching for mealworms offered on the water surface (a–c) or tethered on a nylon thread (d–f) at different distances to horizontal clutter produced by leaves of *H. verticillata* water plants floating on the water surface.

Analysis of echolocation call sequences was conducted using Avisoft SAS-Lab Pro (Version 4.2). Slowed-down signals were re-digitized (sampling rate 22.05 kHz), processed through a FFT, and displayed as color sonograms; spectrograms (FFT 512 points, Hamming window) were generated resulting in a frequency resolution of 646 Hz and a time resolution of 0.893 ms. Measurements were taken with a cursor on screen. We measured seven call parameters and limited our measurement to the second harmonic as it consistently contained the main signal energy of the multi-harmonic calls of *M. macrophyllum*. Based on sono- and oscillograms, we measured pulse duration [ms], pulse interval [ms] (difference between starting time of two consecutive calls), bandwidth [kHz], and peak frequency [kHz] (frequency at maximum amplitude). We also calculated repetition rate [calls/s] (number of calls per time unit), sweep rate [kHz/ms] (bandwidth divided by pulse duration), and duty cycle [%] (percentage of time in which signals are emitted). Measurements were taken at the point where call energy clearly exceeded background noise. This was at a minimum of 25 dB for search and early approach calls, sometimes declining to less (down to about 10 dB) for faint calls prior to capture.

For all statistical tests we used individuals as a statistical unit to avoid pseudoreplication. Herby, we only considered sequences with a good signal-to-noise-ratio and then randomly selected sequences for further analyses. For the first set of experiments, we chose two out of 14–21 echolocation sequences from each individual per experiment. We analyzed and compared flight and echolocation behavior during aerial hawking and assessed potential behavioral variability between hawking of live and dead mealworms. During our second set of experiments in order to assess the influence of clutter on echolocation behavior, we chose one echolocation sequence (out of 9–12) for each individual per trial. Finally, we selected 11 echolocation sequences (out of 65), recorded in 3 nights from bats foraging under natural conditions in the field. This reduced the possibility to include recordings of the same individual several times in the analysis. We then assessed differences in flight and echolocation performance between the confined space of the flight cage and the field. For more details see Weinbeer and Kalko (2007) and Brinkløv et al. (2010).

Following Schnitzler et al. (2003a), we described changes in echolocation behavior and correlated them with characteristic stages in foraging behavior. We thus discriminated between search calls (in *M. macrophyllum* usually regular groups of two calls, rarely a single call), approach calls (usually starting with a group of three up to seven calls and several subsequent groups of varying numbers of calls), and a distinct terminal phase or buzz emitted at a high repetition rate prior to capture (Weinbeer and Kalko, 2007).

To assess echolocation call parameters during foraging stages we calculated means per sequence over search and approach call parameters, respectively. For terminal phase calls however, which changed considerably over the course of the buzz, we separately analyzed the first call, the numerically median call, the call with shortest pulse interval (usually third to fifth last call), and the last call within the buzz sequence. In the first set of experiments with prey suspended in air, we used mean parameter values per individual of the two chosen sequences for statistical analysis to avoid pseudoreplication (Hurlbert, 1984). We then compared these results in a two-factorial Anova design (experiment ∗ individual) with those of Weinbeer and Kalko (2007) to evaluate whether echolocation behavior differs between aerial hawking of tethered prey and trawling from the water surface, while accounting for individual differences in foraging behavior. For the second set of experiments we compared echolocation call parameters between experiments with a two-factorial Anova design (experiment ∗ individual) to assess the influence of clutter on echolocation behavior of individuals. Finally, we compared our results in the flight cage with recordings from the free flying bats in the field in a two-factorial Anova design (experiment ∗ individual) to assess potential differences in flight and echolocation behavior. All values are presented as mean ± *SD*.

RESULTS

FORAGING BEHAVIOR

In our first set of behavioral experiments all nine individual bats readily caught mealworms suspended in the air 20 cm above the smooth water surface (mimicking aerial prey). For unknown reasons, one bat took only a single tethered mealworm at the beginning of the experiments. We thus excluded it from subsequent analyses. In our experiments with live ($N = 68$) and with dead ($N = 65$) mealworms the bats removed them from the thread with 100% capture success. All individuals displayed a stereotypic echolocation behavior similar to trawling *M. macrophyllum* (Weinbeer and Kalko, 2007). When closing in on aerial prey, stages in foraging behavior were tightly linked with characteristic changes in echolocation behavior, with a pronounced shift from search to approach and a distinct terminal phase prior to capture (**Figure 2**). These results strongly suggest that, as it has been shown for trawling *M. macrophyllum* (Weinbeer and Kalko, 2007), echolocation is also the primary sensory cue used by this species to detect, classify, and localize aerial insect prey.

Assessing the influence live or dead aerial mealworms may have on echolocation behavior, we found no significant differences in echolocation call parameters (two-factorial Anova; all $F_{(1,\ 7)} < 3.4$, $0.1 < p < 0.96$) between the two experiments, except for peak frequency of search calls, which was slightly higher in experiments with dead mealworms (55.9 kHz) than with live prey (55.1 kHz; $F_{(1,\ 7)} = 7.5$, $p = 0.03$). However, as this slight difference in frequency was within the range of the frequency resolution (645 Hz) of our analysis, we pooled all data of the two trials for further calculations (**Table 1**).

FLIGHT AND ECHOLOCATION BEHAVIOR DURING TARGET APPROACH

Flight and echolocation behavior prior to detection of mealworms (search phase) was similar, whether prey was suspended in air or placed onto the water surface (**Table 1**, **Figure 2**). In fact, we found no differences in echolocation call parameters between aerial or trawling prey captures (two-factorial Anova; Tukey post-hoc comparison: all $p > 0.06$; $df = 69$) in our flight cage experiments. For detailed description of trawling behavior, see Weinbeer and Kalko (2007).

As indicator for prey detection we took the last search call prior to the beginning of approach calls (Weinbeer and Kalko, 2007). Aerial hawking *M. macrophyllum* detected mealworms at distances of 1–2 m (1.5 ± 0.3 m, $N = 8$). Similar to the observations of trawling *M. macrophyllum*, aerial hunting individuals emitted groups of three to seven approach calls with an intergroup interval of 36.1 ± 2.7 ms ($N = 16$). During their target-oriented approach flight at a speed of 2.4 ± 0.3 ms^{-1} ($N = 8$), bats clearly directed head, ears, and nose leaf toward the prey. In comparison to search calls, approach calls were characterized by decreasing pulse intervals, slightly shorter and decreasing pulse duration, increasingly higher repetition rate and duty cycle, and somewhat increased bandwidth and sweep rate (**Table 1**, **Figure 2**).

At a distance of half a meter or less (mean: 0.5 ± 0.1 m; 0.4–0.5 m; $N = 8$) toward the mealworms suspended in the air, *M. macrophyllum* started to emit a terminal phase of 23 ± 3 calls (range: 16–33 calls, $N = 16$ sequences) that lasted for 203.6 ± 33.3 ms (range: 127–307 ms; $N = 16$). Flight speed was slightly reduced to 2.2 ± 0.3 ms^{-1} ($N = 8$). The terminal phase calls were emitted at a very high repetition rate and characterized by short pulse duration, decreasing bandwidth, increasing duty cycle, and steep sweep rates (**Table 1**). After the first up to 10 buzz calls, *M. macrophyllum* entered the echo-overlap zone at a distance of 0.3–0.4 m ($N = 8$) from the prey, where calls began to overlap with echoes returning from prey (**Figures 2**, **4**).

Just before prey capture, *M. macrophyllum* lowered its tail membrane, approximately perpendicular to its flight direction. It formed a pouch with its large tail membrane stabilized by its large feet, tail, and strong calcars. Head, nose leaf, and ears were directed throughout the approach toward the mealworm. Echolocation stopped a few cm in front of the food. As soon as the distal part of the tail membrane touched the suspended mealworm, the pouch was subsequently closed with the help of feet and calcars. Similar to removal of food from the water surface (Weinbeer and Kalko, 2007), feet and claws were not directly involved in the actual capture of suspended mealworms. The bat then wrapped the mealworm into its tail membrane and briefly pressed it against its abdomen. At that time, head, ears, and nose leaf were moved back into the upright position and the bat resumed echolocation. After the bat had taken the food with its mouth by bending its head quickly into the pouch, *M. macrophyllum* flew to a perch and ate it.

EFFECT OF CLUTTER ON FORAGING AND ECHOLOCATION BEHAVIOR

The second set of experiments revealed that live prey (wiggling mealworms) on the water surface was equally well detected and removed by *M. macrophyllum* when placed at 20 cm (removal rate: 100%, $N = 28$ trials) and 10 cm (97%, $N = 29$ trials) in front of the water plants. However, capture success considerably dropped (23%, $N = 31$ trials) when prey was offered right at the edge (distance 0 cm) of the clutter plot of *H. verticillata* (**Figure 1**). In contrast, when mealworms were suspended 20 cm above the water surface in the air, bats had no difficulties in detecting them in front of (20 cm: 100%, $N = 25$ trials), at the edge of (0 cm: 100%, $N = 24$ trials), or 20 cm over the *H. verticillata* plot (93%, $N = 28$ trials). These results indicate that horizontal clutter of background vegetation negatively affected prey perception and capture success while trawling, but not during aerial hawking of *M. macrophyllum*.

Table 1 | Seven echolocation parameters of *M. macrophyllum* foraging in the flight cage and in the field at BCI, Panamá.

Parameter	Habitat	Search	Approach	Terminal phase			
				F	*M*	*S*	*L*
Pulse duration [ms]	Water	2.6 ± 0.3	2.3 ± 0.2	1.9 ± 0.2	1.5 ± 0.1	1.1 ± 0.1	1.0 ± 0.2
		1.9 – 3.6	1.9 – 2.7	1.5 – 2.4	1.2 – 2.0	0.9 – 1.6	0.7 – 1.5
	Air	2.5 ± 0.2	2.3 ± 0.1	2.0 ± 0.1	1.5 ± 0.2	1.2 ± 0.2	1.1 ± 0.2
		2.1 – 2.8	2.1 – 2.5	1.7 – 2.2	1.3 – 1.7	1.0 – 1.6	0.9 – 1.6
	Field	3.2 ± 0.7**	2.6 ± 0.2**	2.1 ± 0.3*	1.4 ± 0.3	1.0 ± 0.2*	0.9 ± 0.2*
		2.2 – 4.7	2.4 – 2.8	1.6 – 2.5	0.8 – 1.9	0.6 – 1.4	0.7 – 1.4
Pulse interval [ms]	Water	43.0 ± 11.5	19.9 ± 2.0	12.5 ± 1.7	8.1 ± 1.2	6.0 ± 0.5	
		22 – 74	16 – 23	9.0 – 16	6.1 – 11	5.3 – 7.6	
	Air	44.9 ± 14.9	19.1 ± 1.4	12.6 ± 1.2	8.0 ± 1.5	6.1 ± 0.6	
		29 – 81	17 – 22	10 – 14	6.3 – 11	5.4 – 7.6	
	Field	54.5 ± 11.9	17.9 ± 3.3	10.7 ± 1.8*	7.5 ± 1.8	5.8 ± 0.8	
		30 – 66	15 – 22	9.3 – 14	5.7 – 12	5.2 – 7.8	
Repetition rate [calls/s]	Water	24.8 ± 6.4	50.7 ± 5.3	81.5 ± 11.7	125.7 ± 17.6	166.7 ± 13.9	
		14 – 46	43 – 64	61 – 112	91 – 163	132 – 190	
	Air	24.2 ± 6.7	52.5 ± 3.7	80.3 ± 8.3	128.4 ± 21.8	166.6 ± 15.8	
		12 – 35	46 – 58	70 – 97	90 – 158	131 – 185	
	Field	19.4 ± 5.5	57.8 ± 11.4	96.2 ± 17.2	139.5 ± 27.6	174.6 ± 19.6	
		15 – 33	45 – 68	70 – 108	83 – 177	128 – 193	
Band-width [kHz]	Water	23.8 ± 1.8	25.9 ± 2.0	24.9 ± 2.6	21.7 ± 3.1	17.0 ± 2.7	15.0 ± 2.7
		21 – 28	21 – 30	18 – 30	15 – 30	12 – 24	9.9 – 22
	Air	23.7 ± 2.0	26.5 ± 1.6	25.8 ± 2.9	22.6 ± 2.7	18.9 ± 3.5	16.3 ± 3.5
		22 – 28	24 – 30	21 – 31	19 – 28	14 – 25	11 – 22
	Field	25.0 ± 2.4	26.9 ± 2.5	25.1 ± 3.1	21.0 ± 4.2	17.5 ± 2.2	16.4 ± 2.2
		20 – 30	23 – 30	20 – 31	11 – 26	14 – 20	13 – 20
Peak frequency [kHz]	Water	55.2 ± 2.4	54.9 ± 2.5	54.6 ± 2.8	54.4 ± 2.5	54.7 ± 2.5	53.6 ± 2.6
		50 – 59	50 – 60	50 – 63	48 – 59	48 – 58	48 – 57
	Air	55.3 ± 2.2	54.8 ± 2.2	55.0 ± 2.6	53.9 ± 2.0	54.5 ± 1.7	54.3 ± 2.6
		51 – 58	51 – 58	49 – 59	50 – 56	51 – 58	48 – 57
	Field	54.4 ± 1.7	51.9 ± 3.1*	51.9 ± 3.7*	51.7 ± 2.6*	53.3 ± 3.1	50.8 ± 5.3*
		51 – 56	47 – 57	47 – 59	48 – 56	47 – 57	43 – 56
Sweep rate [kHz/ms]	Water	9.4 ± 1.4	11.5 ± 0.8	12.9 ± 1.1	14.4 ± 1.2	14.9 ± 1.9	15.1 ± 2.2
		7.0 – 15	9.5 – 13	10 – 15	11 – 17	12 – 19	11 – 24
	Air	9.6 ± 1.3	11.6 ± 0.8	13.0 ± 1.1	15.2 ± 1.1	15.4 ± 1.6	14.5 ± 1.5
		8.0 – 13	11 – 13	11 – 15	13 – 17	13 – 19	12 – 17
	Field	8.0 ± 1.3	10.5 ± 0.9	11.9 ± 1.1	14.8 ± 1.6	17.3 ± 3.2	18.6 ± 4.1
		5.9 – 11	9.2 – 12	10 – 14	12 – 18	12 – 22	12 – 24
Duty cycle [%]	Water	7.0 ± 1.2	11.9 ± 1.2	15.8 ± 2.2	18.8 ± 2.5	19.1 ± 3.0	
		4.8 – 11	9.8 – 15	13 – 24	13 – 27	15 – 28	
	Air	6.9 ± 1.2	12.3 ± 0.8	15.9 ± 1.5	19.1 ± 3.4	20.5 ± 3.8	
		5.2 – 9.7	11 – 13	13 – 19	15 – 27	16 – 28	
	Field	7.0 ± 1.8	15.3 ± 2.3	20.1 ± 2.8	19.5 ± 4.3	18.0 ± 3.2	
		5.3 – 11	12 – 18	16 – 23	12 – 27	12 – 23	

Presented data (mean ± SD; min–max) are based on measurements taken from 715 search calls, 1610 approach calls, and 360 terminal phase calls of nine bats trawling mealworms floating on the water surface in the flight cage (Weinbeer and Kalko, 2007); 278 search calls, 579 approach calls, and 128 terminal phase calls of eight bats hawking tethered mealworms in the flight cage; and 100 search calls, 137 approach calls, and 44 terminal phase calls of 11 sequences of bats trawling floating natural prey in the field. Significant differences between captive bats and bats in the field are given as $^{*}p < 0.05$ *and* $^{**}p < 0.001$. *Abbreviations for terminal phase: F, first call; M, numerically median call; S, call with shortest pulse interval; and L, last call.*

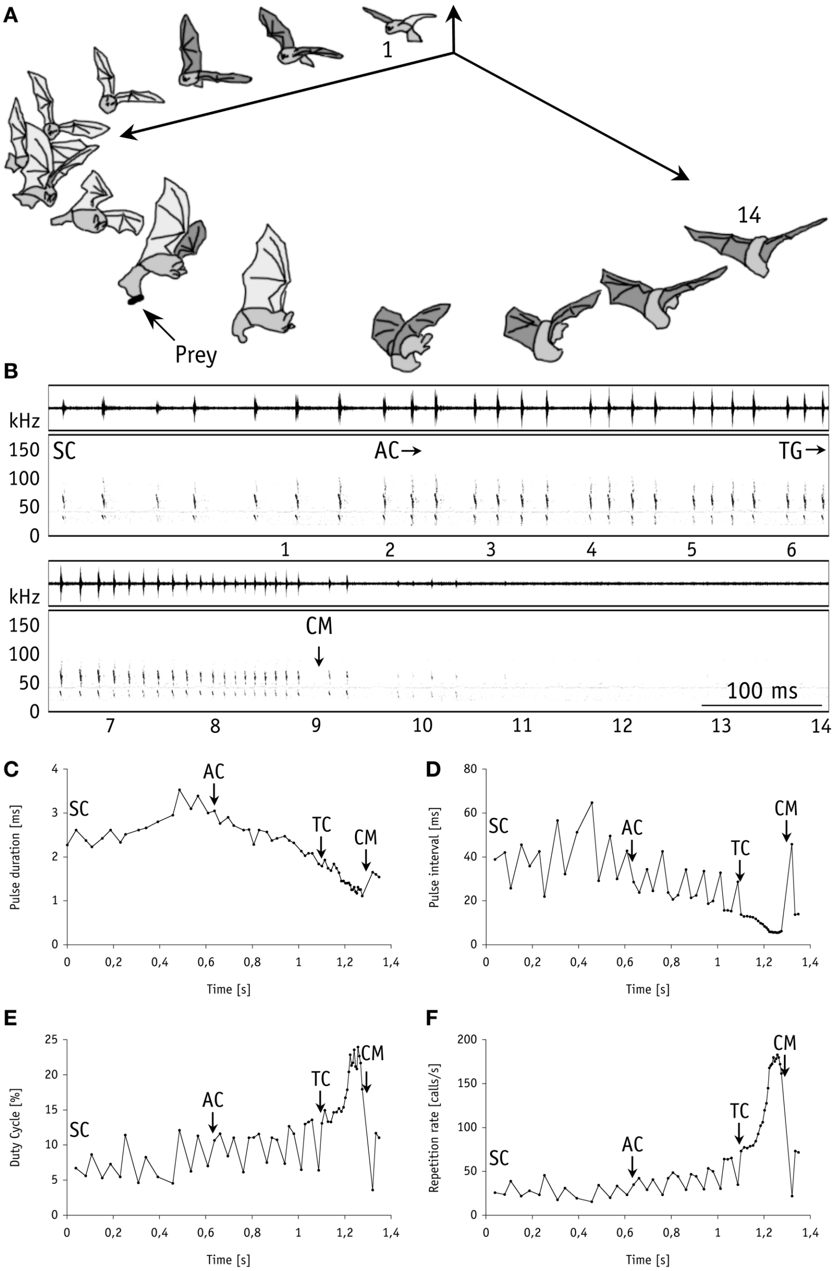

FIGURE 2 | Foraging behavior in 3-dimensional space synchronized with the corresponding echolocation sequence of *M. macrophyllum* approaching and capturing a mealworm suspended in the air above the water surface in the flight cage on BCI, Panamá. (A) 14 images of the bat (temporal resolution: 80 ms). **(B)** Sonogram of the echolocation calls with time signal above; numbers below correspond to images of the bat. Plots of call parameters of the same echolocation sequence including **(C)** pulse duration, **(D)** repetition rate, **(E)** pulse interval, and **(F)** duty cycle. Abbreviations: SC, search calls; AC, start of approach calls; TG/TC, start of terminal group calls; CM, capture of mealworm.

We then compared echolocation behavior of bats in the experiments where mealworms were placed on the water surface at two distances to clutter-producing background (10 and 20 cm, respectively) to test for possible differences in signal parameters. We omitted data from the experiments with mealworms offered at the edge of the clutter plot for further comparison, as the bats only emitted search calls indicating that they had not detected food there. Overall, most call parameters did not vary significantly between the experiments (two-factorial Anova: $F_{(1,\ 8)} < 6.2$; $0.04 < p < 0.95$; **Table 2**). Only the bandwidth of terminal phase calls with shortest pulse intervals was slightly narrower (14.4 kHz versus 15.8 kHz) in experiments with prey closer to clutter ($F_{(1,\ 8)} = 9.4$; $p = 0.02$), and pulse interval of search calls was somewhat longer (44.1 ms vs. 39.0 ms) in experiments with prey

Table 2 | Seven echolocation parameters of nine *M. macrophyllum* foraging in the flight cage on BCI, Panamá.

Parameter	Search	Approach	Terminal phase			
			F	***M***	***S***	***L***
Pulse duration [ms]	2.4 ± 0.3 1.9 – 2.8	2.1 ± 0.1 1.9 – 2.2	1.7 ± 0.1 1.6 – 1.9	1.4 ± 0.2 1.1 – 1.7	1.0 ± 0.1 0.8 – 1.3	0.9 ± 0.1 0.8 – 1.2
Pulse interval [ms]	41.6 ± 10.5 24 – 62	17.9 ± 1.5 15 – 21	11.0 ± 1.2 8.9 – 14	7.9 ± 1.6 6.2 – 11	5.9 ± 0.7 5.1 – 7.8	
Repetition rate [calls/s]	25.6 ± 6.8 16 – 42	56.3 ± 4.9 48 – 66	91.9 ± 9.6 74 – 113	130.7 ± 22.8 87 – 161	172.3 ± 18.0 129 – 195	
Bandwidth [kHz]	22.9 ± 3.1 17 – 29	25.4 ± 1.9 23 – 29	23.5 ± 2.8 18 – 28	19.6 ± 2.6 16 – 24	15.1 ± 2.2 11 – 20	13.6 ± 1.7 11 – 16
Peak frequency [kHz]	56.4 ± 2.2 53 – 60	55.4 ± 2.1 50 – 58	55.9 ± 2.7 52 – 62	54.8 ± 3.2 47 – 59	54.2 ± 2.8 49 – 59	53.2 ± 3.6 47 – 58
Sweep rate [kHz/ms]	9.7 ± 0.9 7.9 – 11	12.3 ± 1.1 10 – 14	13.5 ± 1.5 11 – 16	14.5 ± 1.7 12 – 18	15.3 ± 2.4 10 – 19	15.2 ± 2.0 12 – 19
Duty cycle [%]	6.4 ± 1.3 4.2 – 8.9	12.2 ± 1.2 9.8 – 14	16.0 ± 1.9 13 – 20	17.6 ± 3.0 13 – 24	17.2 ± 2.7 13 – 23	

Presented data (mean ± SD; min–max) are based on measurements taken from 85 search calls, 234 approach calls, and 72 terminal phase calls of bats trawling for mealworms exposed on the water surface near the clutter plot in two experiments. Abbreviations for terminal phase: F, first call; M, numerically median call, S, call with shortest pulse interval; and L, last call.

at larger distance to clutter ($F_{(1,\,8)} = 10.1$; $p = 0.01$). However, as these differences were very small and close to the resolution of our analysis, we pooled all data for **Table 2**.

Calculation of the echo overlap zone (considering sound speeds of 346 m/s at 25°C) revealed that echoes of prey and background clutter overlapped in all trials in which mealworms were presented 10 cm or closer to *Hydrilla* (**Figure 4**). Overlap between background clutter and prey echoes also occurred in trials, where mealworms were 20 cm away from the water plants, if pulse duration exceeded 1.15 ms. Hence, when bats emitted search and approach calls, echoes of mealworms and clutter overlapped, while at the end of terminal phases calls were short enough to avoid overlap effects.

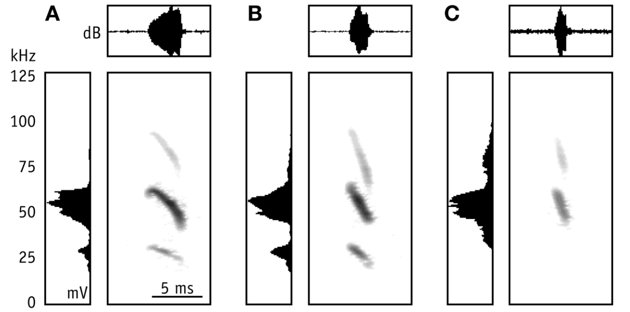

FIGURE 3 | Representative echolocation calls of *M. macrophyllum* foraging in the field above the water surface near the colony on BCI, Panamá. Sonogram (time versus frequency) with oscillogram (time versus amplitude [dB]) above and averaged power spectrum (mV) to the left; values have been normalized. **(A)** Search call; note the short shallow-modulated onset of the call; **(B)** approach call; **(C)** terminal phase call, emitted prior to capture of prey.

FORAGING AND ECHOLOCATION BEHAVIOR IN THE FIELD

While searching for food under natural conditions in the field all bats flew at a higher speed ($3.2 \pm 0.3\,\mathrm{ms}^{-1}$, $N = 6$) than in the flight cage. Nevertheless, prey detection distance of 0.9–2.3 m (1.4 ± 0.5 m, $N = 8$) was comparable to the detection distance of floating or aerial mealworms measured in our flight cage experiments. After a brief pause of 46.3 ± 18.0 ms ($N = 8$ sequences), bats in the field began to emit groups of approach calls (**Figures 2**, **3**) with an inter-pulse interval of 34.9 ± 4.6 ms ($N = 7$) similar to bats in the flight cage. Flight speed remained at $3.1 \pm 0.5\,\mathrm{ms}^{-1}$ ($N = 8$). As in the flight cage, *M. macrophyllum* started to produce a distinct terminal phase (**Figures 2**, **3**) composed of 19 ± 4 (range: 15–26, $N = 11$) calls and a mean duration of 153.6 ± 43.1 ms (99–245 ms, $N = 11$) at a distance of 32–61 cm ($N = 8$) after a short pulse interval of 27.7 ± 3.8 ms ($N = 8$). Flight speed remained high ($3.0 \pm 0.5\,\mathrm{ms}^{-1}$, $N = 8$). After up to 12 buzz calls, *M. macrophyllum* entered the echo overlap zone at a distance of 21–37 cm to the prey with a pulse duration of 1.8 ± 0.3 ms ($N = 8$), where echolocation calls started to overlap echoes returning from prey (**Figure 4**). A few cm before bats reached the food, echolocation stopped for a period of 41.4 ± 13.3 ms ($N = 8$), during which *M. macrophyllum* took the prey from the water surface. Subsequently, the bats resumed echolocation.

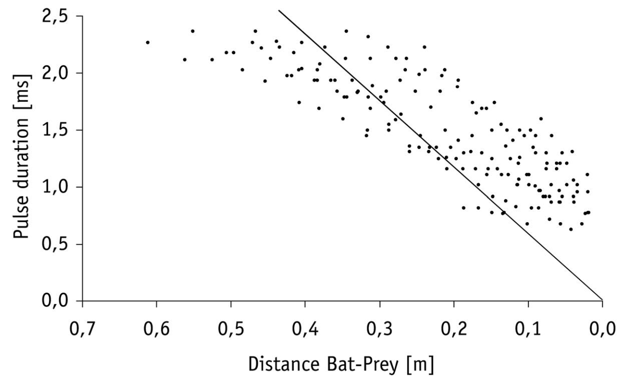

FIGURE 4 | Decrease in pulse duration of terminal phase calls from nine individuals of *M. macrophyllum* while approaching potential food floating on the water surface of Gatún Lake near the shore of BCI, Panamá. The solid line indicates the limit beyond which calls overlap with returning echo from prey.

We found several significant differences in call parameters between bats recorded in the flight cage and in the field (two-factorial Anova; all $F_{(2,\ 69)} < 15.5$; Tukey post-hoc comparison: all $p > 0.0001$; **Table 1**). As a general pattern, pulse duration of search and approach calls was longer in the field than in the flight cage, while terminal phase calls were shorter. Additionally, in the field search and approach calls were emitted at longer pulse intervals, while pulse interval between terminal phase calls was shorter than in the flight cage. Finally, echolocation calls in the field were always emitted at lower peak frequencies than in the flight cage, while bandwidth did not vary significantly (**Table 1**) and most search calls of bats from the field started with a very short, shallow-modulated component (**Figure 3**).

DISCUSSION

FORAGING BEHAVIOR OF *M. macrophyllum*

According to our results, *M. macrophyllum* uses echolocation as the prime sensory cue for finding prey. Furthermore and in reference to our observations in the field, our behavioral experiments revealed that *M. macrophyllum* detects and captures prey equally well in trawling (Weinbeer and Kalko, 2007) and in aerial hawking mode. This behavioral flexibility parallels observations of a variety of trawling bats. The larger of two *Noctilio* species that occur in sympatry with *M. macrophyllum*, *N. leporinus*, trawls prey from the water surface (e.g., Schnitzler et al., 1994) and occasionally performs aerial captures (Übernickel et al. subm.), while the smaller *N. albiventris* frequently forages in trawling and aerial hawking mode (Kalko et al., 1998). Likewise, another sympatric species, the small proboscis bat, *Rhynchonycteris naso* (Emballonuridae), mainly feeds on aerial prey above water bodies, but also takes insects directly from the water surface (unpubl. data). Also various *Myotis* species are known to trawl and to hawk insects in the air (e.g., Britton et al., 1997; Jones and Rayner, 1988, 1991; Kalko and Schnitzler, 1989). The ability of trawling bats to switch their foraging strategy from trawling to aerial hunting while maintaining echolocation as the sole sensorial modality is very likely linked to the similarity of perceptual tasks. Perceptually, foraging above water is rather similar to aerial hawking in (semi-)open space, as the smooth water surface reflects most of the call energy away from a low-flying bat. Hence, over water and in (semi-)open space, little or no clutter echoes interfere with prey perception by echolocation (Boonman et al., 1998; Rydell et al., 1999; Siemers et al., 2001a).

Thus our results confirm that, as it has been shown before in trawling *M. macrophyllum* above open water areas (Weinbeer and Kalko, 2007), echolocation behavior of *M. macrophyllum* strongly resembles echolocation behavior of other trawling and aerial hawking bats (e.g., Jones and Rayner, 1988, 1991; Kalko and Schnitzler, 1989; Schnitzler et al., 1994; Kalko et al., 1998; Zsebok et al., 2013), even when foraging close to background clutter. It however markedly differs from echolocation behavior of other phyllostomid species that typically glean food from vegetation.

Moreover, the distances toward prey at which echolocation behavior of *M. macrophyllum* by changing from search to approach calls reflects target detection (reaction distance), as well as the onset and duration of the terminal phase are similar to those observed in the trawling vespertilionid bat, *Myotis daubentonii*, and aerial hawking pipistrelle bats, *Pipistrellus sp.* (Kalko and Schnitzler, 1993; Kalko, 1995). However, in contrast to other trawling and aerial hawking bats, *M. macrophyllum* enters the signal-echo-overlap zone already at about a distance of 40 cm to prey items. In *M. daubentonii* (Kalko and Schnitzler, 1989) and *P. pipistrellus* (Kalko and Schnitzler, 1993), echolocation stops before entering the echo-overlap zone, as the bats enter this zone only at distances of about 10–20 cm before reaching their prey. This suggests a higher overlap tolerance of phyllostomid echolocation calls with regard to clutter which might be due to a relatively high bandwidth of buzz calls, which potentially facilitates separate processing of call components. A high bandwidth reduces or might prevent potential signal-echo overlap as echoes of the broadband calls can probably be processed in the bat's hearing system as many frequency bands in different channels (Wiegrebe and Schmidt, 1996; Siemers and Schnitzler, 2004; Weinbeer and Kalko, 2007).

EFFECT OF CLUTTER ON ECHOLOCATION AND FORAGING BEHAVIOR

Detection performance of trawling *M. macrophyllum* declined with decreasing distance between the mealworm and the clutter and hence increasing effects of the clutter overlap. As long as prey echoes were only slightly overlapped by clutter echoes (≥10 cm distance of prey to clutter), bats were able to find the mealworms, while prey that was completely buried within clutter-producing background could no longer be detected. This perceptual difficulty was well reflected in echolocation behavior, as *M. macrophyllum* did not emit approach calls and a terminal phase when prey was buried in clutter. Our findings are in accordance with results of *M. daubentonii* for which detection performance decreased with increasing clutter (Zsebok et al., 2013) and ceased foraging in low flight when the cover with duckweed on the water surface became too dense (Boonman et al., 1998).

Interestingly, *M. macrophyllum* did not use other sensory cues to perceive prey buried in clutter, as is typical for most phyllostomid bats. Perhaps, echolocation call structure of the broadband, steep FM calls like those emitted by *M. macrophyllum* increases overlap tolerance. Corresponding neuronal filters are likely to analyze those bands separately, each of which has a shorter duration than the complete call, thus reducing the echo overlap zone (Wiegrebe and Schmidt, 1996). Evidence for this proposition comes from a study of five species of *Myotis* where the tight link between bandwidth and vertical clutter tolerance had been studied (Siemers and Schnitzler, 2004). Species like *M. nattereri* with short, steep FM search calls and a very broad bandwidth of 120 kHz had no difficulty (capture rate: 100%) in finding prey presented at a distance of 5 cm to clutter. In contrast, *M. dasycneme* or *M. daubentonii*, which emit calls of lower bandwidth (44 kHz and 57 kHz, respectively), caught 100% of the offered prey only at 25 cm distance to clutter (Siemers and Schnitzler, 2004). As Zsebok et al. (2013) pointed out, it seems of no relevance to target detection and prey capture attempt whether the clutter producing surface is vertically or horizontally oriented. Our results show that *M. macrophyllum* fits well into this pattern, as it was able to find most prey (capture rate: 97%) at a distance of 10 cm relative to clutter, emitting search calls with a total bandwidth of approximately 70 kHz (Weinbeer and Kalko, 2007). However, while main call energy in *Myotis* was concentrated in the first harmonic, *M. macrophyllum* emitted calls of three and occasionally up to four harmonics (Weinbeer and Kalko, 2007). This may permit *M. macrophyllum* to integrate echo information over several harmonics.

EVOLUTION OF FLEXIBILITY IN FORAGING BEHAVIOR

Flexibility in foraging behavior while maintaining echolocation as the sole sensory mode is likely to grant *M. macrophyllum* access to a wider range of prey, including insects sitting on the water surface or flying somewhat above water. As an example, *M. macrophyllum* often feeds on an abundant, introduced moth, *Parapoynx diminutalis*, (Pyralidae). Its larvae develop in *H. verticillata* plants, where at certain times of the year numerous imagoes emerge. We frequently found scales of *P. diminutalis* in the feces of *M. macrophyllum* (Meyer et al., 2005; Weinbeer et al., 2006). Moths are taken directly from the water surface or caught in mid-air. This efficient exploitation of particular resources based on flexibility in foraging behavior has been found for a wide number of insectivorous bat species that regularly switch between aerial hawking and gleaning from (rough) surfaces (e.g., Schumm et al., 1991; Arlettaz, 1996; Chruszcz and Barclay, 2003).

Foraging flexibility in *M. macrophyllum* may finally be seen in an evolutionary context together with its associated prey detection mode and echolocation behavior. In a postulated evolutionary scenario, extant bats are descended from a late echolocating aerial hawking insectivorous bat. However, it remains unclear whether some groups, such as phyllostomid bats, may have switched several times between aerial hawking and gleaning mode close to or within vegetation (Schnitzler et al., 2003b; Simmons and Geisler, 1998). As most extant leaf-nosed bats produce echolocation calls that are primarily used for spatial orientation, and as they forage mostly in narrow space habitat that hampers use of echolocation for finding food close to or on surfaces, we postulate that *M. macrophyllum* has evolved from this group in a rather unique manner (e.g., Fuzessery et al., 1993; Schnitzler and Kalko, 1998, 2001; Rydell et al., 1999; Arlettaz et al., 2001; Jones et al., 2003).

Indeed, based on molecular data, *M. macrophyllum* is placed near highly derived phyllostomid genera (*Lonchorhina*, *Macrotus*, *Mimon*, or *Trachops*; Freeman, 2000; Wetterer et al., 2000; Lee et al., 2002) that all show the typical, rather uniform echolocation behavior of phyllostomid bats gleaning food within cluttered habitats without emitting a distinct terminal phase. Furthermore, similarities in echolocation and foraging behavior among largely unrelated trawling bats strongly suggest that both foraging and echolocation behavior have evolved independently several times in several families in response to similar ecological conditions rather than *M. macrophyllum* representing a "primitive" form of the Phyllostomidae.

From our experiments in the flight cage and observations in the field we infer that *M. macrophyllum* uses echolocation as a prime sensory mode for finding prey and argue, that this reflects an adaptation to the acoustic characteristics of its main foraging habitat (over water). In addition, *M. macrophyllum* revealed a high flexibility in foraging behavior (trawling and aerial hawking), which is astonishingly similar to other trawling bats. Beyond this, our acoustical analysis showed that *M. macrophyllum* is able to tolerate echo overlap to a certain degree, particularly, when prey is partially buried within clutter. These sensory adaptations attribute *M. macrophyllum* a unique position among leaf-nosed bats, and strongly suggest a convergent evolution of its echolocation behavior with that of other trawling and aerial hawking bats. Thus, in its sensory adaptations, *M. macrophyllum* rather resembles distantly related trawling and aerial hawking bats than closely related Phyllostomids.

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Flexible echolocation behavior of trawling bats during approach of continuous or transient prey cues

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Trawling bats use echolocation not only to detect and classify acoustically continuous cues originated from insects at and above water surfaces, but also to detect small water-dwelling prey items breaking the water surface for a very short time, producing only transient cues to be perceived acoustically. Generally, bats need to adjust their echolocation behavior to the specific task on hand, and because of the diversity of prey cues they use in hunting, trawling bats should be highly flexible in their echolocation behavior. We studied the adaptations in the behavior of *Noctilio leporinus* when approaching either a continuous cue or a transient cue that disappeared during the approach of the bat. Normally the bats reacted by dipping their feet in the water at the cue location. We found that the bats typically started to adapt their calling behavior at approximately 410 ms before prey contact in continuous cue trials, but were also able to adapt their approach behavior to stimuli onsets as short as 177 ms before contact, within a minimum reaction time of 50.9 ms in response to transient cues. In both tasks the approach phase ended between 32 and 53 ms before prey contact. Call emission always continued after the end of the approach phase until around prey contact. In some failed capture attempts, call emission did not cease at all after prey contact. Probably bats used spatial memory to dip at the original location of the transient cue after its disappearance. The duration of the pointed dips was significantly longer in transient cue trials than in continuous cue trials. Our results suggest that trawling bats possess the ability to modify their generally rather stereotyped echolocation behavior during approaches within very short reaction times depending on the sensory information available.

Keywords: approach, prey capture, water surface, reaction time, plasticity, *Noctilio leporinus*

INTRODUCTION

Bats that depend on echolocation to acquire food constantly adjust their echolocation calls to their surroundings and optimize call structure for increased information gain during detection, classification, and localization of prey (Schnitzler and Kalko, 2001). Especially bat species that use more than one prey capture mode in different habitats, e.g., aerial hawking and gleaning (*Myotis lucifugus* and *Myotis evotis*: Barclay, 1991) or from a water surface and in the air (*Myotis daubentonii*: Kalko and Schnitzler, 1989; *Noctilio leporinus*: Schnitzler et al., 1994) may need to adjust their echolocation and flight behavior extremely quickly, in response to the task on hand (Holderied et al., 2008).

A typical aerial hawking insect capture is a reaction to a continuous cue, i.e., to an object that a bat can lock its center of attention onto and home in on (e.g., Ghose et al., 2009; Surlykke et al., 2009; Moss and Surlykke, 2010). Such detection events are typically followed by modifications of echolocation behavior that are remarkably consistent: the animals switch from search mode to approach mode by decreasing pulse duration and pulse interval (e.g., Griffin, 1958; Simmons et al., 1979; Schnitzler et al., 1994; Kalko et al., 1998; Schnitzler and Kalko, 2001). The approach phase may be divided into an initial part and a terminal part. The latter is characterized by emission of usually one but sometimes two groups that are composed of many calls (Schnitzler and Kalko, 2001; Melcón et al., 2007). This terminal part is essential for continuously updating the information on the exact location of the prey and in most species may be subdivided into two components, final buzz I with successively shortening pulse intervals and final buzz II with very short but invariant pulse intervals (e.g., Kalko and Schnitzler, 1989; Siemers and Schnitzler, 2000; Melcón et al., 2007). The emission of echolocation calls typically ceases shortly before prey contact and is resumed after completion of the capture attempt (e.g., Schnitzler and Kalko, 2001).

Just as aerial hawking bats react to flying prey, trawling bats may perform stereotyped capture attempts upon detecting potential insect prey floating on the water surface that provides a continuously detectable acoustic cue. However, they have also developed the ability to forage on water-dwelling prey (e.g., fish, shrimp) (e.g., Brooke, 1994; Blood and Clark, 1998; Siemers et al., 2001; Aihartza et al., 2008). Water-dwelling prey may provide only temporary acoustic cues (hereafter: transient cues), i.e., a short disturbance that disappears within about 50–100 ms after breaking the water surface (Schnitzler et al., 1994). Trawling bats, such as the Greater Bulldog bat *N. leporinus*, recognize these stimuli as cues for prey and react with the emission of an approach phase and a capture attempt by dipping their feet near the center of the expanding ripples in the water ("pointed dips", Schnitzler et al., 1994).

We hypothesized that bats with such flexible hunting behavior are likely to also possess adaptive plasticity in their echolocation behavior (e.g., Schnitzler and Kalko, 2001). Therefore, we presented *N. leporinus* with continuous and transient prey cues and tested whether and how the animals adapted their echolocation and flight behavior when approaching these targets. We compared the bats' behavior in both situations using ultrasound recordings with synchronized high-speed video.

We wanted to pinpoint a stable point in time for the onset of the approach phase in continuous cue trials, indicating the instant in time prior to prey contact that allows the bat to easily perform all necessary behavior in the remaining time prior to prey contact, similar to the wire avoidance task with *Myotis lucifugus* in Grinnell and Griffin (1958). Additionally, we wanted to assess a minimal reaction time between the onset of the transient stimulus and the onset of the approach phase and expected to find values around 50–60 ms similar to earlier reported minimal reaction times for e.g., *Myotis nattereri* and *Eptesicus fuscus* (Webster, 1967; Masters et al., 1985; Melcón et al., 2007).

We anticipated that the disappearance of the transient cue during the approach would have an effect on the echolocation behavior of the bat, because prey item localization is not possible anymore. To detect modifications of the approach behavior during the approach of a transient cue, we compared several call parameters between continuous and transient cue approaches, i.e., pulse intervals, pulse durations, and call composition (relation between duration of quasi-constant frequency (QCF) and frequency modulated (FM) components).

We expected the duration of the dip to be longer in transient cue trials than in continuous cue trials, due to the uncertainties the bats are facing when trying to grasp an undetectable prey item. Furthermore, we expected to find faster resumption of call emission after contact when the capture attempt has failed (Britton and Jones, 1999) so that the bat may achieve fast updates of information after a failed prey capture (Ghose et al., 2009).

Our results may provide a valuable contribution to the ongoing discussion of how quickly bats are able to adapt their echolocation behavior while approaching different cues and how they adjust their echolocation when a situation changes before completion of the approach.

MATERIALS AND METHODS

STUDY SITE AND ANIMALS

We caught two male and one female *Noctilio leporinus* on Barro Colorado Island (BCI), a field station of the Smithsonian Tropical Research Institute (for details on the study area refer to Leigh, 1999) during two field stays between November 2009 and May 2010. After capture we allowed each bat to habituate for one night to the flight cage before we began task related training and experiments in the second night of captivity. We kept the bats individually and fed them with small fish, mealworms (larvae of *Tenebrio molitor,* Coleoptera), and occasionally locally caught bushcrickets. For supervision of nutritional status we monitored the weight of daily food intake ($\bar{x} = 24$ g; range 15–32 g) as well as body weight of each bat. We released all bats in healthy condition with weight equal to the weight at capture ($\bar{x} = 57$ g) or slightly increased ($\bar{x} = +0.7$ g). All animals were released close to their capture site in the night after trial completion.

Permission of scientific collecting was provided by Autoridad Nacional del Ambiente (ANAM) and Ministerio de Desarrollo Agropecuario (MIDA). Our experiments complied with the national animal care policies (IACUC No. 2008-10-06-24-08).

EXPERIMENTAL SETUP

We performed all experiments in a flight-cage (12 × 5 × 2 m) with an artificial pond (7 × 1.5 m) and a roost at one corner of the flight cage (**Figure 1**). The camera was positioned to film the instant of prey capture while the microphone was at the side of the pond opposite the perch and 40–50 cm above the water surface. In all tasks we offered prey objects that consisted of a piece of fish (weight $\bar{x} = 0.8 \pm 0.3$ g), mounted at varying distances from the perch ($\bar{x} = 3.5 \pm 0.4$ m) in order to avoid habituation of the bat to one single prey location and hence to increase the need for precise prey localization through echolocation. All three bats mastered the continuous cue task during the first night of training and learned within two more nights to approach also our transient cues.

We either presented a continuous or a transient cue at a time. In preparation for continuous stimulus trials, we lowered a curtain between the roost and the pond to prevent the bat from detecting the prey object prior to leaving the roost. First we placed the prey slightly protruding from the water surface, i.e., for 3–5 mm, then we raised the curtain. A trained bat would immediately leave the perch, fly 5–20 cm above the water surface in a straight line toward the prey and attempt capture (sample flight-path, **Figure 1**).

To provide the stimulus for the transient cue task, we used a device similar to the "artifish" used by Schnitzler et al. (1994). It consisted of a small plastic tube (Ø 4 mm) connected to a small air pump (LifeTech 3500) and a custom-made control device (scientific electronic workshop, University of Ulm, Germany). Upon being powered it produced small water splashes

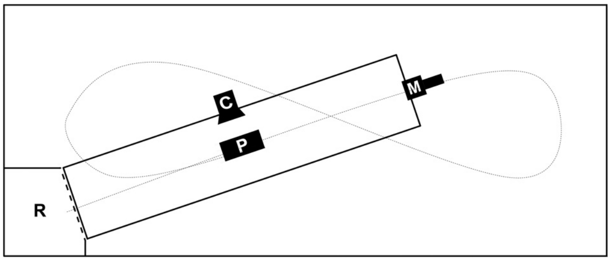

FIGURE 1 | Scheme of trial set up in the flight cage (12 × 5 × 2 m); C, high-speed camera filming the moment of prey capture; M, microphone position during all trials pointing over the length of the pond (7 × 1.5 m) toward the roost; P, prey area, varying in distance to roost (*d* = 3.5 ± 0.4 m); R, roost; short dashed line, curtain—raised during continuous cue trials; long dashed line, approximate trial trajectory of trained bats.

of $\bar{x} = 267 \pm 75$ ms duration in intervals of 3 s. Neither the free end of the tube nor the prey item mounted close to it protruded from the water surface. This guaranteed acoustical undetectability of the prey until the "artifish" produced a stimulus that broke the water surface. The position of the transient cue in the pond varied in the same area as the continuous cue. At the beginning of the transient cue trials we encouraged the bats to fly and they generally started immediately to search for prey objects at the water surface. While a bat was flying we activated the "artifish". We triggered video and audio recordings whenever the bats reacted to the stimulus by dipping at the prey location while flying toward the microphone (**Figure 1**).

Whenever the bats dipped at the prey position, but lost the prey item while pulling it out of the water we scored this as a failed trial in both tasks.

DATA RECORDING AND ANALYSIS

We recorded the behavior of the bats with a high-speed video camera (CamRecord 600 × 2, Optronis, Kehl, Germany) set to a frame rate of 850 fps and a shutter time of 1/3000, using the manufacturers' software (Camcontrol V4.04, Optronis, Kehl, Germany). We recorded echolocation calls directly onto the hard disk of a laptop, using a condenser microphone (CM16/CMPA, Avisoft bioacoustics, Berlin, Germany) connected to a sound interface (116 Hm or 416 H, Avisoft bioacoustics) with a sampling rate of at least 300 kHz, using Avisoft software (Avisoft Recorder, version 3.3 to version 3.4.2, Avisoft bioacoustics, Berlin, Germany). Audio and video recordings were triggered synchronously by using a manual trigger device connected to both systems. We controlled both recording systems with the same laptop (Lenovo IBM 3000N200T8300 XP pro, Mainz, Germany).

We synchronized and analyzed audio and video data with custom-made software (Highsync, Version 0.94, Slomotec, Dr. Frank and Hella Gabler GbR, Frankfurt, Germany) and corrected for sound travel time to the microphone considering ambient temperature (recorded with a data-logger for temperature, humidity, and pressure, MSR Electronics GmbH, Model: 145, Henggart, Switzerland) and distance between camera position and microphone. For a detailed audio analysis we used SasLab Pro (version 5.2.06, Avisoft bioacoustics).

We tested two situations with two possible outcomes each: successful (1) and failed (2) continuous cue trials and successful (3) and failed (4) transient cue trials. 36 trials (2 tasks × 2 outcomes × 3 bats × 3 repetitions) entered our data analysis. For statistical comparisons we performed *t*-tests and Mann–Whitney-*U*-tests in SigmaStat 3.5 (Systat Software Inc., Chicago, IL 60606, USA), unless stated otherwise. Initially, we compared the selected parameters within one task across successful and failed trials. Only when these tests revealed no significant differences across trials in both tasks, the data per task were pooled to allow the use of the full dataset for comparison across tasks.

To enable comparisons across trials and tasks we required a reference point that allowed an alignment of all sequences. For this we used (a) the moment of contact between the bats' feet and prey in continuous cue trials and equivalently (b) the instant when the bats' feet passed the location of the water splash in transient cue trials. For all time-based analyses we defined these instants as zero and present all time information relative to this point of reference. Events occurring before the prey contact therefore scored negative time values.

For the acoustic analysis of echolocation calls we took into account that bats decrease the pulse amplitude successively throughout an approach (Hartley et al., 1989; Surlykke and Moss, 2000; Boonman and Jones, 2002). To compensate for systematic errors in measurements based on amplitude we normalized all calls (Holderied et al., 2008) to 75% of relative sound intensity. For measurements we used the automated measurement function of SasLab Pro set to a threshold of −40 dB relative to maximum amplitude and to measure peak frequencies at time intervals of 0.3 ms. We analyzed the audio data in a flat top spectrogram window, with an FFT length of 1024, 96.87% overlap, and a resulting reading accuracy of 293 Hz and 0.11 ms. We analyzed all echolocation calls emitted between −0.9 s before prey capture and ca. 0.3 s after prey capture. For each call we extracted pulse interval, pulse duration and the duration of QCF and FM components. We defined the moment of the switch between QCF and FM components within one call as the first of three 300 Hz intervals that were steadily declining in frequency.

The confined space of flight-cages generates increased pulse-echo overlap in comparison to field situations, and bats generally respond to this situation by using shorter calls during orientation flight (Suthers, 1965; Surlykke and Moss, 2000). We therefore obtained reference values of orientation flight in the cage by analyzing calls that were emitted between −0.9 s and −0.6 s before prey contact in all 36 continuous and transient cue trials and calculated for each individual the mean and standard deviations for pulse duration, pulse interval and QCF and FM components.

We defined the onset of the approach phase per bat as the beginning of the first call that had shorter pulse duration and pulse interval than the previously determined reference values minus one standard deviation (**Table 1**, arrows 2 in **Figure 2**, dashed lines in **Figure 4**). We defined the end of the acoustic approach phase as being at the end of the shortest call in the sequence (arrows 3 in **Figure 2**, **Figure 3**) (Holderied et al., 2005). We defined final buzz II as existent in those trials where a minimum of two successive pulse intervals showed a pulse interval of <7 ms (**Figure 3**). For measuring the maximum sound pressure level emitted by *Noctilio leporinus* in the flight-cage, we used the same equipment in a similar set-up as described in Brinkløv et al. (2011).

Table 1 | Reference values for pulse duration (PD) and pulse interval (PI) for each bat during orientation phase.

Individual	PD mean (±*SD*)	PI mean (±*SD*)
Bat 1	9.4 (±0.5)	52.0 (±18.9)
Bat 2	9.1 (±0.8)	49.5 (±15.5)
Bat 3	8.5 (±0.6)	51.2 (±18.5)

By definition, the approach phase in a trial began when both pulse duration and pulse interval fell below the threshold values calculated as mean value minus one standard deviation (SD).

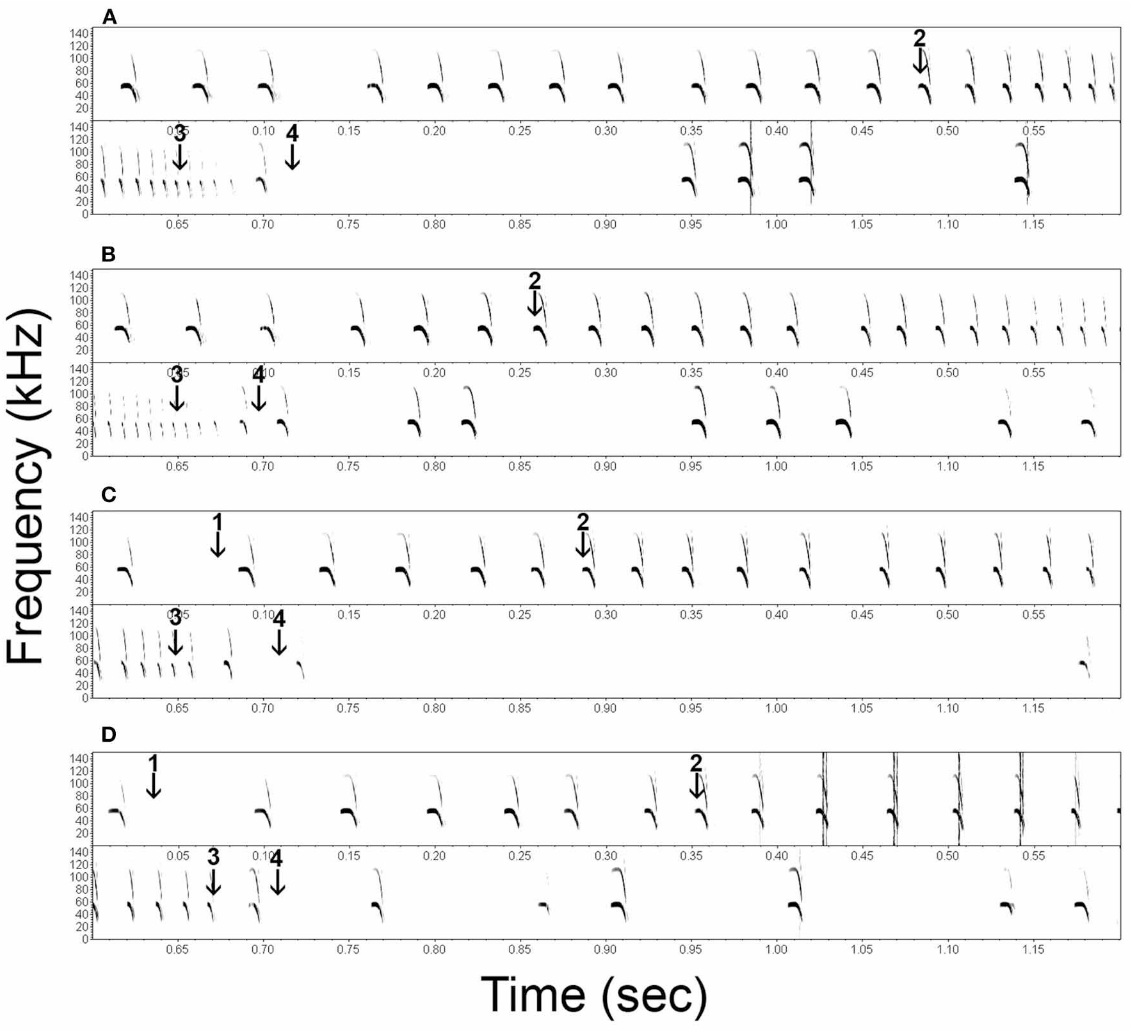

FIGURE 2 | Representative spectrograms of the echolocation behavior of *Noctilio leporinus* (bat 1) during the performance of a successful (A) and failed (B) approach to a continuous cue and successful (C) and failed (D) approaches to a transient cue. Arrows indicate the onset of the transient stimulus (1), the onset of the approach phase (2), the end of the approach phase (3) and the instant of first prey contact (4).

COMPARISON OF BEHAVIOR

We compared echolocation behavior of bats approaching transient cues to bats approaching continuous cues. We tested for significant differences in onset and end of the approach phase relative to prey contact across tasks.

For a better understanding of the timing of bats reacting to transient cues we also extracted from the video recordings the onset of the stimulus relative to prey contact in each trial and approximated the minimum reaction time by measuring the time intervals between the onset of a transient stimulus and the onset of the approach phase in the transient cue trials.

We analyzed and compared the changes in echolocation call components (QCF and FM) during the overall pulse duration reduction until the end of the approach phase. For this, we compared the timing of the first call without a QCF component within call sequences and we compared the onset of FM component reduction, defined as the instant when the FM component duration fell below mean duration in orientation flight minus one standard deviation ($\bar{x} = 4.6 \pm 0.5\,\text{ms}$). To translate the changes in temporal echolocation behavior, as defined, into a distance to prey scale, we used video observations on flight speed of *N. leporinus* in the flight cage, to approximate the distances when the changes took place. Additionally, we tested if the presence or absence of final buzz II differed significantly across tasks (Chi-square-test) and we compared minimal pulse intervals across tasks. To investigate if the duration of the dip, i.e., the duration of contact between feet and water, differed across tasks, we calculated for each trial the time difference between instant of first contact between feet and water and the moment when the feet lost contact with the water, and compared this duration across tasks. Furthermore, we observed the degree to which the bats hit the exact location of the transient cue after it had already disappeared.

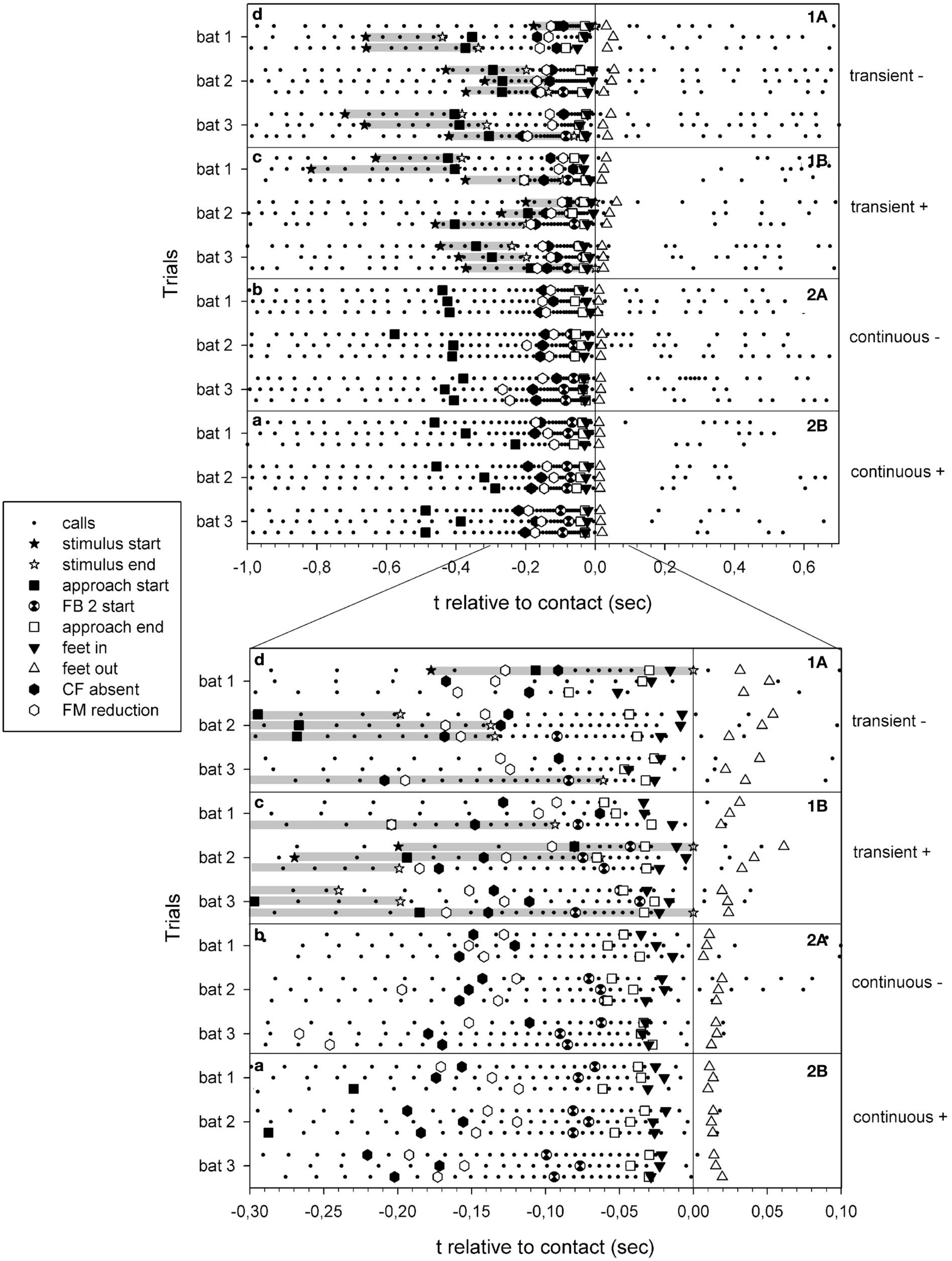

FIGURE 3 | Summary of echolocation phases of all selected trials of failed (A) and successful (B) approaches to the site of transient cues (1) and continuous cues (2). The trials are presented as a function of time relative to prey contact/or water splash location arrival (zero). Each dot represents the onset of a call. Gray bars: duration of transient cues.

Calls occurring after the end of the approach phase were classified as post buzz calls type 1 and type 2. Type 1 calls had the same overall structure as final buzz II calls, and consisted only of a low intensity FM component that was longer in duration than the shortest call that defined the end of the approach phase. Type 2 consisted of a QCF and a FM component, mostly with higher intensity, similar to orientation phase calls in the flight cage. Either type 1 or type 2 calls, or a combination of both were always present shortly before, during, and/or shortly after prey contact. We documented the emission of post buzz calls type 1 and type 2 and how call continuity was related to success or failure of the capture attempts.

RESULTS

ECHOLOCATION: ORIENTATION PHASE

Measurements of call parameters for the three individuals during the orientation phase ranged consistently about 9 ms for pulse durations and pulse intervals of 51 ms (**Table 1**). Duration of QCF components was 4.5 ms (median; quartiles 25%: 3.9 ms, 75%: 5.1 ms), and FM components lasted 4.5 ms (median; quartiles 25%: 4.2 ms, 75%: 4.8 ms). There was no significant difference between the durations of the two components (Wilkoxon signed rank test $W = 1579.0$; $P = 0.32$; $n = 213$).

APPROACH OF THE PREY CUE

Analysis of differences across successful and failed trials within tasks revealed no significant differences. The onsets of transient stimuli varied between −817 ms and −177 ms before prey contact, but revealed no significant differences in the time interval between the onset of the transient stimuli and contact to prey (t-test, $P = 0.574$) between successful and non-successful approaches. Furthermore, we found no significant differences in the parameters onset of the approach phase (continuous cue: Mann–Whitney-U-test, $U = 33.0$, $P = 0.536$; transient cue: t-test, $P = 0.620$), end of approach phase (continuous cue: t-test, $P = 0.578$; transient cue: Mann–Whitney-U-test, $U = 37.0$, $P = 0.791$), minimal pulse duration at the end of the approach phase (continuous cue: t-test, $P = 0.832$; transient cue: t-test, $P = 0.557$), instant of QCF component elimination (continuous cue: t-test, $P = 0.050$; transient cue: t-test, $P = 0.536$), instant of first FM component reduction (continuous cue: t-test, $P = 0.175$; transient cue: Mann–Whitney-U-test, $U = 42.0$, $P = 0.930$), minimum pulse interval at the end of the approach phase (continuous cue: t-test, $P = 0.285$; transient cue: Mann–Whitney-U-test, $U = 46.5$, $P = 0.625$), first feet-water contact (continuous cue: t-test, $P = 0.378$; transient cue: t-test, $P = 0.499$), and last feet-water contact (continuous cue: t-test, $P = 0.984$; transient cue: t-test, $P = 0.236$). As we found no significant differences between successful and failed tasks in the mentioned parameters, we pooled the data and compared across tasks.

As expected, we found differences in the transient cue approach sequences compared to those with the continuous cue. The onset of the approach phase in continuous cue trials were rather stereotypic and began significantly earlier ($\bar{x} = -410 \pm 79$ ms, approximately −2.2 m) than in transient cue trials ($\bar{x} = -294 \pm 105$ ms, approximately −1.7 m) (t-test; $P = 0.001$). Variability was much lower in continuous cue trials (coefficient of variance = 19.3%) than in transient cue trials (coefficient of variance = 35.7%). The time difference between the onset of the transient cue and the instant of contact to prey was $\bar{x} = -466 \pm 185$ ms (range: −817 to −177 ms).

The reaction time, determined as the interval between the onset of the transient cue and the onset of the approach phase, was $\bar{x} = 171.5 \pm 106.2$ ms. Shortest reaction time was 50.9 ms.

The time difference between the end of the approach phase and the moment of prey contact was similar in both tasks (Mann–Whitney-U–Test, $U = 174.0$; $P = 0.716$). The feet were inserted into the water at similar instances across tasks as well (Mann–Whitney-U-test, $U = 191.0$, $P = 0.367$). Overall the feet were inserted into the water ($\bar{x} = -24.6 \pm 9.8$ ms) significantly after the end of the approach phase ($\bar{x} = -43.0 \pm 14.6$ ms) (t-test; $P = 0.001$) (**Figure 4**).

The reduction of overall pulse duration resulted in minimal values of $\bar{x} = 2.5 \pm 0.4$ ms at the end of the approach phase (**Figure 4**) and did not differ significantly across tasks (t-test, $P = 0.083$). The consistent decrease of pulse duration after the beginning of the approach phase mainly occurred in the QCF part of the call. It gradually disappeared while the FM component stayed largely unchanged. In continuous cue trials the QCF component was completely eliminated at −158 ms (median, quartiles 25%: −179 ms, 75%: −149 ms, approximately −0.9 m) before prey contact. The FM component remained stable in the approach phase until −149 ms (median, quartiles 25%: −173 ms, 75%: −132 ms, approximately −0.9 m) before prey contact. In transient cue approaches the QCF component was eliminated at $\bar{x} = -130 \pm 36.1$ ms (approximately −0.8 m) before contact, while the FM component remained unchanged until late in the approach phase at $\bar{x} = -144 \pm 32.1$ ms (approximately −0.9 m) before prey contact. The data for complete reduction of the QCF and FM components from transient cue trials may be affected by the random encounter of the regularly occurring transient cue stimuli by the bat.

Minimum pulse interval per trial was significantly shorter in continuous cue trials (median = 6.1 ms, quartiles 25%: 5.8 ms, 75%: 6.5 ms) than in transient cue trials (median = 6.8 ms, quartiles 25%: 6.1 ms, 75%: 7.1 ms) (Mann–Whitney-U-test, $U = 233.0$; $P = 0.025$) (**Figure 4**). Because of longer minimal pulse intervals in transient cue trials, final buzz 2 was significantly less frequent in transient cue trials (7 out of 18) than in continuous cue trials (13 out of 18) (Chi-square = 4.05, $P = 0.0442$).

PREY CONTACT

In all 36 trials the bats dipped at and passed the cue location within less than the span of a single foot. In reaction to the transient cue the bats dipped their feet at the location of the water splash while it was still occurring, or shortly after. Following successful spearing of prey that was hidden under the water surface, the bats proceeded to transfer it in flight from their feet to their mouths.

As expected, we found a significant difference in the dip duration between the two tasks, which lasted significantly

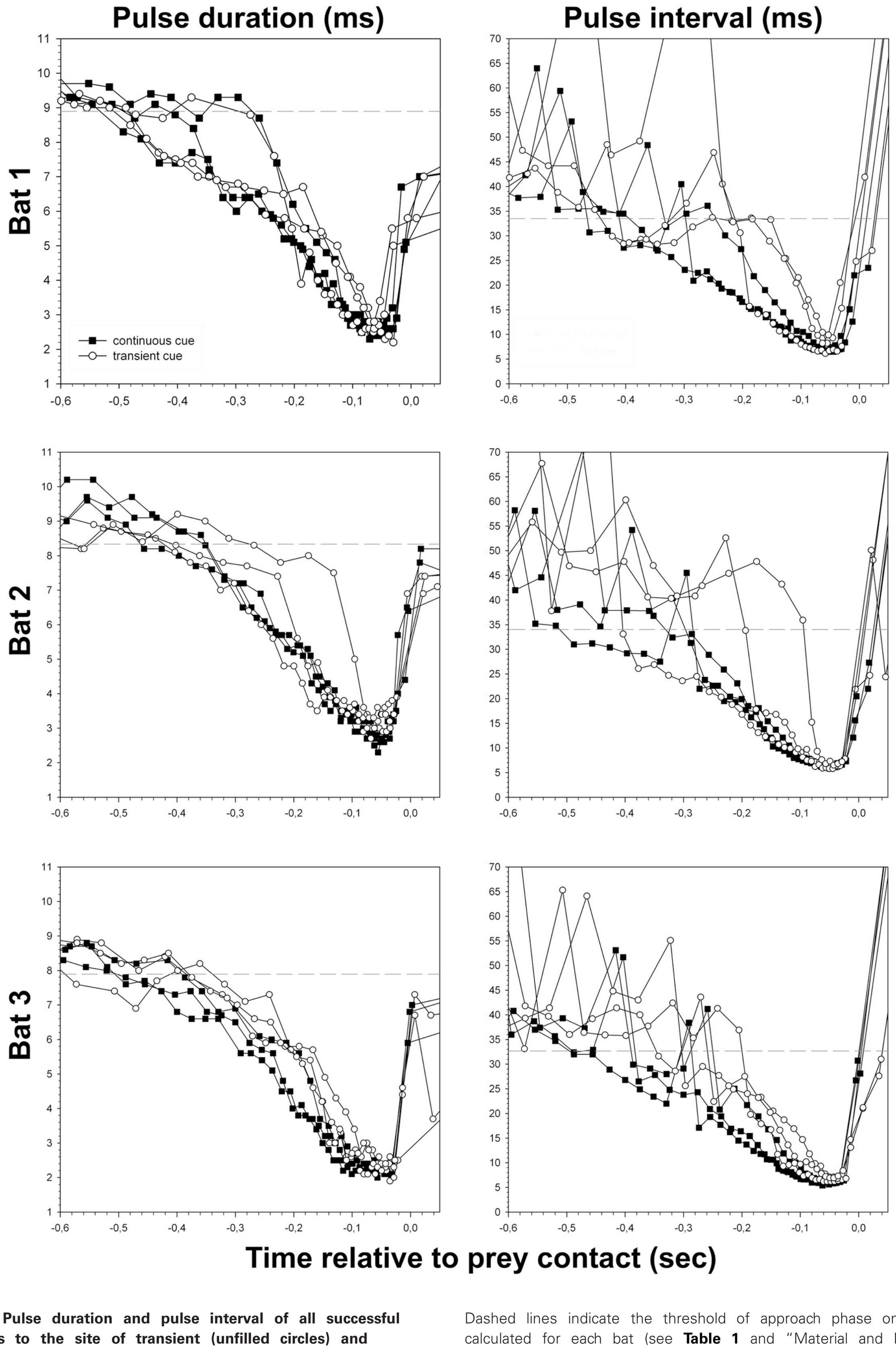

FIGURE 4 | Pulse duration and pulse interval of all successful approaches to the site of transient (unfilled circles) and continuous cues (filled squares), plotted separately for each bat. Dashed lines indicate the threshold of approach phase onset calculated for each bat (see **Table 1** and "Material and Methods" section).

longer during transient cue trials (median = 56.75 ms, quartiles 25%: 46.9 ms, 75%: 66.9 ms) than during continuous cue trials (median = 39.35 ms, quartiles 25%: 35.1 ms, 75%: 46.2 ms) (Mann–Whitney-*U*-test, $U = 282.0$, $P = 0.001$). A closer look revealed that dip duration was not significantly different up to prey contact (see above), but dip duration after prey contact was significantly longer in transient cue trials (median = 32.2 ms, quartiles 25%: 23.8 ms, 75%: 41.8 ms) compared to continuous cue trials (median = 13.4 ms, quartiles 25%: 10.8 ms, 75%: 15.5 ms) (Mann–Whitney-*U*-test, $U = 320.0$, $P = 0.001$).

AFTER PREY CONTACT

In both successful and failed capture attempts *N. leporinus* kept emitting echolocation calls after the end of the approach phase (**Figure 2**, calls between arrows 3 and 4, and shortly after arrow 4). Post buzz calls type 1 are emitted between the end of the approach phase and prey contact. Type 2 calls are emitted shortly before, during and shortly after prey contact.

Successful prey captures resulted in a pause of echolocation call emission while the bats transferred prey from their tail membrane to their mouths (**Figure 3**). In failed trials, the bats paused either only shortly in emission of echolocation calls or continued calling without pause (**Figures 2**, **3**). In the latter we found a gradual transition from post buzz calls to orientation phase calls.

DISCUSSION

It has been known for some time that bats modify their echolocation behavior depending on the task on hand (e.g., Schnitzler et al., 2003; Holderied et al., 2008; Moss and Surlykke, 2010), but comparisons of echolocation behavior of a single bat species performing prey captures under different conditions remains scarce (Faure and Barclay, 1994), in particular in response to transient cues.

Here we compared the echolocation and dip performance of the trawling bat *Noctilio leporinus* when reacting to two different types of cues presented at a water surface. Trawling bat species may take continuously floating insects from the water surfaces but may also successfully attack transient targets, such as briefly surfacing small fish or crustaceans (Blood and Clark, 1998; Siemers et al., 2001; Aihartza et al., 2008). We asked whether the approach phase is a stereotypic behavior, or if it is specifically adapted to each cue suggesting a prey item, and focused on the similarities and differences of the bats' behavior across both tasks.

APPROACH OF THE PREY

As expected, all bats showed a clear approach phase in their echolocation behavior when coming closer to both types of cues, but we also found specific differences between the task-related echolocation behavior.

In continuous cue trials, we found a relatively stable onset of the approach phase at $\bar{x} = -410$ ms/−2.2 m, whereas the onset of approach phase during transient cue trials occurred later ($\bar{x} = -294$ ms/−1.7 m). The rather late onset of the acoustic approach phase in both tasks and the high sound pressure levels *Noctilio leporinus* uses in the field (max. 142.7 dB source level, Surlykke and Kalko, 2008), suggest a discrepancy between the distance of prey detection and the instant when the bats started to react to the cues indicating prey. The stimulus should have been detectable in the continuous cue trials at ca. 4.1 m distance from the prey (Stilz and Schnitzler, 2012, online calculator with the following settings: point reflector, dynamic range of 80 dB assuming a hearing threshold of 20 dB, 56 kHz, 26°C, and a humidity of 90%) with *N. leporinus* calling in our flight cage at a maximum intensity of 100 dB sound pressure level, measured 1 m before the mouth. Considering this calculated detection distance, *Noctilio* probably already detected the continuous cue while leaving the roost/perch but did not need to alter its echolocation behavior until −410 ms/−2.2 m before prey contact, a similar reaction distance as found for *Myotis lucifugus* avoiding wires (Grinnell and Griffin, 1958).

The difference in the values and variabilities of the approach phase onsets for the two tasks is influenced by the random onset of the transient cue stimulus relative to the bat's position. When exposing a free-flying bat to a transient cue we were not able to control for the bat's distance to the cue location. The transient cue water splash was triggered and the bat that was flying somewhere in the flight cage started to adapt its echolocation behavior when it was in a favorable position for cue detection. It is noteworthy that even in the six trials with stimulus onsets more than −410 ms before prey contact (**Figure 3**), we did not find approach phase onsets earlier than in the range of approach onset of continuous cue trials. In the remaining trials the transient stimuli had occurred close to or less than −410 ms before prey contact and a bat can only react after it perceives a stimulus, resulting in overall shorter approach phase onsets.

It is interesting that the shortest reaction time between stimulus onset and the onset of the approach phase was as short as 50.9 ms. This result corroborates a minimal reaction time of 47–63 ms for *Myotis nattereri* (Melcón et al., 2007).

CALL PARAMETERS DURING THE APPROACH

The differences in the call parameters during the approach phases of the two tasks were most likely due to the fact that the bats could steadily home in on the continuously detectable object (Surlykke et al., 2009), while the transient cue appeared and disappeared over time. In the continuous cue trials, all bats showed a rather stereotypic echolocation behavior, consisting of a stereotypical onset of approach phase and emission of final buzz I and in most cases also final buzz II, just as has been described for many aerial hawking bats prey captures (e.g., Pipistrelles and some vespertilionids: Schnitzler and Kalko, 2001; *Molossus molossus*: Mora et al., 2004). In contrast, our transient cue disappeared while bats were still approaching. The remaining circular waves on the water surface are unlikely to be perceived by the bats because they are non-breaking waves and therefore unlikely to be detectable through echolocation (Schnitzler et al., 1994). After the disappearance of the short-lived water splash, the bat changed its behavior from a typical approach to a prey object in a way similar to the echolocation behavior reported when *Myotis nattereri* approaches a landing site (Melcón et al., 2007). Similar to landing *M. nattereri*, our *N. leporinus* employed during the approach phase in transient cue trials longer pulse intervals, causing the final buzz II to be suppressed. Such prompt adaptations of call parameters to changes in conditions of the environment has also been reported for *Eptesicus fuscus* avoiding broadcast-echo ambiguity (Hiryu et al., 2010). We propose that bats, approaching a stable two dimensional water surface, without any specific object to home in on, require a lower information flow than aerial insect pursuit and capture with a prey object potentially moving in three dimensions (Schnitzler and Kalko, 2001; Melcón et al., 2007).

In contrast to the parameters mentioned above, the reduction in pulse duration, first by shortening the QCF component and only late in the approach phase by reduction of the FM component, is a stereotypical behavior (Schnitzler et al., 1994; Kalko et al., 1998). QCF components are adaptations that facilitate fluttering target detection (e.g., Schnitzler et al., 2003), detection of prey movement relative to the echolocating bat, and long distance detection of weak echoes (Simmons et al., 1975; Schnitzler and Kalko, 2001). Broadband FM components, in contrast, provide advantages for precise target localization (e.g., Schnitzler et al., 2003). Coming closer to a prey object at some point the bat enters the zone of pulse-echo overlap (e.g., Siemers and Schnitzler, 2000; Schnitzler and Kalko, 2001). As FM components are well suited for exact target localization at short distances, it is not surprising that the QCF component is reduced first (Schnitzler et al., 1994; Kalko et al., 1998). Assuming a speed of sound of 346.39 m/s at 25°C, an approximate end of the approach phase at $\bar{x} = -43$ ms/approximately 35 cm distance to prey, and minimal pulse durations of 2.5 ms, the bats had entered the zone of pulse-echo-overlap shortly before the end of the approach phase. In earlier studies *N. leporinus* was found to enter the zone of pulse-echo overlap at a distance of 0.4 m from the prey (Hartley et al., 1989). For other species there are similar findings (*Eptesicus fuscus*: overlap in the last 60 ms or 18 cm, Wilson and Moss, 2004).

PREY CONTACT

In both tasks, the end of the approach phase occurred at $\bar{x} = -43.0$ ms before prey contact (**Figures 3**, **4**). We argue that at this point the bats had acquired all information needed for the capture attempt. Further calls, emitted shortly after the end of the approach phase but before, during, or shortly after prey contact, may serve a different purpose. Unlike other species (e.g., *Pipistrellus* sp.: Kalko, 1995; *Eptesicus fuscus* and *Myotis septentrionalis*: Wilson and Moss, 2004) that stop calling after the end of the approach phase, *N. leporinus* continues to emit post buzz calls type 1 and type 2 (**Figure 2**). Post buzz calls type 2 have previously been described in *N. leporinus* (Wenstrup and Suthers, 1984; Hartley et al., 1989).

Based on a reaction time of ~50 ms, the last part of final buzz II and post buzz calls type 1 and/or 2 occur so close to the time of prey contact that processing of new information and initiation of appropriate reactions would not be possible in time to serve for prey capture. Possibly the emission of these calls is a mechanism that ensures the availability of updated prey or environmental information after a failed capture attempt (Schnitzler and Kalko, 2001; Melcón et al., 2007; Ghose et al., 2009). Also, the increase in pulse amplitude in post buzz calls type 2 indicates a shift of attention from a close prey object to the bat's larger surroundings (Hartley et al., 1989). A similar shift of acoustic gaze before task completion has been observed in *Eptesicus fuscus* (Surlykke et al., 2009).

In our selected trials the water splash was vertical and the bats in our transient cue trials always dipped at the spot where the splash had occurred, suggesting the use of a spatial memory for prey capture (Moss and Surlykke, 2010). Interestingly, in some trials that were excluded from further analysis the water splash was not vertical but slanted, with the water hitting the surface at some distance from the "artifish" tube. In those cases the bats dipped up to several centimeters away from the location of the "artifish" near the point of water fall back to the surface (K. Übernickel, unpublished data). This indicates that the bats dip at the location of the cue latest in time, but this assumption would need further experiments.

AFTER PREY CONTACT

As expected, the bats' feet were inserted into the water at approximately the same point in time before anticipated prey contact in both tasks, but were dragged significantly longer through the water after passing the transient prey position than when reacting to the continuous cue. This behavior might illustrate the uncertainty of the bat about the submerged prey that is likely to be near the surface but at some distance from the initial position during the transient cue.

After a capture attempt, echolocation behavior continues in a differing manner, depending on hunting success or failure. While bats briefly ceased call emission after successful captures during the transfer of the prey into the mouth, this pause is considerably shorter in failed attempts (Britton and Jones, 1999), or may not be present at all (**Figure 3**). In extreme cases there is a gradual transition from post buzz calls to orientation phase calls, similar to the situation of an aborted buzz and the subsequent gradual transition back to search or early approach phase calls (Holderied et al., 2008).

CONCLUSION

In conclusion, our results indicate that trawling bats possess the ability to modify their otherwise stereotyped echolocation behavior during approaches, within very short reaction times, depending on the sensory task. Even when an acoustic target disappears during an approach, they are still able to adapt their behavior and complete the task, dipping at the site of the transient cue based on spatial memory and dragging for a longer distance, presumably based on former experiences.

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Trawling bats exploit an echo-acoustic ground effect

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A water surface acts not only as an optic mirror but also as an acoustic mirror. Echolocation calls emitted by bats at low heights above water are reflected away from the bat, and hence the background clutter is reduced. Moreover, targets on the surface create an enhanced echo. Here, we formally quantified the effect of the surface and target height on both target detection and -discrimination in a combined laboratory and field approach with *Myotis daubentonii*. In a two-alternative, forced-choice paradigm, the bats had to detect a mealworm and discriminate it from an inedible dummy (20 mm PVC disc). Psychophysical performance was measured as a function of height above either smooth surfaces (water or PVC) or above a clutter surface (artificial grass). At low heights above the clutter surface (10, 20, or 35 cm), the bats' detection performance was worse than above a smooth surface. At a height of 50 cm, the surface structure had no influence on target detection. Above the clutter surface, also target discrimination was significantly impaired with decreasing target height. A detailed analysis of the bats' echolocation calls during target approach shows that above the clutter surface, the bats produce calls with significantly higher peak frequency. Flight-path reconstruction revealed that the bats attacked an target from below over water but from above over a clutter surface. These results are consistent with the hypothesis that trawling bats exploit an echo-acoustic ground effect, in terms of a spatio-temporal integration of direct reflections with indirect reflections from the water surface, to optimize prey detection and -discrimination not only for prey on the water but also for some range above.

Keywords: *Myotis daubentonii*, echo-acoustic mirrors, target detection, target discrimination, echo enhancement, trawling bats, ground effect

INTRODUCTION

In course of evolution, bats, as the only airborne mammals, adapted to a large variety of habitats. The species of this ecologically highly diverse group provide many morphological, physiological as well as behavioral adaptations e.g., of their sensory-motor system (Schnitzler and Kalko, 2001). Echolocating bats emit ultrasonic sounds and listen to the returning echoes reflected by objects in the environment. This enables bats to orient and hunt in complete darkness allowing prey detection, localization, and identification. But the biosonar system is prone to interferences. When bats use echolocation e.g., during foraging they have to deal with sound attenuation and masking effects. Amongst others, attenuation can be caused by atmospheric absorption losses that especially have a strong impact on high frequencies as they are used by bats (Lawrence and Simmons, 1982). Items close to the object of interest can create masking effects that impede prey detection (Fenton, 1990; Suemer et al., 2009; Bates et al., 2011). This so-called clutter interference can appear e.g., when hunting close to the ground or foliage. Hence, bats are not only morphologically adapted to their habitats (e.g., by wing shape) (Norberg and Rayner, 1987), but also by their echolocation signals (Schnitzler and Kalko, 2001; Siemers and Schnitzler, 2004; Wund, 2005). The differences in the echolocation call parameters (e.g., frequency, call duration, call intensity) are species-specific and also habitat-dependent. For example in vespertilionid bats, species that hunt in free airspace emit loud, narrowband echolocation calls to detect prey from a larger distance, whereas species that hunt near vegetation emit broadband echolocation calls to catch prey objects that are only a few centimeters in front of a clutter producing background (Schnitzler et al., 2003). Additionally the structure of echolocation signals can also differ with the behavioral task. In insectivorous bats for example, the echolocation signals during search, approach, and final buzz phase are very different (Schnitzler and Kalko, 2001).

One particularly interesting group consists of bats hunting almost exclusively above water surfaces. These so-called "trawling bats" hunt at low heights above water and capture fish or insects directly from or close to the surface. Water bodies like lakes, ponds, or streams are favorable hunting habitats for bats as the high abundance of insects provides a profitable food source (Zahn and Maier, 1997; Warren et al., 2000; Ciechanowski, 2002). In previous studies it was found that bats of this ecotype prefer to hunt over calm water compared to water e.g., covered by plants like duckweed, artificial objects, or turbulent, rippled water

(Von Frenckell and Barclay, 1987; Mackey and Barclay, 1989; Boonman et al., 1998; Rydell et al., 1999; Siemers et al., 2001b; Siemers and Schnitzler, 2004). Two laboratory studies revealed that in the three European trawling-bat species' (*Myotis capaccinii*, *M. dasycneme*, and *M. daubentonii*) capture success was increased, compared to a clutter surface, when prey was presented on a smooth surface (linoleum screen) that mimicked the reflection characteristics of calm water. It was concluded that since the water surface acts as an acoustic mirror, echolocation calls emitted by bats are reflected away in acute angles from the bat. This creates an echo-image without, or just low clutter echoes and thus increases search efficiency as the prey echo is acoustically conspicuous (Siemers et al., 2001b, 2005). The search image for these bats was defined as "small and isolated echo-reflecting objects on or above an acoustically smooth surface" (Siemers et al., 2001a,b). This theory does not exclude inedible objects e.g., small leaves or debris on a water surface. However, one would expect efficient prey discrimination during flight to be beneficial to avoid catching inedible prey. But, in actively hunting bats no discrimination between edible and inedible objects that fit the general search image could be observed so far (Barclay and Brigham, 1994; Siemers et al., 2001b). Siemers et al. (2001b) showed that under semi-natural laboratory conditions trawling bats did not discriminate between a mealworm and a dummy presented on a linoleum screen.

The trawling bat *Myotis daubentonii* often hunts over rivers and streams (Jones and Rayner, 1988) providing a unidirectional water flow that often contains inedible objects as well as drifting prey. In a field study it was shown that *M. daubentonii* switches between trawling of prey from the water surface and aerial hawking (Todd and Waters, 2007), depending on the amount of clutter on the water surface.

Since previous studies were mainly designed to investigate prey detection on acoustic mirror and clutter surfaces without testing discrimination performance in detail, this study was designed to test prey detection *and* -discrimination. As the previous studies were conducted in the field, the participating animals behaved under natural conditions, but the participating number is an unknown factor. Whereas studies conducted in the laboratory allow control over the number of animals, but are limited in their imitation of natural surroundings. To benefit from both study types we formally quantify in the current study the effect of surface structure on both prey detection and -discrimination and on the echolocation behavior in a combined laboratory and field approach.

The main objectives of our study were to investigate the effect of the surface structure on the attacking and discrimination performance of the bats as well as flight path and the sonar vocalization features. These behavioral measures are discussed with respect to echo-acoustic features of the surface structures.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

The species used in this study was the microchiropteran Daubenton's bat, *Myotis daubentonii*. It is found throughout Europe, foraging for insects above water surfaces using short (<5 ms), broadband frequency sweeps (95–25 kHz) for echolocation (Kalko and Schnitzler, 1989).

LABORATORY EXPERIMENT

Animal housing

Laboratory experiments were conducted in July and August 2011 in the Max Planck Institute of Ornithology in Seewiesen, Germany. Data from five individuals of 12 h time shifted Daubenton's bats were recorded. The experiments were conducted under license of the responsible authorities and complied with German laws (LLUR 515/5327.74.1.6).

Experimental setup

In the experiment a mealworm (larvae of *Tenebrio molitor*) and a dummy (1 mm black plastic disc with a diameter of 2 cm) were presented simultaneously. Both targets were hanging from easily exchangeable, variable-length nylon threads (Ø 0.15 mm) that were attached via small solenoids to a horizontal bar (**Figure 1**). The bar itself was suspended from the ceiling. This allowed an easy manipulation of the presented targets, e.g., target height (by variable lengths of nylon threads) and position (left or right side). The distance between the two targets was 1.2 m. The two targets were presented above either an artificial surface floating on the water or the water itself. The artificial surface measured 1.2 × 2.4 m. The targets were positioned such that each was hanging above the center of one half of a surface area with a minimum distance of 60 cm to the midline and the edges. The size of the experimental room was 3 × 7 × 3.5 m.

The experiment was monitored with synchronized normal- and high-speed video under infrared-light illumination and audio recordings. The normal-speed (25 frames/s) recordings were made by a single camera (WAT-902H2 Ultimate, Watec Co. LTD, Higashine, Japan) by means of the surveillance software (USB120 Server, Digiprotect, Frankfurt, Germany) to record the whole experimental process on the computer. The two high-speed digital video cameras (MV1-D1312I-160-CL-12, Photonfocus, Lachen, Switzerland; 100 frames/s, resolution 1312 by 1024 pixels, with specially developed software by Rauscher GmbH, Olching, Germany) recorded the last 5 s before a capture attempt. These high-speed recordings were used for reconstructing the flight path

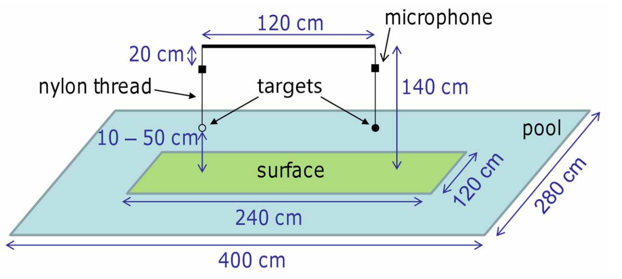

FIGURE 1 | Setup of the Field and Laboratory experiments. In the two-alternative forced-choice paradigm the bat had the opportunity to attack one of the targets (mealworm or dummy). The surface beneath the targets was covered either with artificial grass or smooth PVC, or the place was left clear for the water surface. The two targets were always presented at the same height which was 10, 20, 35, or 50 cm above the surface. The horizontal bar holding the targets and microphones was attached to the ceiling of the Laboratory or, for the Field experiment, to a fishing rod anchored to the ground.

later on. The illumination was supported by two custom made stroboscopic flash lights (Department of Animal Physiology, University of Tübingen, Germany).

Acoustic signals were picked up by two ultrasound microphones (Knowles SPM0204, Itasca, IL, USA) that were attached 20 cm below the horizontal bar, i.e., vertically above the two targets. Echolocation calls were amplified and digitized with an Ultralite-mk3 (MOTU, Cambridge, UK) at a sampling rate of 192 kHz and recorded with Adobe Audition 2.0 (Adobe Systems Inc., San Jose, CA, USA) on the computer.

Experimental procedure

In the experimental conditions target height and surface type were varied. The surface types were defined as clutter surface (artificial grass matting with a height of 3 cm) or as smooth surface (water). Above the two different surfaces the targets were presented at four different heights (10, 20, 35, and 50 cm). This resulted in eight different experimental conditions which were presented following a pseudo-random protocol. The position (left or right) of the targets was also randomized.

Before each trial, both targets were hidden by two 70 cm high paper tubes while attaching them to the setup. This prevented bats from identifying and attacking the targets before trial start. In a trial, both targets were always presented simultaneously and at the same height. As the targets were suspended from nylon threads, they were not perfectly stationary, specifically, they often rotated slowly around their vertical axis.

FIELD EXPERIMENT

The Field experiment was conducted under license of the responsible authorities (Referat für Umwelt und Gesundheit, München, 641-304/P-12/7).

Recording sites and experimental setup

The field recording site was a shallow branch of the river Würm, located in Munich-Pasing, Germany (48° 8′ 0.59″ N/11° 26′ 52.37″ E, water depth: 10–20 cm). Data recording took place on 10 evenings between April and October 2011. The experiments were performed shortly after sunset when the first bats started hunting at the recording site. Depending on bat activity, recording sessions lasted about 3 h per night. To fit the requirements of the field research site, a slightly adapted version of the laboratory setup was used in the Field experiment (**Figure 1**). The horizontal bar holding the nylon threads with the targets was suspended from a fishing rod that was anchored to the ground. For video acquisition, a single high-speed digital video camera [Basler A602f, Ahrensburg, Germany, 95 frames/s with a Pentax H612A (TH) objective lens, Pentax Ricoh Imaging Co., Ltd., Tokyo, Japan] was used. The camera was positioned about 2 m from the targets and ∼50 cm above the water surface. Red light illumination (two Philips IR PAR38E 150W, Amsterdam, Netherlands) was used to supply sufficient light for the camera. The microphones and their position were exactly the same as in the laboratory. Audio and video data were recorded in a 5 s ring buffer system implemented in MATLAB 7.5 (The MathWorks, Inc., Natick, MA, USA).

Experimental procedure

In the Field experiment, an additional, smooth surface type (PVC) was used with similar acoustic reflection properties as smooth water. The PVC board (1.2 × 2.4 m) was used as a control condition to rule out the possibility of potential performance changes of the bats being merely due to the artificial surface. Moreover, the water from the river was not smooth but, due to the irregular floor beneath the shallow, flowing water, the surface had small, regular waves, and ripples.

Unlike in the Laboratory experiment, only three different target heights were applied: 20, 35, and 50 cm. The presentation of these nine different conditions (three heights times three surfaces) followed a pseudo-random protocol where in successive nine trials each condition was presented once. Like in the laboratory, the position (left or right) of the targets was also randomized.

Before each trial, the bat species hunting at the setup were identified visually and acoustically by means of their echolocation calls with a Mini-3 Bat Detector (Ultra Sound Advice, London, UK). Later, this was verified by both video and sound analyses. Data analysis (see below) was the same as for the Laboratory experiment, except that the single camera did not allow flight-path reconstruction, and acoustic data from the field was not evaluated.

DATA ANALYSIS

Attacking performance

A trial began when a bat initiated an attack or when it had circled around one or both of the targets at least three times. An executed attack was registered when the bat performed a final buzz and touched one of the targets or the threads. Later, the audio and video recordings of each trial were analyzed to correct for any wrong observations during the trials.

The data from each individual obtained in the laboratory was summarized and the attacking performance was calculated as the ratio of the number of attacks (independent of whether it was the dummy or the mealworm) divided by the number of trials where a bat initiated a trial according to the above criteria. In the water surface conditions, the attacking performance was always 100% independently of the target height (see results below), therefore it needed no statistical evaluation. For the statistical evaluation of the performance in the grass surface conditions, a General Linear Mixed Model (GLMM) was fitted on the arcsine transformed attacking performance data (as independent variable) with factors height (fixed effect) and individual (random effect).

As for the field results, it was not possible to distinguish different individuals; therefore only one performance value was calculated in each condition. In the water and PVC surface conditions, the attacking performance was maximal (100%) independently of the height (no statistics needed). To evaluate the effect of the height in the grass surface condition a Fischer's exact test was applied. All the statistical computations in this study were conducted in Statistica 8.0 (Statsoft Inc., Tulsa, OK, USA) and in MATLAB.

Discrimination performance

To calculate the discrimination performance only those trials were used in which an attack had been executed. An attack toward the mealworm was defined as a correct decision, an attack toward the dummy as a wrong decision. The discrimination performance was calculated as the ratio of correct decisions divided by all attacks in each condition.

For the laboratory results a GLMM was built on the arcsine transformed discrimination performance data (as independent variable) with the factors target height (fixed effect) and individual (random effect). This was done for the water and the grass surface conditions separately. The data obtained at the 10 cm target height conditions was omitted, as only one individual once attacked the targets offered at this height in the grass surface condition.

For the field results, the height effect was tested with the Fischer's exact test for all three surface conditions on the performance data.

A binomial test was used to test whether the probability of the mealworm choice was above 50% chance level. This was done separately for the Laboratory and the Field experiment on the pooled data.

Flight path analysis

The high-speed video recordings of the Laboratory experiment were used to reconstruct the flight paths for the trials of the 35 cm target height conditions. The calculations were made using the freely available DLTdv3 program written in MATLAB (Hedrick, 2008). After the flight path reconstruction the median and the quartiles from the water and the grass surface condition were calculated. This was done separately for each frame relative to the capture moment for the graphical presentation. The average flight height for each path was calculated and a GLMM was applied to test the effect of the individuals (random factor) and the surface (fixed factor).

Call analysis

Calls were analyzed with a custom written MATLAB program based on a program provided by Holger Görlitz. Calls were first high-pass filtered at 20 kHz. The frequency spectrum was then obtained by computing a 1024-point FFT (fast Fourier transform) over a Hanning window. Before calculating the frequency parameters the spectrum was fitted with an 18th-order polynomial to smooth out the ripples caused by constructive and destructive interferences between a call and reflections from the water surface. These interferences create higher and lower magnitudes, respectively, which are smoothed out by the polynomial fit. There was a continuous, narrow-band disturbance from a power supply in the recordings. For this narrow frequency range, the measured spectral magnitude was replaced by a linear interpolation. From the fitted spectrum, peak frequency, bandwidth and the −20 dB lower and upper cut-off frequencies were calculated. Due to reflections from the water, the analysis of the temporal call parameters was impeded. Depending on the pulse intervals (PIs), calls were separated into either Approach (15 ms $<$ PI $<$ 30 ms) or Buzz I phase (6.5 ms $\leq$ PI $\leq$ 15 ms). Kalko and Schnitzler (1989) measured a PI of 55–65 ms at the beginning of the Approach phase and 12–8 ms at the end. Here we used a rather narrow window to categorize the approach calls to ensure non-Approach calls were excluded. Buzz II calls with a PI shorter than 6.5 ms were not analyzed as the decreasing amplitude of the calls, the water reflections and the short PI impeded the analysis. In the following, Buzz I is referred to as Buzz.

To test the significance of the difference in peak frequency between the water and grass condition we applied a GLMM taking the surface as fixed factor and the identity of the individuals as random factor for each height (20, 35, and 50 cm) and phase (Approach and Buzz) separately (altogether six tests). We excluded the data from the 10 cm target height condition from this analysis, as we had only one recording in which the target was attacked. We did not analyze the echolocation calls obtained in the field, as the analysis of the laboratory data showed a highly significant individual effect for peak frequency (due to the lacking identity of the recorded bats in the field).

Ensonification and impulse response analysis

To quantify the structural properties of the surfaces, the PVC, and the grass matting were ensonified to obtain their impulse responses (IR). The IR is the echo reflected from an object when the object is ensonified with an acoustic impulse (Dirac impulse) of theoretically infinite shortness and infinite amplitude (Weissenbacher and Wiegrebe, 2003). The IR was calculated by cross-correlating the recorded echo with the original signal in the time domain.

A disc of the respective material (PVC or grass) with a diameter of 30 cm was positioned at a distance of 90 cm to an ultrasonic speaker (Matsushita EAS 10 TH 800D, Osaka, Japan), and a ¼ inch ultrasonic microphone (Brüel & Kjær 4135 with 2671 preamplifier and 2610 measuring amplifier, Nærum, Denmark) which was attached coaxially at the speaker front. The discs were ensonified from 10 different angles between 90° (sound impinging perpendicularly on the disc) and 0° (sound propagating parallel to the disc) in 10° steps. To measure the IR, white noise with a cut-off frequency of 96 kHz was created in MATLAB, sent to the DA/AD converter (MOTU Ultralite-mk3; sampling frequency 192 kHz), amplified (Toellner Toe 7606, Herdecke, Germany), and played via the ultrasonic speaker for the duration of 40 s. Simultaneously the echo was recorded by the ultrasonic microphone. Spectrograms of the IRs were calculated using a 64-point FFT over a Hanning window and an overlap of 95%.

RESULTS

ATTACKING PERFORMANCE

In the laboratory 347 trials were conducted with five individuals for eight conditions (four target heights, two surface types). For three individuals, data were obtained for four different target heights (10, 20, 35, and 50 cm). For two individuals, data were obtained for three different heights (20, 35, and 50 cm). After initiating a trial, all bats attacked one of the targets above water (**Figures 2A–E**, blue bars) independent of the target height. Above the grass surface, however, the performance deteriorated with decreasing target height (**Figures 2A–E**, green bars). The GLMM showed a significant effect of target height $[F_{(4,\ 10)} = 20.0,\ p < 0.001]$ but also an effect of the individual $[F_{(3,\ 10)} = 8.4,\ p = 0.003]$, meaning that the individual attacking

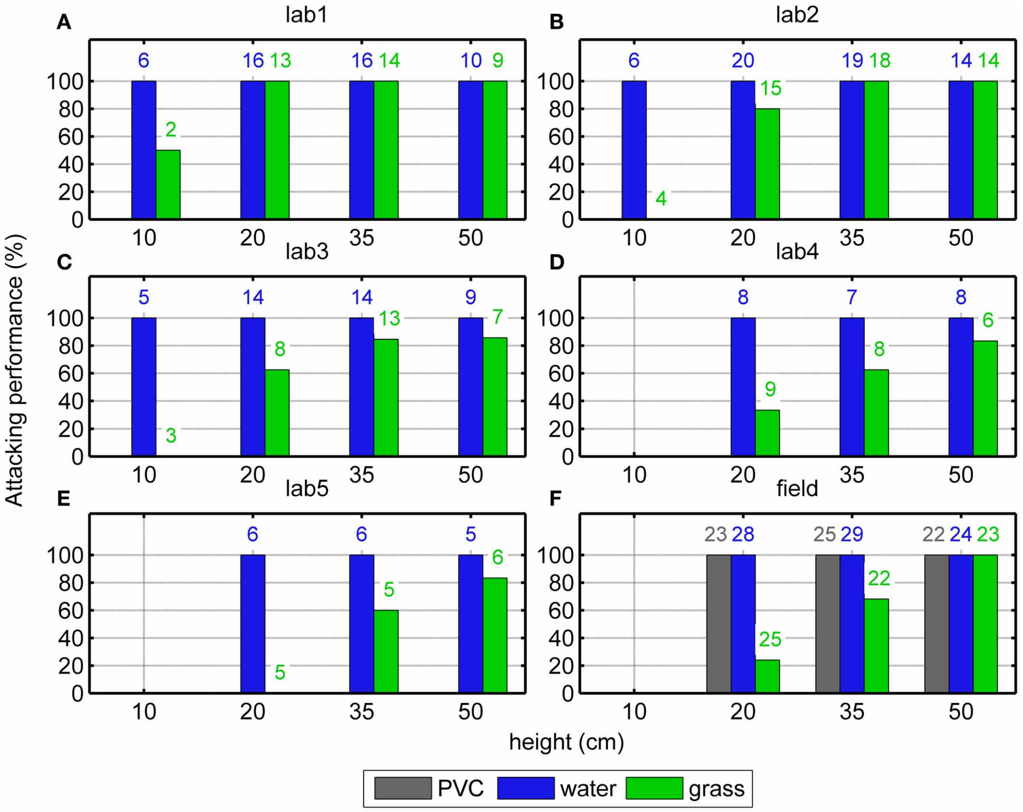

FIGURE 2 | Attacking performance above different surfaces at different heights. The results from 5 bats in the laboratory ("lab1-5", **A–E**) and from the field ("field", **F**) show that the bats always attacked one of the targets when it was above water or PVC (blue and gray bars). In contrast, above grass (green bars), the bats' attacking performance drastically diminished with decreasing height. The numbers of the successful trials are shown on the top of the bars.

performances above the grass surface differed significantly from each other.

In the Field experiment (218 trials) three different surfaces (PVC, water, or grass) and three different target heights (20, 35, or 50 cm) were presented. The same pattern of results as in the Laboratory experiment was observed: above water or PVC, the attacking performance was always 100% independently of target height (**Figure 2F**, blue and gray bars). However, above the grass surface, the attacking performance decreased monotonically with decreasing target height (green bars in **Figure 2F**, Fischer's exact test, $p < 0.001$).

DISCRIMINATION PERFORMANCE

In the Laboratory experiment, data from six different conditions [three target heights (20, 35, or 50 cm) above two surface types (water or grass)] were used to evaluate the bats' discrimination of the mealworm from the disk dummy. In general, the bats attacked the mealworm more often than the dummy, regardless of height and surfaces. While the average discrimination performance across the five bats in the laboratory was only 66% correct (206 correct trials out of 313), this performance is statistically significant because of the high number of trials (One-sided Binomial Test, $p < 0.001$). The GLMM analysis shows no significant difference in the overall (height independent) discrimination performance between water and grass surfaces [GLMM, $F_{(1,\ 27)} = 0.64$, $p = 0.43$]. Also, discrimination performance did not deteriorate significantly with decreasing height of the targets above water [blue bars in **Figures 3A–E**, $F_{(2,\ 8)} = 1.1$; $p = 0.37$]. However, discrimination performance deteriorated significantly with decreasing height of the targets above the grass surface [green bars in **Figures 3A–E**, $F_{(2,\ 7)} = 11.2$; $p = 0.007$].

In the Field experiment, data from nine different conditions [three target heights (20, 35, or 50 cm) times three surface types (PVC, water, or grass)] were used. Similar to the Laboratory experiment, the bats attacked the mealworm significantly more often regardless of height and surface (One-sided Binomial Test, $p < 0.001$, **Figure 3F**). However, in none of the surface type conditions an effect of target height was found (Fischer's exact tests, $p = 0.40$ with PVC; $p = 0.93$ with water and $p = 0.81$ with grass). There was also no significant difference between the surfaces (Fischer's exact test, $p = 0.075$).

FLIGHT PATH ANALYSIS

The bats' flight paths at the 35 cm target height conditions were reconstructed based on the laboratory video recordings of the last 4 s before capture. The median flight height above the grass surface was about 20 cm higher than above water (**Figure 4**). The

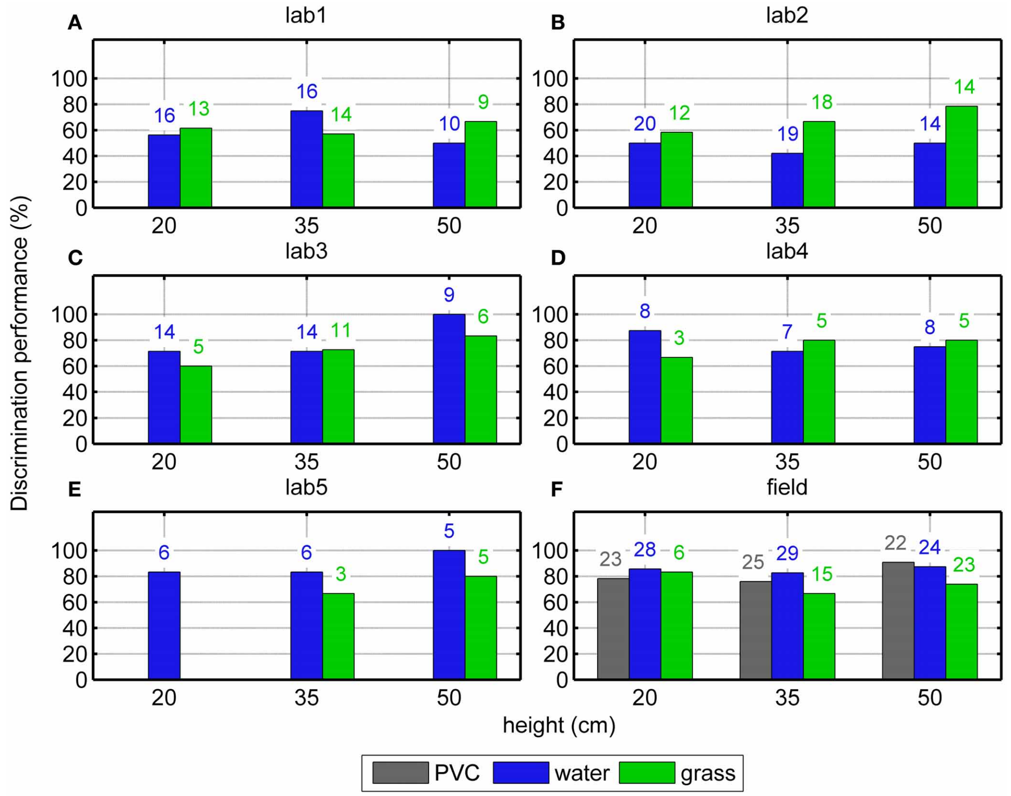

FIGURE 3 | Discrimination performance above different surfaces at different heights. Results from 5 bats in the laboratory ("lab1-5", **A–E**) and from the field ("field", **F**) are shown. There is a statistically significant decrease of the discrimination performance in the laboratory animals over grass; however we have found no such significant relationship in the field. The number of the trials in which we observed an attack are shown on the top of the bars.

median flight heights show that in the grass surface condition, the bats approached the target slightly from above, whereas in the water condition, the bats approached the target from below. The GLMM showed a significant surface effect [$F_{(1,\,47)} = 48.9$, $p < 0.001$], but no individual effect [$F_{(4,\,47)} = 1.26$; $p = 0.30$] on flight height.

CALL ANALYSIS

Two hundred and forty-six echolocation call sequences from Approach phases and 221 sequences from Buzz phases were analyzed in the laboratory recordings. On average, Approach phases contained 13.1 ± 0.6 calls and the Buzz phases contained 9.0 ± 0.34 calls (median ± standard error). The calls' peak frequency was analyzed for Approach and Buzz phase separately. When the targets were presented low above the grass surface, the bats increased the peak frequency of their calls significantly (**Figure 5**).

The GLMM analysis reveals significant differences in peak frequency between the water and grass surfaces conditions at a target height of 20 cm [GLMM, $F_{(1,\,71)} = 38.5$, $p < 0.001$ in Approach and $F_{(1,\,65)} = 12.8$; $p < 0.001$ in Buzz phase] and of 35 cm [$F_{(1,\,82)} = 12.2$; $p < 0.001$ in Approach phase and $F_{(1,\,68)} = 11.5$; $p = 0.001$ in Buzz phase]. No significant differences were found when the targets were 50 cm above the surfaces [$F_{(1,\,58)} = 1.2$; $p = 0.28$ in Approach phase and $F_{(1,\,53)} = 0.5$; $p = 0.47$ in Buzz phase].

ENSONIFICATION, IMPULSE RESPONSES

Two 30 cm discs made of either PVC or artificial grass were ensonified at different angles (**Figure 6**). At an ensonification angle of 90° (perpendicular ensonification, top row of **Figure 6**) the IR of the PVC is sharper and louder than that of the grass matting. However, at ensonification angles between 30 and 70°, the IR of PVC is weaker than that of the grass matting, especially at frequencies higher than about 50 kHz. Additionally the IR of the grass matting at these ensonification angles is temporally expanded. At a very small angle (10°) there is hardly any difference between the two surfaces.

DISCUSSION

In our study we found that for the bat *M. daubentonii*, the detection and discrimination of prey objects decreases at low heights above a clutter surface. This deterioration in psychophysical performance is accompanied by significant increases in both flight height and increases in the peak frequency of the bats' sonar emissions. The good agreement of the data from the Laboratory- and Field experiments corroborates the ecological relevance of the current tasks for the animals in the wild.

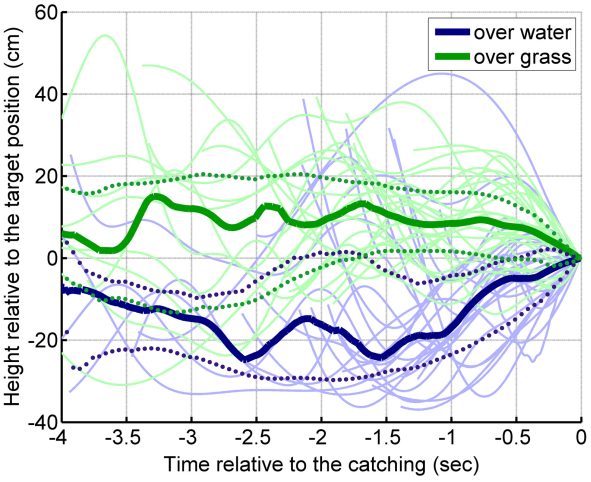

FIGURE 4 | Flight height of the bats in the last 4 s before making a capture at a target height of 35 cm. The moment of the capture is shown at 0 s (on the right side of the graph). On average, the bats flew around 10 cm above the target height when they were presented above grass (thick green line). When the targets were presented above water, the bats flew about 10–20 cm below the target height (thick blue line). The strong continuous lines show the median of the flight paths, the dotted lines show the upper and lower quartiles.

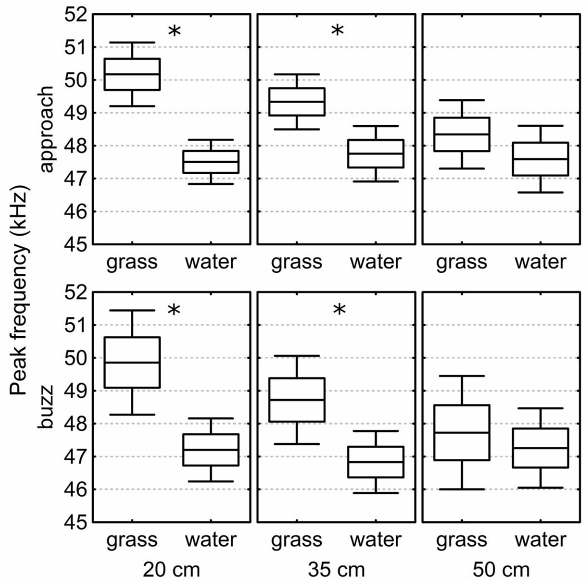

FIGURE 5 | Peak frequency of the echolocation calls above grass and water in the laboratory. In both, Buzz and Approach phase, and at target heights of both 20 and 35 cm, the peak frequency was significantly higher above grass than above water. At 50 cm height we found no significant differences in the peak frequency between the two surfaces. The box-plots show the mean, the standard error, and the confidence interval. Stars indicate significant differences ($p < 0.001$) (see call analysis section).

In the following we will discuss the data, first with respect to the performance of the bat, i.e., target detection and -discrimination, and second with respect to the behavioral adaptations of the bats, i.e., flight path and echolocation behavior.

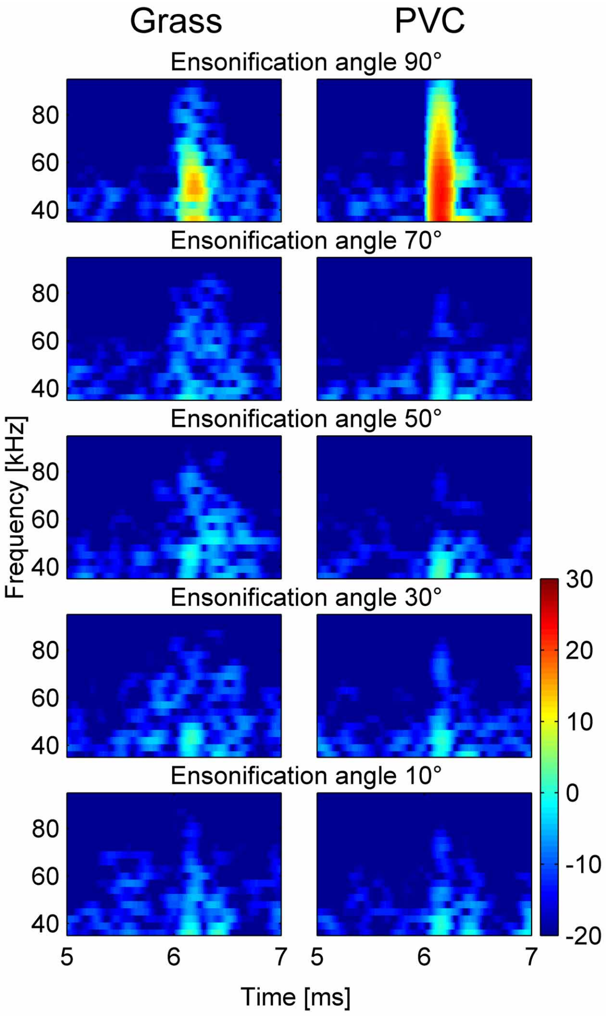

FIGURE 6 | Spectrograms of the impulse responses of the artificial grass and PVC surface at different ensonification angles. At an angle of 90° the impulse response of the PVC is stronger and smoother than that of grass (top row). At angles between 30 and 70° (second to fourth row) the impulse responses of the grass surfaces are longer and contain more high frequencies than those of the PVC surface. At 10° there is hardly any difference between the two surfaces.

TARGET DETECTION

In the Laboratory and in the Field experiments, the animals always executed an attack after they had initiated a trial when targets were presented 50 cm above any surface. However, with decreasing target height, the bats attacked less often above the grass surface (**Figures 2A–F**, green bars) while they still executed attacks above water. The current 2 AFC setup required the bats to find the one thread from which a mealworm is hanging. Unlike in a natural detection task, the general structure of the setup will indicate for the bats where to search for potential prey. Nevertheless, we observed that especially at very low heights above the grass surface, the animals attacked much less frequently.

In an experiment where *M. daubentonii* were trained to catch a mealworm suspended in front of a vertical clutter surface, Siemers and Schnitzler (2004) also found a significant decrease in attacking performance when the target distance was 25 cm or less. Thus, the attacking impairment appears independent of the absolute orientation of the surface (horizontal or vertical).

A particular case of target detection above surface is when the target is on the surface itself. Siemers et al. (2001b) found in their experiment that mealworms which were placed on smooth horizontal linoleum were readily caught, however, when mealworms were placed on the clutter screen, they were almost never caught. Accordingly, Boonman et al. (1998) found that higher duckweed density on natural water surfaces correlates with lower catching success of the bats from the surface. Moreover, bats prefer open waters against waters covered with duckweed. Rydell et al. (1999) also found reduced bat activity above rippled water. Von Frenckell and Barclay (1987) showed that bats' (*M. lucifugus*) foraging activity is higher above calm water than above turbulent water. We have found that attacking performance above the smooth water in the laboratory was the same as above rippled water in the field. These data indicate that although the water in our Field experiment was not smooth, its echo-acoustic reflection properties did not impair the bats' performance. Both literature- and our current findings thus indicate that a clearer definition of clutter is required: the surface tension of a rippled water surfaces acts as a spatial low-pass filter preventing sharp edges on the water surface. Any solid structure protruding from a water surface, however, will produce sharp edges in the surface structure. The artificial grass used in the current experiments consists mainly of such sharp edges. Also the clutter screen used by Siemers and Schnitzler (2004) and the duckweed vegetation of Boonman et al. (1998) included regular sharp edges. Thus, as soon as the background structure includes sharp edges, attacking performance of the bats is dramatically reduced. The question how sensitive the bats' sonar system is to such surface discontinuities has never been formally addressed.

The ensonification experiments showed that the grass surface created stronger echoes, especially at high frequencies, when ensonified at acute angles which represent angles used by bats hunting at low heights above a surface. It is likely that these echoes deteriorate the bats' perception of the three-dimensional shape of the target, and thus lead to the decrease in attacking and discrimination performance with decreasing target height.

Mackey and Barclay (1989) showed that both echo-acoustic clutter and the water-generated noise reduced foraging activity of the bats. By using the artificial grass, we can rule out a detrimental effect of the water-generated noise in our data. Also Siemers and Schnitzler (2004) used a "silent" clutter surface. These data indicate that echo-acoustic clutter introduced by sharp edges is much more likely to limit capture performance for most natural water surfaces.

Schnitzler and Kalko (1998) suggested that prey detection close to a clutter background is determined by the "clutter-overlap zone." This zone is defined as that prey-clutter distance at which the clutter echo overlaps with the prey echo, and thus inhibits detection. For *M. daubentonii* with a call duration of 1–1.5 ms, the clutter-overlap zone would be around 17–25 cm. Here, we show that detection performance already decreases at a distance of 35 cm to the clutter surface. Thus, a simple distinction in "Detection in the overlap free window" and "No detection in the clutter-overlap zone" is not sufficient to explain the observed hunting performance.

TARGET DISCRIMINATION

In Siemers et al. (2001b) naïve *M. daubentonii* did not spontaneously discriminate between mealworms and dummies (metal and rubber reflectors). The bats had to capture mealworms on a smooth or clutter linoleum screen. They readily captured mealworms on the smooth screen and repeatedly attacked the dummies placed in the same manner. Thus, the following search image was proposed: "small and isolated echo-reflecting objects on or above an acoustically smooth surface." Our results indicate that when challenged in a two-alternative forced-choice task bats show the ability to discriminate correctly between a mealworm and a similar-sized dummy. However, in nature bats are rarely confronted with such a defined task and it is more often the case that bats have to discriminate between different kinds of objects and prey, e.g., between leaves or little twigs and insects floating on the water surface. Thus, the suggested search image is reasonable, but not generally valid. Boonman et al. (1998) suggested that Daubenton's bats discriminate edible from inedible objects by analyzing changes in the spectrum of subsequent echoes. These changes are evoked by either moving targets, or by the bats moving around the stationary targets, when the targets have aspect-dependent reflection characteristics. In our study, both targets, the mealworm and the dummy, were moving (typically rotating slowly) and thus created changes in the spectrum over subsequent echoes. Yet the bats were still able to discriminate the mealworm from the dummy. Hence, *M. daubentonii* has to have a more sophisticated echo analysis than just analyzing a sequence of echoes which change in their spectral content over time from an echo sequence which is spectrally invariant over time.

FLIGHT PATH

Flight paths illustrated in **Figure 4** show that above water, the bats fly very close to the surface and attack the prey from below. This behavioral strategy appears to maximize the echo-acoustic enhancement effect (Siemers et al., 2001b, 2005): the lower the height of the bat above the water, the smaller the elevational angle between the direct echo from the prey to the bat and the indirect echo from the prey via the water to the bat. Moreover, when the bat flies close to the water surface, the echo-delay difference between the two echo paths is minimal. As the perceptual echo enhancement will increase with both decreasing angular difference and decreasing temporal difference, the observed flight behavior strongly supports the hypothesis that bats exploit the additional echoes from the water surface to detect and possibly also identify the prey item. As it is true for the aerodynamic ground effect, the increased acoustic impedance of the water surface facilitates the generation of additional prey echoes. Thus, the animals appear to exploit an echo-acoustic ground effect through the spatio-temporal integration of direct echoes from the prey with indirect echoes via water surface. Note, however that this enhancement comes at the expense of misleading spatial cues in the echo, because the indirect echo via the water surface signals the wrong elevation of the prey. To avoid this problem bats could resign to precedence-like auditory strategies, where accurate localization is dominated by the first sound. Precedence effects in the vertical plane have been described in human psychophysics (Litovsky et al., 1997).

Above a clutter surface, the bats flew significantly higher. Increasing the flight height will increase both the angular and temporal differences between the direct echo and the scattered indirect echo (cf. **Figure 6**) via the clutter surface. Thus, the observed increase in flight height is consistent with the hypothesis that echoes from the clutter surface are not useful for the bat and the bat tries to separate those echoes (both in terms of echo delay and elevational angle) from the direct echoes.

The bats' increased flight height could also be an indication that they fail to properly determine their height above the surface due to the increased and diffuse reflections caused by the clutter surface. As a consequence, they increase the flight height to avoid colliding with the surface as the roughness may indicate a higher likelihood of objects protruding high enough to interfere with the flight path.

Another possible explanation for this adjustment of flight height may lie in echo-acoustic flow-field information. Bhagavatula et al. (2011) showed that, based on visual flow-field information, budgerigars adjusted their flight trajectory always to be closer to that wall which evoked a smaller visual image motion. In our experimental paradigm, the echo-acoustic image motion above artificial grass would be stronger than above water. It is conceivable that such echo-acoustic flow-field information resulted in an adjustment of the flight trajectory to a larger height in the grass condition.

ECHOLOCATION BEHAVIOR

We analyzed calls from 467 sequences from the Laboratory experiment. Above grass, the bats significantly increased the peak frequency of their echolocation calls with decreasing target height. We stress that these changes in echolocation are small (~3 kHz), but due to the correlation with height and surface, are likely to be a behavioral response of the bat to the surface. Brinklov et al. (2010) showed that *Macrophyllum macrophyllum* increases its peak frequency in a cluttered environment compared to open space. Since the width of the sonar beam is mainly determined by the frequency, these changes in the bats' echolocation calls lead to narrowing of the sonar beam. Suemer et al. (2009) found that *Eptesicus fuscus* tends to increase the second harmonic of its echolocation signals when challenged with a spatial unmasking task. This suggests that a narrow sonar beam is likely to be advantageous when hunting in a cluttered environment, for it reduces the number and intensity of off-axis echoes, and thus increases signal-to-noise ratio.

Due to the downward frequency-modulated structure of the *M. daubentonii* echolocation calls, the increase of call peak frequency is likely to be correlated with decreased call duration. While, due to the strong water reflections picked up by the microphones, an analysis of temporal call parameters appears impossible in our hands, a putative decrease in call duration would further facilitate the temporal separation of prey- and clutter echoes as discussed above (see flight path section).

CONCLUSIONS

The present data provide new behavioral insight into the sophisticated hunting strategies recruited by bats hunting over water. Specifically, the data show that bats not only reliably detect targets above water but can also discriminate targets. When the water surface is covered with a clutter surface (in our case artificial grass, often vegetation in nature), the bats hunting performance, both in terms of detection and discrimination, decreased significantly with decreasing distance to the surface. Also the flight- and ensonification pattern is significantly changed: in contrast to flight over a clutter surface, the bats chose very low flight paths over water which allow for optimal spatio-temporal integration of direct echoes from the prey with indirect echoes via the water surface. This echo-acoustic strategy is analogous to trawling bats exploiting an aerodynamic ground effect (Norberg and Rayner, 1987; Aldridge, 1988; Jones and Rayner, 1991), i.e., the higher impedance of a smooth surface for the lift of an object moving above water. The suggested combination of spatio-temporal integration and precedence-like localization can be viewed as trawling bats not only exploiting an aerodynamic but also an echo-acoustic ground effect.

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The noseleaf of *Rhinolophus formosae* focuses the Frequency Modulated (FM) component of the calls

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Bats of the family Rhinolophidae emit their echolocation calls through their nostrils and feature elaborate noseleaves shaping the directionality of the emissions. The calls of these bats consist of a long constant-frequency component preceded and/or followed by short frequency-modulated sweeps. While Rhinolophidae are known for their physiological specializations for processing the constant frequency part of the calls, previous evidence suggests that the noseleaves of these animals are tuned to the frequencies in the frequency modulated components of the calls. In this paper, we seek further support for this hypothesis by simulating the emission beam pattern of the bat *Rhinolophus formosae*. Filling the furrows of lancet and removing the basal lappets (i.e., two flaps on the noseleaf) we find that these conspicuous features of the noseleaf focus the emitted energy mostly for frequencies in the frequency-modulated components. Based on the assumption that this component of the call is used by the bats for ranging, we develop a qualitative model to assess the increase in performance due to the furrows and/or the lappets. The model confirms that both structures decrease the ambiguity in selecting relevant targets for ranging. The lappets and the furrows shape the emission beam for different spatial regions and frequency ranges. Therefore, we conclude that the presented evidence is in line with the hypothesis that different parts of the noseleaves of Rhinolophidae are tuned to different frequency ranges with at least some of the most conspicuous ones being tuned to the frequency modulated components of the calls—thus yielding strong evidence for the sensory importance of the component.

Keywords: rhinolophus, chiroptera, emission, formosae, ranging, noseleaf, furrows, lappets

INTRODUCTION

Various bat species emit their echolocation calls through their nostrils (Nowak, 1994). In these species, the nostrils are often surrounded by leaf- or spear-like structures called noseleaves. Noseleaves have been shown in experiments (Hartley and Suthers, 1987) and in acoustic simulations (Vanderelst et al., 2010) to act as baffle and to focus the emission beams of bats. The most elaborate noseleaves are found in the Horseshoe bats (Rhinolophidae) and the Old World Leaf-Nosed bats (Hipposideridae). Bats of both families emit echolocation pulses consisting mostly of a single narrow constant frequency (CF) component that is often preceded and/or followed by a very short frequency modulated (FM) component (See Jones and Teeling, 2006; Schnitzler and Denzinger, 2011, for a review).

Zhuang and Muller (2006) argued based on acoustic simulations, that the furrows (see **Figure 1**) on the noseleaf of the Rufous Horseshoe Bat *Rhinolophus rouxii* function as resonance cavities de-focusing the emission beam at the lowest frequencies contained in the FM component of the call. Recently, we attempted to replicate this study but found only partial agreement. In accordance with Zhuang and Muller (2006), the results of our study indicate that the furrows affects the emission beam most for frequencies in the FM component of the echolocation call. However, we found that the noseleaf furrows, in accordance with the functionality of noseleaves in other bat species (Hartley and Suthers, 1987; Vanderelst et al., 2010), aid in focusing the emission beam (Vanderelst et al., 2012). Apart from the disagreement of the two studies about the specific effect of the furrows (i.e., focussing vs. de-focussing), both studies found that the noseleaf furrows of *R. rouxii* act on the emission beam at the frequency range of the FM part of the call. These results suggest that the lancets of Rhinolophids are morphological structures adapted to the FM component of the calls yielding direct evidence for the importance of the FM component for the sonar system of these bats. This is somewhat unexpected, as Rhinolophids are otherwise known for featuring a wide range of anatomical and physiological specializations tuned to processing the CF component of their call (Reviewed in Schnitzler and Denzinger, 2011).

As the evaluation of the function of noseleaf furrows was performed only for a single species, the generality of this result

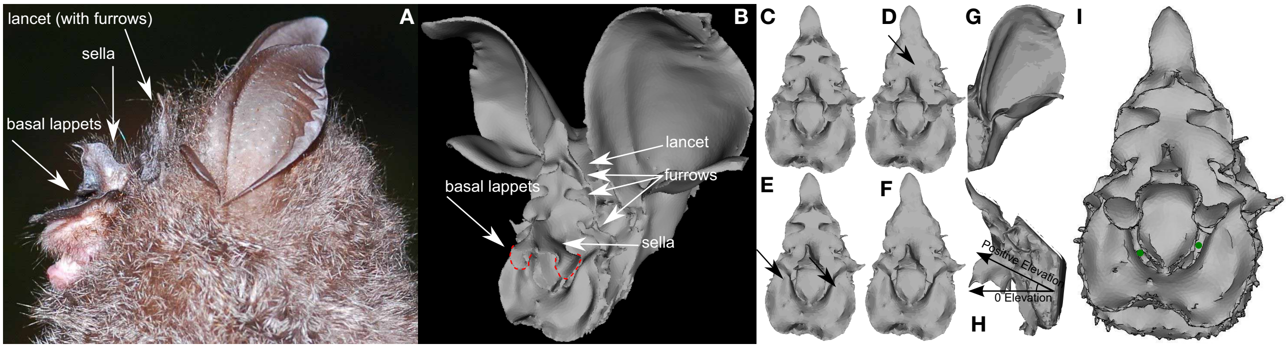

FIGURE 1 | Rendering of the 3D models of the noseleaf and pinna of *Rhinolophus formosae* used to simulate the emission and hearing beam patterns. (A) Photo of *R. formosae* (courtesy of Chun-Wei Hsie). **(B)** Rendering of complete head model from which the noseleaf model **(C–F)** and the pinna model **(G)** were derived. In panels **(A,B)** the different parts of the noseleaf have been indicated. In panel **(B)** the basal lappets have been circled. **(C)** Original noseleaf model. **(D)** Model with filled furrows (see arrow). **(E)** Noseleaf model with removed basal lappets (see arrows). **(F)** Noseleaf model with both filled furrows and removed basal lappets. **(G)** Pinna model. Note that the rendered pictures **(C–F)** and **(H)** are not aligned but have been rotated to facilitate viewing of the features of the noseleaf. **(H)** Model indicating the reference position of the noseleaf model. **(I)** Rendering of the noseleaf with the position of the virtual receivers indicated by green dots. The supporting material provides movies of the rotating models.

remains to be confirmed. In this paper, we investigate the effect of the furrows in the noseleaf of the Formosan Wooly Horseshoe Bat *Rhinolophus formosae* (Sanborn, 1939). Specifically, we seek further support for the hypothesis that the furrows in Rhinolophids play a dominant role for the FM frequency range of an emitted call. In addition to furrows, the noseleaf of *R. formosae* also features two flaps (the base of the sella has a pair of circular basal lappets, see **Figure 1**) partially covering the nostrils. We hypothesize that these flaps aid in focusing the emission beam by interacting with the emitted sound field. Furthermore, we suggest that if these flaps influence the sound field primarily at the FM frequencies, it provides further support for the hypothesis that particular noseleaf structures of Rhinolophids are not tuned to the CF but to the FM component. Finally, this paper introduces a formal model to quantify the functional relevance of noseleaf structures in bats, e.g., furrows and basal lappets in the case of *R. formosae*, based on the current understanding of the function of the FM component.

METHODS

MODEL CONSTRUCTION AND SIMULATION

We used the Boundary Element Method (BEM) to simulate the directionality of the echolocation system of *R. formosae*. As we have reported in detail on this method and its validation elsewhere (De Mey et al., 2008; Vanderelst et al., 2012), we will only describe the simulation method briefly. Using BEM to simulate the sound field around a bat's head requires the construction of a detailed mesh model of the head morphology. A single specimen of *R. formosae* was collected by mist-netting in Kenting, Taiwan, in 2010. We first preserved the specimen in 95% ethanol, and later in a sealed and air-proofed specimen box, well cushioned with wet cotton cloth during shipping to maintain constant humidity levels and preserve the natural shape of the outer ears and the facial structures. The head of the specimen of *R. formosae* was scanned with a MicroCT machine using a resolution of 70 μm. After reconstructing the shadow images, an initial mesh model of the complete head was obtained using a set of standard biomedical imaging tools (see **Figure 1** for renderings of the models).

Current computational facilities allow to simulate models containing up to 35,000 triangles. The noseleaf of *R. formosae* is a very complex structure consisting of two rows of furrows and basal lappets overhanging both nostrils. Furthermore, in comparison to most other echolocating bats, *R. formosae* is a relatively large species, with adults averaging around 21 g in body mass and 58 mm in forearm length (Lee et al., 2012). Hence, to construct a model of sufficient detail of the noseleaf, we made a separate model of the noseleaf to simulate the emission directionality of *R. formosae*. As the pinna is also quite large compared to that of most other echolocating bats, we again made a separate model of the left pinna of the bat. The mirrored left ear (and its sensitivity pattern) was used as a replacement for the right ear.

The initial noseleaf and pinna models were subjected to several rounds of smoothing and remeshing to reduce the number of triangles in the models to about 30,000. The maximum edge length of the final models was 0.6 mm. At 80 kHz, the highest frequency employed in the simulations, an edge length of 0.6 mm results in a sampling of 7 nodes per wavelength (4.2 mm) which was sufficient to obtain stable simulation results. From the original noseleaf model, two additional models were derived. In one model we filled the furrows in the upper part of the noseleaf , whereas in the other noseleaf model, we removed the basal lappets of the sella that overhang the nostrils (see **Figure 1** for renderings and the supporting material for movies of the models).

To simulate the emission beam pattern we placed a virtual receiver in both nostrils of the noseleaf model (see **Figure 1I**). Placing receivers in the noseleaf model to simulate the emission beam pattern is warranted by the reciprocity principle (Pierce et al., 2008) and enhances numerical stability of the simulations (Moller and Cutanda Henriquez, 2009). To obtain the emission beam pattern, the complex sound fields of the left and the right nostril are summed and the magnitude of this sum is reported.

Simulating the directional hearing sensitivity was done by placing four virtual receivers in the ear canal of the pinna model. Virtual omnidirectional sources are placed on a sphere with a diameter of 1 m around the bat noseleaf model. The sources are spaced 2.5° apart covering −90 to 90° in both azimuth and elevation (i.e., 5329 sources). Placing the sources in this regular configuration allows for easy preprocessing of the data. This configuration, however, does not sample the sound field on the sphere uniformly. Therefore, we resampled both the emitted sound field and the hearing directionality at 528 equally spaced positions during the processing of the data using the Recursive Zonal Equal Area Sphere Partitioning Toolbox (Leopardi, 2006). In processing the emission beam pattern, we assume that all the emitted sound energy stays within the frontal hemisphere, i.e., negligible amounts of energy are radiated backward, requiring the normalization of the emission beam patterns of the bats per frequency f,

$$\int_{\Omega} p^2_{f,\,\phi,\,\theta} \cdot d\phi d\theta = 1 \tag{1}$$

with p denoting the magnitude of the emission strength for frequency f in direction (azimuth $= \phi$, elevation $= \theta$) and Ω the frontal hemisphere.

To assess the roles of the furrows and the flaps in focusing the emission beam, we calculate the average gain $\bar{g}$ for the normalized emission beam patterns,

$$\bar{g} = \frac{\int_{\Omega} g_{f,\,\phi,\,\theta}}{\Omega} \tag{2}$$

with the gain for a particular direction and frequency given by

$$g_{f,\,\phi,\,\theta} = 10 \cdot \log_{10} \frac{p^2_{f,\,\phi,\,\theta}}{\max_{\Omega} p^2_{f,\phi,\theta}} \tag{3}$$

In accordance with Schnitzler and Grinnell (1977) and Firzlaff and Schuller (2004), the model was oriented such that the horseshoe of the noseleaf was vertical (see **Figure 1H** for an illustration of the coordinate system used in this paper).

CALL RECORDINGS

In order to asses the frequency range of the FM components of the calls of *R. formosae,* recordings were collected from 7 individual bats and 159 calls were extracted and analyzed. Echolocation calls of *R. formosae* individuals were recorded every evening between the 5th and 12th of October 2010, in the Guijijaou Experimental Forest and Hengchun Tropical Botanical Garden (HTBG, 120°48′E, 20°58′N, ca. 450 ha in area and 200–300 m in elevation; Taiwan Forestry Research Institute), Kenting, Taiwan. Recordings were started around sunset in the prime activity period of the bats (Lee et al., 2012), and lasted until around 23:00. Echolocation calls were recorded using a condenser microphone (microphone capsule CM16, CMPA preamplifier unit, Avisoft Bioacoustics, Berlin, Germany) and digitized with a real time ultrasound acquisition board (UltraSoundGate 116, Avisoft Bioacoustics, Germany; 375 kHz sampling rate, 16 bit resolution) connected via USB port to a laptop computer (Eee PC, ASUS, Taiwan). We walked along different trails in the forest and around different edge or open sites to avoid sampling the same individuals.

RESULTS

ACOUSTIC RECORDINGS

In total, 159 calls of high quality from seven individuals were chosen to be analyzed (Spectrograms were calculated using a 256 sample FFT with 75% overlap and Hanning windowing). **Figure 2** displays the spectrograms of two call sequences of *R. formosa.* Only 4% of the analyzed calls had neither a leading (FM_1) nor a trailing (FM_2) FM part. Of all calls ($n = 159$), 18% missed a leading (FM_1) and 10% a trailing (FM_2) FM component. Where present, the lowest frequency of the FM parts was extracted using AviSoft SaSLab Pro (Raimund Specht, Berlin). In addition, the frequencies of the CF part and the intensity of both FM and CF parts were registered. The resulting data for the seven bats are plotted in **Figure 2**. The FM parts were 15–20 dB weaker than the CF parts. Our acoustic recordings indicate that *R. formosae* uses calls with a CF of about 43 kHz and FM parts spanning from 36 to 43 kHz. This frequency range covers the first overtone of the bat's calls. The other harmonics were detectible in the recordings but were typically 30–40 dB weaker.

SIMULATIONS

Figure 3 displays the simulated emission beam patterns for selected frequencies and the different noseleaf models. The emission beam consists of a single mainlobe located little below the horizontal plane (i.e., around −15° elevation). Filling the furrows increases the gain of the emission beam pattern for high elevation positions (around 60° elevation). The flaps reduce the gain of the emission beam pattern in a circular area around the mainlobe. The average gain of the emission pattern shows a global minimum in the frequency range coinciding with that of the calls of *R. formosae*. Removing either the basal lappets or filling the furrows results in an increase in the average gain (see Equation 2) and thus a loss in directivity. The effect of filling the furrows and removing the lappets on the average gain is largest in the frequency range of the calls of the bat. However, the largest effect is not found around the CF frequency (42–43 kHz) but in the frequency range of the FM part of the call. The effect of filling the furrows is largest for 40 kHz. For the lappets, the effect is largest for 36 kHz.

The simulation results were confirmed by acoustic measurements using 3D printed versions of the original noseleaf model and the model without lappets and filled furrows. In the measurements, the structures also focussed the beam most strongly around 36 kHz (results provided as supporting material).

QUANTIFICATION OF THE NOSELEAF FUNCTIONALITY

The finding that characteristic substructures of the noseleaf of *R. formosae* have the largest acoustic effects at the frequencies of the FM part of the calls suggests that the FM component is an integral and important part of the calls of CF/FM bats. These bats are assumed to detect, identify and locate prey based on the frequency modulations of the CF component of the echo caused by fluttering prey. Listening for frequency modulations in the echo using a highly specialized hearing apparatus makes

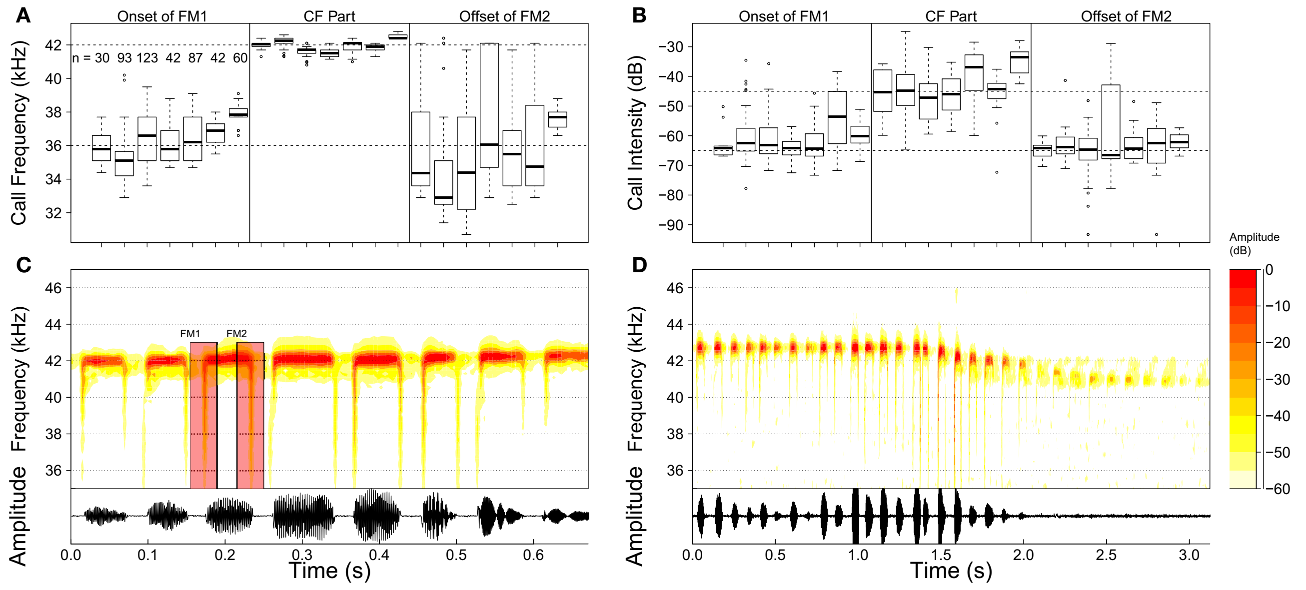

FIGURE 2 | (A) Boxplots for the frequency of the FM and CF parts for each of the seven bat specimens whose calls were analyzed. Open circles denote outliers defined as data points that lie below $Q_1 - 1.5 \times (Q_3 - Q_1)$ or above $Q_3 + 1.5 \times (Q_3 - Q_1)$ (with Q_n the nth quartile). The number of calls analyzed for each individual are displayed in the leftmost section of the panel. **(B)** Similar but for call intensity. **(C,D)** Two spectrograms of call sequences of *Rhinolophus formosae*. **(C)** calls of a perched bat. The frequency modulated parts (FM_1 and FM_2) of one of the calls are indicated. **(D)** calls of a perched bat taking off. Only the strongest first overtone is shown in this figure.

these bats highly robust with respect to clutter echoes (Schnitzler and Denzinger, 2011). Echoes originating from stationary vegetation can be effectively filtered out by the hearing apparatus and the Doppler shifted parts of the echo contain sufficient localization information (Vanderelst et al., 2011). Conversely, the bat has no mechanism to reliably filter out any FM echoes generated by clutter objects. Hence, featuring morphological adaptations to focus the beam in the FM part of calls makes sense considering the echolocation strategies of these CF/FM bats. As reviewed by Schnitzler and Denzinger (2011), the FM parts of the calls of Rhinolophus bats are assumed to be used predominantly for measuring range. Therefore, we developed a model quantifying the effectivity of clutter rejection of the lappets and furrows in a ranging task.

We will assume that ranging only requires the FM part of the echo from a target object. From radar theory (Skolnik, 1980) it is known that both detection probability and ranging accuracy depend upon the energy in the received signal. Hence, we assume the bat estimates the energy in this FM part of the echo E,

$$E = 10 \cdot \log_{10} \int_f p_f^2 \tag{4}$$

with p_f the magnitude of the Fourier transform of the sound pressure level at frequency f. Having reduced each echo to a scalar energy estimate, the simplest strategy for the bat is to interpret the echo with the highest energy as coming from the object onto which it has centered its beam, which coincides more or less with the flight direction of the bat. Such a mechanism will result in selecting the correct echo most of the time. However, the mechanism breaks down in the presence of strong clutter reflectors. The probability of selecting the incorrect clutter echo $C_{\phi,\theta}$, originating from azimuth ϕ and elevation θ as the one coming from the target object can be written as,

$$P(E_{T,H} < E_{C,H}|C_{\phi,\theta}) = 1 - \int_{-\infty}^{0} L \cdot \int_{-\infty}^{0} R \tag{5}$$

The energy of both the target and the clutter echo depend on the spatial sensitivity H of the bat (i.e., combination of emission beam pattern and auditory spatial sensitivity). In Equation (5), L and R denote the normally distributed energy of the clutter echo arriving at the left and right ear, respectively. This is, $L = N(\hat{E}_{\phi,\theta}|l, \sigma)$ and $R = N(\hat{E}_{\phi,\theta}|r, \sigma)$ with $\hat{E}_{l,\phi,\theta}$ and $\hat{E}_{r,\phi,\theta}$ the expected energy of a clutter echo coming from azimuth ϕ and elevation θ and arriving at the left and the right ear, respectively, given the spatial sensitivity of the left (l) or the right (r) ear. In Equation (5), we assume that the reflector strength of objects in the environment is normally distributed with a standard deviation given by σ.

Using Equation (5), we can calculate, for each of the noseleaf models and their respective emission beam patterns, the probability that an echo arriving from a given location will interfere with the correct selection of the FM echo coming from the target for a given value of σ. In addition, we calculated the difference in the probabilities for the models in which the furrows were filled or the flaps were removed.

Selecting values for σ should preferably be done based on empirical measurements of the variation in the energy of echoes returning from a large sample of different plants. However, to the best of our knowledge, no such estimates have been published.

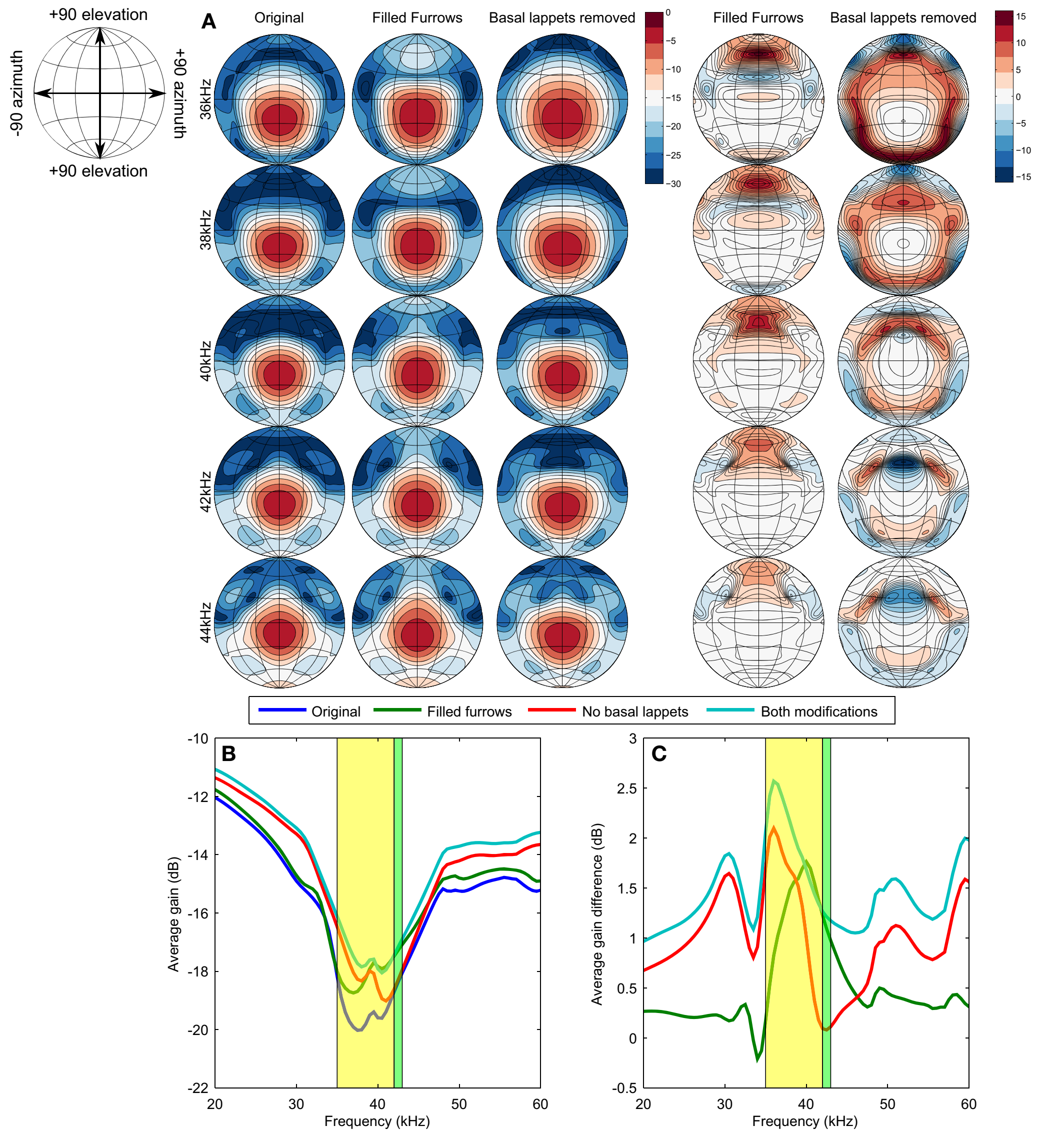

FIGURE 3 | (A) Simulated emission beam patterns for the original model and the models with filled furrows or without flaps (contours are spaced 3 dB apart). The two right most columns show the difference in gain ($\bar{g}$) between the original model and the two altered models (contours are spaced 1 dB apart). **(B)** Average gain of the emission beam pattern as function of frequency. The frequency ranges of both the FM and the CF components are shaded in yellow and green, respectively. The maps in panels **(A,B)** are Lambert azimuthal equal-area projections centered around zero azimuth and elevation. The parallels and meridians are 30° apart. See the top left inset for the definition of the axes. **(C)** similar as **(B)** but showing the average gain difference with the original model as a function of frequency.

Therefore, we extrapolate the value for σ from measurements we collected earlier as well as data provided by Ralph Simon (University Ulm). This data set consists of echoes collected from fluttering insects and a number of flowers from different aspect angles. Earlier we showed, based on these data, that the variation in energy for a narrow frequency band could be adequately modeled using a normal distribution with a standard deviation of about 10 dB (see supplementary material of the paper by Reijniers et al., 2010). The variation in energy of a broadband echo can be approximated based on this data by making an adequate assumption about the number of independent frequency channels an echo will stimulate in the cochlea of the bat. This can be estimated using the Equivalent Rectangular Bandwidth (ERB). Extrapolating the formula for calculating ERB values given in Moore and Glasberg (1983). for the bat's frequency range, a frequency channel with center frequency $f = 40$ kHz corresponds with an ERB value of 4.3 kHz. This implies that the frequency range of the FM component (about 7 kHz) can be modeled by about 2 independent frequency channels. Assuming 2 independent frequency channels and a standard deviation of 10 dB per frequency channel yields a standard deviation of about 14 dB for the energy of an echo. Based on this extrapolation, we evaluate the model for a wide range of σ values around 14 dB, i.c. from 10 to 25 dB.

Figure 4 displays the interference probability $P(E_{T,H} < E_{C,H}|C_{\phi,\theta})$ as a function of azimuth and elevation as well as the increase in error probability associated with having either furrows, flaps or both structures removed. Irrespective of the value of σ, the probability of confusing a non-target FM echo with the FM echo is the largest in a ellipsoid area around azimuth 0 and elevation −15°. The furrows reduce the probability confusing the target and non-target echo mostly in an area between 0 and +30° azimuth. The flaps reduce the confusion in a circular area around −15° elevation.

As demonstrated above, the furrows and the basal lappets alter the emission beam pattern mostly in a frequency range coinciding with the FM part of the call. To confirm that the furrows and the basal lappets cause the largest reduction in the probability of confusion in the same frequency range, we calculate the expected angular error due to the removal of the basal lappets and the filling of the furrows across the frontal hemisphere for a range of frequencies and values of σ. In particular, we calculate the increase in angular error resulting from the removal of the furrows and the flaps for frequency ranges given by $[f_{\text{low}}, f_{\text{low}} + \Delta f]$ with f_{low} ranging from 30 to 50 kHz and Δf set to 7 kHz (the range spanned by the FM component). The increase in error is given by the expected distance in degrees $E(\zeta)$ between the strongest echo and the target echo using Equation (6). In this equation, $G(\phi, \theta)$ gives the arc length in degrees between the direction of the target echo and the direction of the interfering echo.

$$E(\zeta) = \int^{\phi,\,\theta} P(E_{T,H} < E_{C,H}|C_{\phi,\theta}) \times G(\phi,\theta) \qquad (6)$$

Figure 5 shows that the effect of the both the furrows and the lappets is largest in a frequency range coinciding with the FM range. In agreement with the effects of both structures on the average gain, the effect of the furrows is maximal for a frequency range starting at a somewhat higher frequency than the effect of the basal lappets. The effect of the furrows is maximal for the range (37, 44 kHz) while the effect of the lappets is largest for the frequency range (35, 42 kHz).

CONCLUSION

Simulating the effect of both the furrows and the flaps of the noseleaf of *R. formosae* on the emission pattern, we find that both structures aid in focusing the emission beam. The largest effect

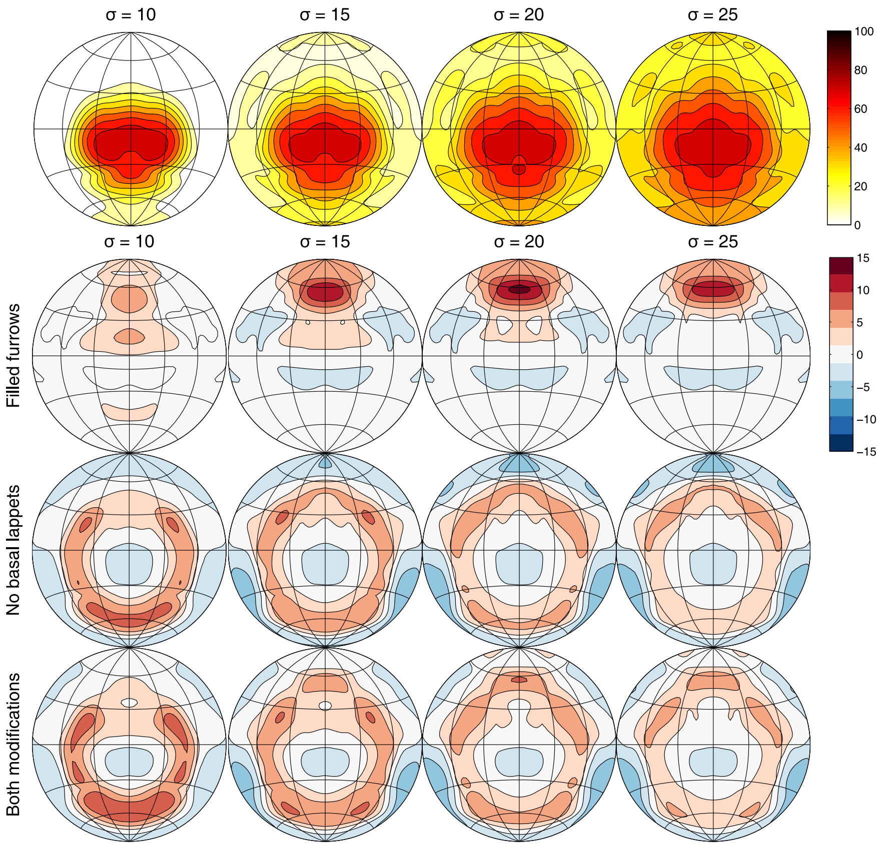

FIGURE 4 | Row 1: The probability of the energy of an FM echo coming from a clutter object being higher than an echo coming from the target position as a function of azimuth and elevation (Contours spaced 10% apart) and for different values of σ. As specified in the main text, the reflector strength of objects in the environment is assumed to be normally distributed. The standard deviation of this distribution is given by σ. These plots show the area in which confusion between the target echo and an interfering echo is most likely. **Rows 2–4:** the gain in the probability of confusion by removing either furrows, basal lappets or both (Contours spaced 2% apart). Plots show the averages across the frequency range of the calls (i.e., from 36 to 43 kHz).

of these structures is not found around the CF frequency but in the frequency range of the FM parts of the calls. Interestingly, the frequency at which the effects of each structure is largest differs: the lappets have the largest impact at somewhat lower frequencies than the furrows. However, the structures are not only complementary in terms of frequency. The effects of both structures are also spatially complementary as the furrows influence the beam mostly for high elevations while the lappets focus the beam in a circular area around the main beam.

Currently, the best supported hypothesis about the functionality of the FM component is that it is used in ranging. The model presented in the paper suggests that the lappets and furrows increase the bat's ranging accuracy by suppressing echoes coming from peripheral targets. Moreover, the model showed that the angular error in selecting the target echo is reduced most efficiently for frequency ranges coinciding with the FM component.

In summary, the evidence presented here and elsewhere (Zhuang and Muller, 2006; Vanderelst et al., 2012) suggests a division of labor between different substructures of the noseleaves of Rhinolophidae with various morphological structures shaping the soundfield at different frequencies. Moreover, at least some of the most conspicuous features seem to be tuned to the FM component.

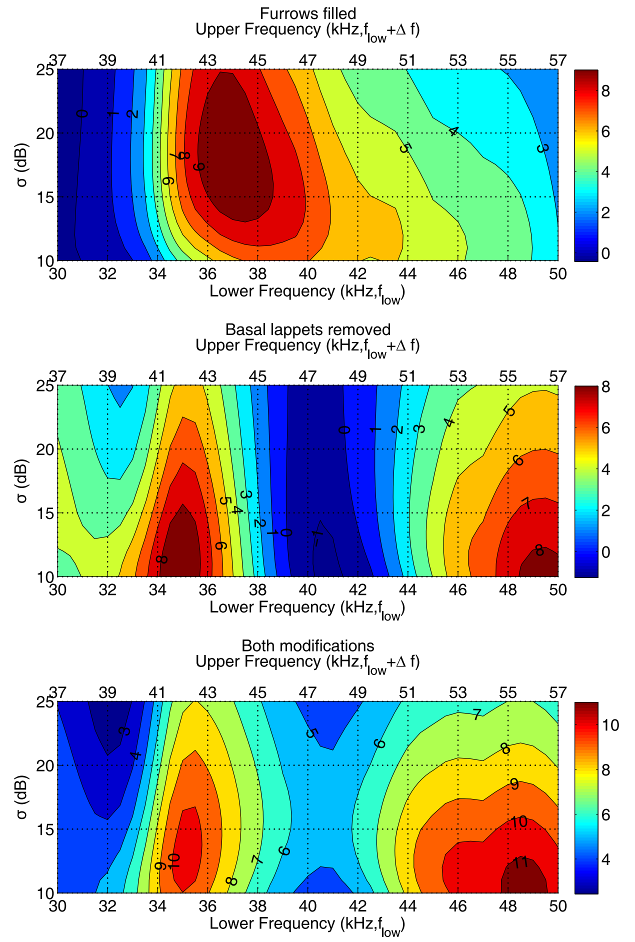

FIGURE 5 | The expected angular error E(ζ)(in degrees) between the strongest echo and the target echo averaged across the frontal hemisphere for different frequency ranges and values of σ and different noseleaf models. The lower x-axis depicts the lower frequency of the interval. The upper x-axis shows the upper frequency.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/Integrative_Physiology/10.3389/fphys.2013.00191/abstract

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Echolocation intensity and directionality of perching and flying fringe-lipped bats, *Trachops cirrhosus* (Phyllostomidae)

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The Neotropical frog-eating bat, *Trachops cirrhosus*, primarily hunts stationary prey, either by gleaning on the wing, or in a sit-and-wait mode hanging from a perch. It listens passively for prey-generated sounds, but uses echolocation in all stages of the hunt. Like other bats in the family Phyllostomidae, *T. cirrhosus* has a conspicuous nose leaf, hypothesized to direct and focus echolocation calls emitted from the nostrils. *T. cirrhosus* is highly flexible in its cognitive abilities and its use of sensory strategies for prey detection. Additionally, *T. cirrhosus* has been observed to echolocate both with closed and open mouth. We hypothesize that its flexibility extends to echolocation call design. We investigated the effect of hunting mode, perching or flying, as well as the effect of mouth opening, on the acoustic parameters and directionality of the echolocation call. We used a multi-microphone array, a high-speed video camera, and a microphone-diode-video system to directly visualize the echolocation sound beam synchronized with the bat's behavior. We found that *T. cirrhosus* emits a highly directional sound beam with half amplitude angle (HAM) of 12–18° and DI (directionality index) of ~17 dB, among the most directional bat sonar beams measured to date. The directionality was high both when flying and when perching. The emitted intensity was low, around 88 dB SPL at 10 cm from the mouth, when hanging, but higher, around 100 dB SPL at 10 cm, when flying or just before take-off. Our data suggests that the limited search volume of *T. cirrhosus* sonar beam defined by the high directionality and the rather low intensity of its echolocation calls is adapted to the highly cluttered hunting habitat and to the perch hunting mode.

Keywords: echolocation, directionality, intensity, sonar beam, perch hunting

INTRODUCTION

Echolocation is one of the key adaptations enabling the successful and rapid radiation of bats. Bats emit high frequency signals and use the returning echoes to orientate in darkness, to detect and localize prey, and to find roosts. There is considerable variation in echolocation call design across bat species, and a large number of studies have shown that within species, individuals can flexibly adapt the time- and frequency features of their echolocation calls to the situation and task at hand (e.g., Neuweiler, 1989; Schnitzler and Kalko, 2001). Recent data demonstrate that this flexibility also extends to the intensity (Brinkløv et al., 2010) and directionality of the sonar signal (Surlykke et al., 2009b; Jakobsen et al., 2013). Intensity and directionality are critical in defining the sonar search volume, i.e., the volume of space ahead of the bat in which objects are ensonified with sufficient sound energy to reflect detectable echoes. Some bats hunt in a sit-and-wait hunting mode, hanging from a perch and scanning the surroundings for potential prey by rotating the head and body. Perch hunting is often seen in rhinolophid bats (Neuweiler et al., 1987; Jones and Rayner, 1989), but also in other families, e.g., Phyllostomidae (Weinbeer and Kalko, 2004), Megadermatidae (Audet et al., 1991), and Nycteridae (Fenton et al., 1987). Because echolocation call production can be coupled with wing beats, it may require close to no extra energy to produce echolocation sounds in flight (Speakman and Racey, 1991). However, overall flight costs are high and perch hunting is far less costly energetically than continuous flight (Voigt et al., 2010). It is, however, unknown whether perch hunting poses special constraints on the echolocation, thus, promoting adaptive changes to intensity and directionality as well as other acoustic features of the echolocation calls.

The fringe-lipped bat, *Trachops cirrhosus* (Phyllostomidae), occurs in the Neotropics, from southern Mexico to southern Brazil (Reid, 1997). It roosts in hollow trees and flies a short distance (1–2 km) to its foraging grounds, where it gleans prey over a relatively small area (3–4 ha) (Kalko et al., 1999). *T. cirrhosus* uses both continuous flight and perch hunting when foraging. Radio-telemetry studies found that *T. cirrhosus* makes long flights (>2 min) early in the evening, hunting on the wing along streams and over ponds, likely predominantly for frogs, i.e., túngara frog [*Engystomops* (formerly *Physalaemus*) *pustulosus*]. Later in the night, when frog calling activity decreases, *T. cirrhosus* switches to perch hunting, sallying from a perch in short flights of less than 1 min, presumably hunting insect prey, such as forest katydids (Kalko et al., 1999). *T. cirrhosus* relies primarily on prey-emitted acoustic cues to detect and localize its prey, and can use species-specific frog mating calls to assess prey palatability (Tuttle and Ryan, 1981). It has been suggested that *T. cirrhosus* can detect the loud, conspicuous calls of túngara frogs and other preferred frog species even while on the wing, but when listening for katydid wing beat or landing sounds, or eavesdropping on their faint, high frequency and often low duty cycle calling song, a hang-and-wait strategy is more effective (Kalko et al., 1999).

Eavesdropping on prey-generated acoustic cues has been well documented in *T. cirrhosus*, both in field and flight cage experiments (Ryan et al., 1982). However, even though *T. cirrhosus* primarily uses passive listening to detect and localize its prey, it produces echolocation calls throughout the hunting approach (Barclay et al., 1981). Flight cage experiments show that it can use echolocation and spatial memory (Page and Ryan, 2008) to detect prey that falls silent upon approach, and can use both echolocation and chemical cues in the final stages of prey assessment (Page et al., 2012). *T. cirrhosus* emits typical phyllostomid calls, consisting of short, multiharmonic sweeps of low intensity. In confined space, such as the laboratory, most energy is in the third (sweeping from 110 kHz down to 80 kHz) and fourth harmonic and call duration is less than 1 ms. The cruising pulse rate is around 25 Hz, but in the final phase before attacking their prey the rate increases to around 80 Hz (Barclay et al., 1981).

A member of the phyllostomid family of leaf-nosed bats, *T. cirrhosus* has a prominent nose leaf, extending from the base of the nostrils. Nose leaves of phyllostomids are fairly similar in overall shape but differ greatly in size (Vanderelst et al., 2010). It is generally accepted that phyllostomids emit echolocation calls through the nostrils. In all probability, the nose leaf, which is not found in mouth-emitting bat families like e.g., Vespertilionidae or Emballonuridae, has a role in shaping and steering the sonar sound beam (Hartley and Suthers, 1987; Vanderelst et al., 2010). However, directionality has rarely been measured directly, and new data from flying *Carollia perspicilliata* (Brinkløv et al., 2011) demonstrated a narrower sonar beam when flying than earlier data from sitting bats had indicated (Hartley and Suthers, 1987). Thus, phyllostomid bats, like vespertilionids, may have the ability to flexibly modify their beam shapes to adapt to a given situation (Surlykke et al., 2009b; Jakobsen and Surlykke, 2010; Jakobsen et al., 2013). Since *Trachops* hunts while hanging from a perch as well as on the wing, it offers an excellent opportunity to study whether sonar search volume (intensity and directionality) is adapted to hunting strategy. In addition to listening for the sounds of its prey, we also frequently observed that *T. cirrhosus* opened its mouth while echolocating from a perch. Several other phyllostomid species have also been observed to open the mouth while echolocating (Tschapka, page 11 in LaVal and Rodrìguez-H, 2002), which might influence the sound emission by changing the emission site or altering the head-related transfer function. Thus, a second purpose of this study was to determine if *T. cirrhosus* adds an extra level to its sonar flexibility by being able to echolocate both through the nostrils and through the open mouth.

MATERIALS AND METHODS

ANIMALS

We captured bats on Barro Colorado Island, in Soberanía National Park and the areas surrounding Gamboa, Panama, using mist nets set along small streams and ponds at dusk. We recorded echolocation calls from 6 *T. cirrhosus* with a mean capture weight of 32.5 g (range 28–37 g) and mean forearm length of 58.8 mm (range 57.2–60.7 mm). We measured the length of the lancet of the nose leaf for 9 other individuals (**Figure 1**) from the tip of the nose leaf to the center of a line connecting the two nostril centers (mean ± SEM: 9.2 ± 0.3 mm), as measured in Brinkløv et al. (2011). In 2006, we recorded from two females, in 2008, from two males, and in 2012, from two males. All bats were held and tested in screened, outdoor flight cages. Bats from 2006 and 2008 were tested in a 4.5 × 4 × 2.5 m flight cage on Barro Colorado Island; bats from 2012 were tested in a 5 × 5 × 2.5 m flight cage in Gamboa. We recorded the bats in two behavioral situations: (1) while they were hanging from their perch, a short branch in the upper corner of the flight cage ca. 1.75 m above the floor, scanning the environment by turning head and body, and (2) while they were flying toward a loudspeaker placed on the floor of the flight cage ca. 2.5 m horizontal distance from the perch (**Figure 2**).

SOUND RECORDINGS

We recorded all bats with arrays of ¼″ (G.R.A.S) microphones (without grids) amplified by G.R.A.S. 12AA or Avisoft ¼″ power modules. We digitized the signals at 250, 500, or 300 kHz per channel using either an IOTech Wavebook or an Avisoft USGH (Avisoft Bioacoustics, Berlin, Germany), and stored data on a laptop computer. In 2006, we used four microphones in a T-shaped array with approximately 30 cm distance between microphones placed above the loudspeaker on the floor. In 2008, we used 10 microphones with ca. 30 cm distance, 8 G.R.A.S. ¼″ on a linear

FIGURE 1 | *Trachops cirrhosus* has a nose leaf with a lancet that is 9 mm from tip to nostrils. The large ears and the characteristic tubercles around the chin and lips are also clearly visible.

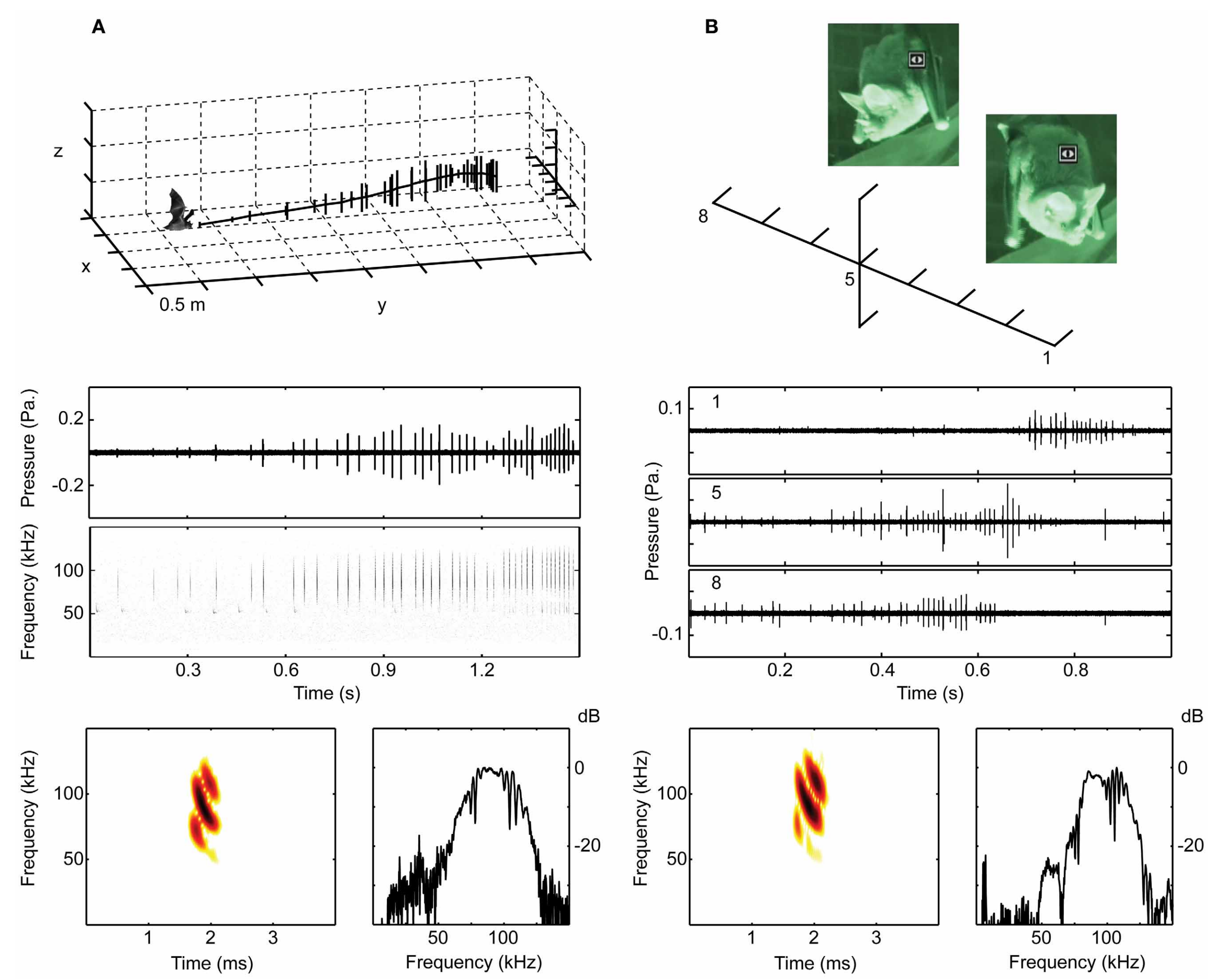

FIGURE 2 | The bats were recorded with microphone arrays when hanging from a perch or when flying. (A) Shows the set-up in 2012 with 11 microphones in a cross-shaped array, 7 horizontal and 3 above and 1 below the center microphone. The middle panel shows oscillograms and spectrograms of the echolocation calls emitted in the flight illustrated in the upper panel, where each vertical line is a call. The lower panel shows a spectrogram and a spectrum of one of the calls from the same sequence. **(B)** Shows a recording from 2008 of a perching bat. The 10 microphone array had 8 microphones on a horizontal line at the height of the bat's mouth. The middle panel shows oscillograms of a single echolocation sequence, recorded simultaneously on microphones 1, 5 and 8. Stills from the infrared video illustrate the bat turning its head from right to left. Due to the high directionality of the sonar beam, the calls are only visible on the channels at which the bat is aiming, i.e., channel 8 when the bat is facing right in the beginning and channel 1 when the bat is facing left in the end of the trial. Below are shown spectrum and spectrogram of one of the calls from this sequence.

horizontal line and an Avisoft condenser microphone (CM16) above and below the 5th microphone, which was 90 cm in front of the bat and at the same height as its mouth. In 2012, we used 11 ¼″ G.R.A.S. microphones with 25 cm distance in a cross shaped array, 7 horizontally and 3 above and one below the center (4th) microphone. The array was ca. 5 m from the wall on which the bats perched (**Figure 2**).

VIDEO RECORDINGS

All trials were conducted in red (25W red light bulb) and infrared light (Wisecomm IR045 LED and Conrad infrared spot) to minimize the bat's use of vision. All trials were video-recorded. In 2006, we used a Sony nightshot DCR-SR45 camcorder. Bats were presented with speakers broadcasting the calls of túngara frogs, a preferred prey species, and rewarded with a prey item on the speaker. In 2008, we recorded bats hanging from a perch with two Sony nightshot DCR-SR45 camcorders. One video focused on the bat's head and the other on an array of diodes connected directly via custom build amplifiers to a second 4 × 4 array of microphones (Knowles) spaced by 46 cm horizontally and 26 cm vertically. The minimum distance to the bat was 1 m translating into a resolution of around 20–30°. The diodes had 16 steps of light from green over yellow to red over a 30 dB range, and were adjusted to just emit green light at background noise. Thus, the diodes corresponding to the microphone(s) ensonified by the bat would emit orange or red light according to the sound level on the microphone. We combined the footage from the two camcorders into one movie using an Extron PIP 422 Picture-in-Picture Processor thus giving us on-line synchronized feedback simultaneously about the bat's head and nose leaf position, mouth opening, and sonar beam aim. In 2008, we additionally recorded the perching bats with high-speed video (CamRecord 600, Optronis, Germany) at 500 frames per second. The high-speed video was synchronized to an Avisoft Ultrasound recording system using an Avisoft condenser microphone (CM16, Avisoft Bioacoustics, Berlin, Germany) and a one-channel Avisoft USG digitizer. The high speed video and synchronized sound was stored on a laptop computer. Both video systems were used to find sequences where the mouth was clearly open, and the diodes were used to control that the sonar beam hit the diodes in the directions the head and nose leaf was aiming.

In 2012, we recorded sounds from two bats, both when they were perching and when they were flying toward a speaker broadcasting frog calls for a food reward. In contrast to the previous recordings, the bats would perch at a relatively random location on the cage wall and thus approach the speaker from a variation of directions. The bats were video recorded with a Sony HDR-CX550V camcorder, which was adjusted to the bat's position before each trial.

ANALYSES

We estimated source levels and directionality in flight for the two bats recorded in 2006 and the two bats recorded in 2012. We obtained the bats' flight paths using the arrival time differences at the microphones to localize the bats at the time of each echolocation emission. We determined source levels and directionality only from calls where the beam aim was in the center of the microphone array. Because of the array configuration we obtained only horizontal directionality from the bats recorded in 2006 and 2008, but both directions for the 2012 data. Using the estimated positions, we calculated source levels (emitted intensity referenced to 10 cm from the bat's mouth measured in dB SPL rms) by adding transmission loss (geometric spreading loss and atmospheric attenuation) and microphone directionality (Brüel and Kjær, 1982) using the method described in Jakobsen et al. (2012).

We estimated source levels and directionality for perching bats with either open or closed mouth for the two bats recorded in 2008 and for the two bats recorded in 2012. In 2008, the array allowed for determinations of the horizontal directionality, but only indications of beam aim in the vertical plane. We used the camcorder video combined with the diode display to guide us to sequences, where the bat's mouth was either clearly open, or clearly closed, and analysed the acoustic behavior in detail by using the high speed video and ¼″ microphone recordings.

Directionality can be quantified as half amplitude angle (HAM) or directivity index (DI). HAM is the off-axis angle, where the amplitude of the signal has declined by 6 dB. DI compares on-axis sound pressure with the sound pressure of an omnidirectional emitter producing a signal of equal energy. For all estimates of source level and directionality, we estimated beam-aim by fitting a 2nd order polynomial to the recorded beam pattern, using the peak of the polynomial as a proxy for beam aim. For details see Brinkløv et al. (2011). We calculated the DI as in Møhl et al. (2003):

$$DI = 10\log_{10}\left(\frac{2}{\sum_{i=1}^{N}(b_i \times \sin(\nu_i) \times \Delta\nu)}\right)$$

where b_i is the i'th sample of an interpolation of the measured beam pattern, ν_i is the angle, between 0 and π radians, and $\Delta\nu$ is the angular interval between interpolation points. N is the number of samples. The expression assumes rotational symmetry. To obtain the interpolation of the measured beam pattern, we pooled the measured relative sound pressures (both horizontal and vertical) into 1° bins and averaged them. We then extrapolated the measurements to obtain the complete sound field around the bat by fitting a second order polynomial to the average of the measured beams (**Figures 3**, **4**).

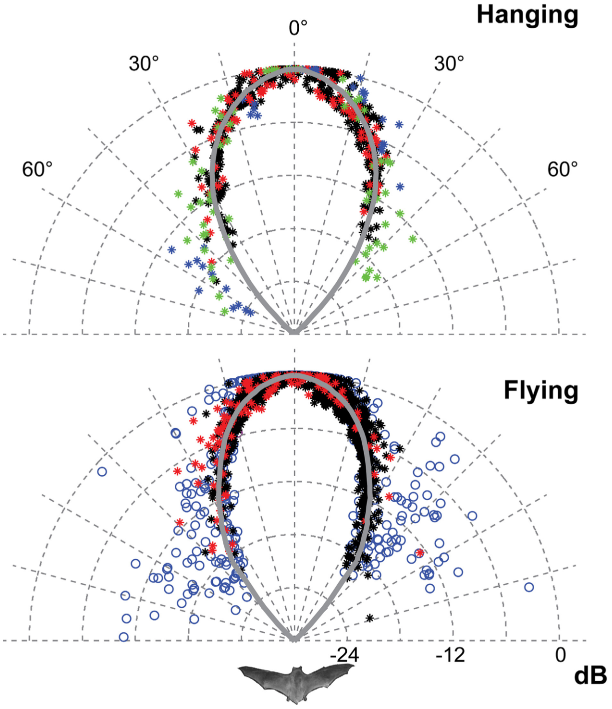

FIGURE 3 | The horizontal directionality when flying and hanging. The data for hanging bats are from 2012 (red and black) and from 2008 (blue and green). The data for flying bats are from 2012 (red and black) and from 2006 (blue). Each point is the normalised amplitude in that direction. The curves are the average of the measured values pooled in 1° bins and extrapolated using a fitted second order polynomial. Only data from 2012 was used for the traces and DI estimates because of the higher degree of control of beam aim. The sonar beam is very directional, both when flying and hanging. HAM was 18°.

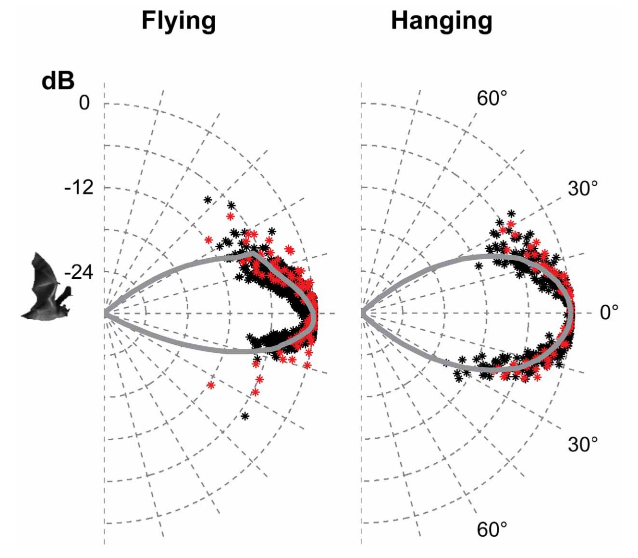

FIGURE 4 | The vertical directionality when flying and hanging. The data are from the two bats recorded in 2012, red for one bat and black for the other. Each point is the normalised amplitude in that direction. The curves are the average of the measured values pooled in 1° bins and extrapolated using a fitted second order polynomial. The beam was symmetrical in the vertical direction when hanging with HAM of 18°. When flying, the beam was more directional than when hanging, and asymmetrical with HAM in the upwards direction of 15° and 12° in the downward direction.

RESULTS

ECHOLOCATION SOUNDS WHEN FLYING AND HANGING FROM A PERCH

When leaving the perch and flying toward the loudspeaker the bats always echolocated and we never observed opening of the mouth. The echolocation calls had main energy in the third harmonic with F_{peak} (the frequency with maximum energy) at ca. 90 kHz, and less energy in the second and fourth harmonic (F_{peak} at 60 and 120 kHz, respectively). The calls were short, between 0.3 and 0.9 ms, and repeated with irregular pulse intervals of 30–120 ms when the bat was far away from the loudspeaker. At closer range the sonar pulses were grouped with increasing pulse number and decreasing pulse interval within the groups, which were 70–100 ms long, consisting of 3–10 pulses. Within groups the pulse interval was relatively constant (**Figure 2A**).

Since the source level decreased as the bat approached the loudspeaker and simultaneously got closer to the floor, we calculated source levels from calls emitted, when the bats were still far enough from the microphone array. At this distance we also got good S/N on all four or all eleven microphones. The maximum source levels (referenced to 10 cm) were recorded immediately after the bat left the perch and were 103 ± 3 dB, and 99 ± 4 dB for the two bats in 2006 and 102 ± 3 dB and 99 ± 3 dB for the two bats in 2012.

When on the perch, the bats often hung silently for long periods, but they echolocated when they lifted their heads and started scanning the surroundings, turning the head and the whole body while rapidly moving the pinnae of the ears back and forth. A typical emission pattern is illustrated in **Figure 2B**. Bats emitted trains of pulses with pulse intervals ranging from 20 to 120 ms, often 30–50 ms. The pulse duration was the same as when flying, i.e., around 0.5–0.8 ms and again the main energy was in the third harmonic with F_{peak} at 85–90 kHz. The apparent amplitude modulations (**Figure 2B**, middle panel) are not due to changes in emitted sound level, but reflect the bats' rapid scanning movements combined with the directionality of the calls. From the video we determined approximate scanning angles. The body turned ca. 45° from extreme to extreme, in addition the head turned an extra ca. 45°, thus totaling ca. 90° turn of head aim angle. While perching the source level was 86 dB ± 10 dB SPL and 88 dB ± 7 dB SPL for the two bats recorded in 2008. Right before taking off from the perch they emitted more intense calls, with source levels ca. 10 dB louder: 99.7 ± 3.4 dB for the two 2008 bats, demonstrating that they control the emitted amplitude over a large dynamic range. In 2012, the source levels when the bats were perching were estimated to be higher, 102 ± 2 dB and 98 ± 4 dB SPL. The difference is likely to be caused by the much longer distance from the hanging bat to the microphone array in 2012 (**Figure 2**), only allowing for recording of the loudest calls directed toward the array.

DIRECTIONALITY OF THE ECHOLOCATION SOUNDS

We determined the directionality in three different situations: (i) flying, (ii) hanging from the perch with closed mouth, and (iii) hanging with open mouth. We never observed any of the six bats flying with open mouth, but video from 2008 showed several sequences where the bats had open mouth while echolocating from the perch (**Figure 2B**).

Only the horizontal directionality could be extracted from the 2006 and 2008 data, but both vertical and horizontal directionality were determined from the 2012 data (**Figures 3** and **4** red and black data points). Estimates of beam shapes and statistical analyses were performed on the 2012 data, where we recorded with many microphones, but the values for 2008 confirm the measurements and are plotted in the same graphs (**Figure 3** blue and green data points). The beam was narrow with a horizontal HAM of 18°, both when flying and hanging (**Figure 3**). In the vertical direction the measured directionality of the sonar beam was slightly narrower when the bats were flying than when hanging. HAM was ca. 18° both up and down when hanging, but when flying HAM in the upward direction was 15°, and only 12° in the downward direction (**Figure 4**). DI for the combined data-set, was 16 dB when hanging and 17 dB when flying. When all data from both scenarios were pooled, DI was 17 dB. DI estimated using only the vertical directionality data was 17 dB for hanging bats and slightly more directional, 19 dB, for flying bats. Hence, the data indicated a narrower and more asymmetrical beam when the bats were in flight (**Figure 4**) although the differences between directionality from hanging and flying bats were not statistical significant.We regressed angle (absolute value) against sound pressure (Pa) for each bat's echolocation calls produced while flying and while hanging and found no difference in the slope of these two lines for either bat (two- and one-tailed tests for difference between two population regression coefficients, $P > 0.05$ for all).

The data from bats hanging and echolocating with open mouth is somewhat inconsistent. One of the two bats recorded in 2008 emitted calls with open mouth that were indistinguishable from the calls emitted with closed mouth, for all acoustic parameters measured: spectrum, amplitude, and directionality. In contrast, when the other 2008 bat emitted calls with open mouth, there was an additional pronounced peak in the spectrum around the first harmonic (the fundamental) at 30 kHz, which was not seen when this bat echolocated with the mouth closed. The directionality at 30 kHz was as expected much broader than at 90 kHz with HAM of 45° (**Figure 5**). We did not have synchronized video documentation in 2012 to allow us to know exactly when the bats had open mouth, but we did not record any signals with a prominent fundamental from either of the two, neither when hanging nor when flying.

DISCUSSION

The recordings from all six *T. cirrhosus* showed typical phyllostomid echolocation calls, i.e., short, multiharmonic calls with most energy at high frequencies, around 90 kHz, in the third harmonic, and often of relatively low output intensity. Barclay et al. (1981) reported a peak frequency closer to 75 kHz. However, distance, off-axis recordings, as well as microphone directionality will all low-pass filter the sounds. Here we report spectral characteristics of calls recorded on-axis and compensated for those low-pass effects, implying that the emitted F_{peak} is really around 90 kHz. The calls are very directional both while flying (DI = 17 dB) and while perching (DI = 16 dB).

The narrow sonar beam of *T. cirrhosus* corroborates data from *Carollia perspicillata*, (HAM 16° horizontally and 14° vertically and DI = 17 dB, calculated from the original data) the only other phyllostomid species for which directionality has been measured in freely flying bats (Brinkløv et al., 2011). *C. perspicillata* is somewhat smaller than *T. cirrhosus* (41–45 mm vs. 57–65 mm forearm length and $\sim$18 g vs. $\sim$30 g) but the lancet of the nose leaf is almost the same size (8 mm vs. 9 mm) (Brinkløv et al., 2011). Both bat species emit very similar echolocation signals, with F_{peak} around 90 kHz. Measurements from anesthetized *C. perspicillata* (Hartley and Suthers, 1987) as well as modeled directionality from *Phyllostomus discolor* (Vanderelst et al., 2010) demonstrate that the high directionality in the vertical plane is due to the extended nose leaf whereas the two nostrils determine directionality in the horizontal plane. Presumably this holds for *T. cirrhosus* too, since the overall shape and size of the nose leaf are quite similar in all three species. Given the similarity in nose leaf morphology and echolocation call features it is not surprising that the sonar beam directionality is similar in *C. perspicillata* and *T. cirrhosus*. Still, it is important to note that the nose leaf is not the sound emitter, but instead likely functions as a baffle, and thus its exact size is not expected to affect the sound field as much and as predictably as the size of the emitter. In mouth-emitting bats the emitter size appears to be the gape size and thus mouth-emitting bats presumably have more mechanical control over directionality (Jakobsen et al., 2013) than nostril-emitting bats. However, we are still far from understanding the functional significance of motor control of nose leaf shape for sonar directionality (Hartley and Suthers, 1987; Feng et al., 2012). Vanderelst et al. (2010)'s model predicted a sonar beam that was symmetrical in the horizontal direction, but asymmetrical in the vertical direction, with a main lobe, which was wider above than below the acoustic axis. This is in accordance with our results for flying *T. cirrhosus,* whereas we found the vertical directionality to be symmetrical for hanging bats. The difference in sonar beam shape

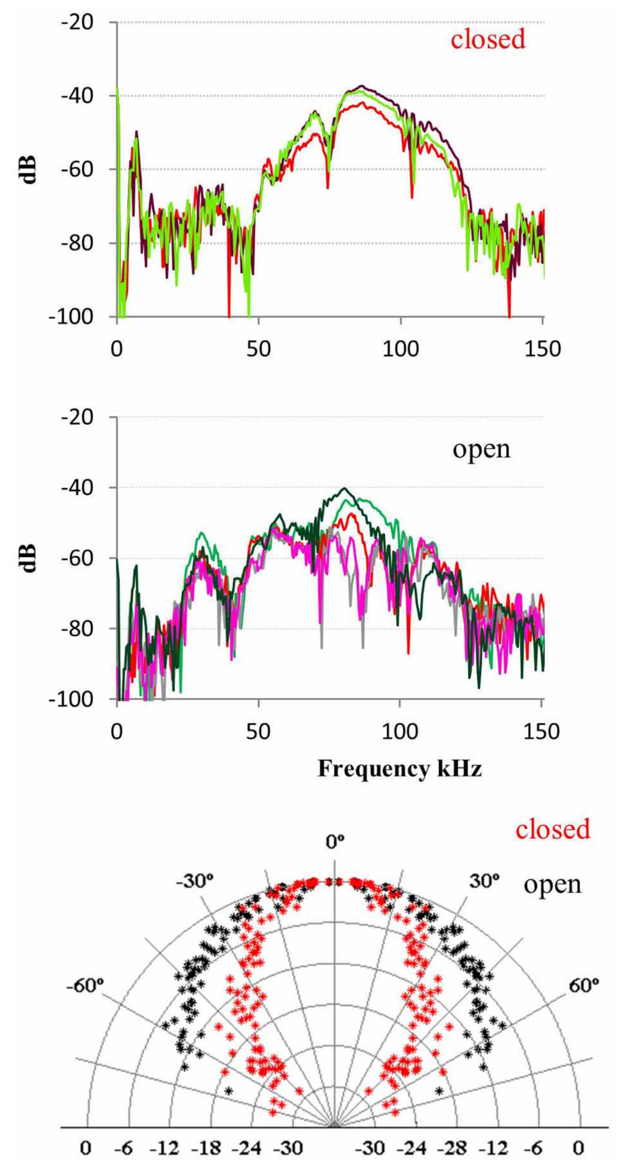

FIGURE 5 | Calls emitted with closed and open mouth. When one of the bats recorded in 2008 emitted calls with closed mouth (upper panel) it had most energy in the third harmonic (F_{peak} 90 kHz) and some in the second and fourth harmonic. When it emitted calls with open mouth (middle panel) it also emitted substantial energy in the first harmonic (F_{peak} 30 kHz). The effect of this was that the bat added a component with broad directionality (lower panel, black data points) when it called with the mouth open, compared to the directionality calculated for closed mouth calls with most energy around 90 kHz (red points).

between hanging and flying bats was not statistically significant, probably due to the limited number of bats and data. Potentially, it indicates active control of the beam by bending the nose leaf in the vertical direction as has also been suggested for another phyllostomid bat, *Macrophyllum macrophyllum* (Weinbeer and Kalko, 2007), but further investigations are needed to clarify. If motor control of the nose leaf functions in dynamic active adaptation of the sonar beam axis and directionality in phyllostomid bat, in particular in flight, this once more emphasizes the importance of verifying models and measurements based on static morphological data with measurements from live naturally behaving bats.

Our data does not provide a clear conclusion to whether mouth opening is part of beam control. The data on one bat very clearly showed addition of lower frequency and thus a broader component of the beam, but data from only one bat is far from conclusive. If more data should show this to be of functional significance, it would add yet another level of flexible control of sonar search volume in *T. cirrhosus* or perhaps more generally in phyllostomids that open the mouth while echolocating.

While our data was not sufficient to show a significant difference between flying and hanging, it did show unequivocally that the beam is very narrow under all circumstances, similar to the beam of flying *Carollia perspicillata*. Although *T. cirrhosus* is carnivorous and *C. perspicillata* is frugivorous, they both take predominantly stationary prey in dense clutter, so in some respects their foraging ecology and demands on their echolocation systems are quite similar. An advantage of a very narrow beam is that it provides inherent directional information: if the energy is focused in a narrow angle around the axis of the sound beam, off axis objects will only be ensonified with low intensity sound and their echoes will be much reduced, leaving salient echo information to come from the direction of the sonar beam axis. Narrow beams thus also reduce the load on the processing system. Interestingly, the opposite adaptation is seen in vespertilionid bats, which broaden the beam in confined space (Surlykke et al., 2009b; Jakobsen et al., 2013). This difference might reflect that we have not yet understood the function of directionality. However, it might also be evidence of the enormous diversity of echolocating bats. Bats of different families have different strategies for detecting insects close to background vegetation: bats that use frequency modulated echolocation calls (FM bats) shorten the calls in closed habitats to make discrimination easier along the time axis, whereas bats that produce constant frequency calls (CF bats) produce extremely long narrow banded calls to discriminate between prey and background along the frequency axis (Schnitzler, 1967; Neuweiler, 1989; Moss et al., 2011). Along the same lines, we hypothesize that bats, depending on their phylogeny, hunting habitat, and prey type, use different strategies to deal with clutter. Phyllostomid bats hunting stationary prey may benefit from a narrow beam to decrease the load on the processing system and focus on the important target, whereas vespertilionid bats hunting primarily moving prey may broaden the beam to prevent the prey from escaping out of the echolocation beam (Goerlitz et al., 2010; Jakobsen and Surlykke, 2010) and also to "keep an eye" on the clutter in order not to collide while pursuing erratic prey.

Radio-tracking studies suggest that *T. cirrhosus* often switches from gleaning to perch-hunting (Kalko et al., 1999). Perch hunting is thought to reduce the energy consumption compared to constant flight (Neuweiler et al., 1987; Voigt et al., 2010). Another advantage of perch hunting is the possibility of using a wider search angle when hunting prey. Our data indicates a wide search angle (ca. 90°) for *T. cirrhosus* although not quite as wide as the 200° estimated for rhinolophid bats (Neuweiler et al., 1987). In addition, scanning may reduce clutter. Bats sample their environment sequentially (Surlykke et al., 2009a), and when scanning perch hunting bats sequentially ensonify objects within a wide angle of directions. By integrating the input over time, they can create an auditory scene in great detail with much less off-axis clutter than a broader beam covering the same total angle would provide.

Finally, an underappreciated advantage of perch hunting might be an improved signal-to-noise ratio, since there is no wind noise from flight. Wind noise has never been measured for flying bats, but has been estimated to increase detection thresholds from the standard mammalian threshold of 0 dB SPL to around 20 dB SPL (Surlykke and Kalko, 2008). Stationary bats with large ears have been shown to have minimum hearing thresholds below 0 dB SPL (down to −20 dB SPL) (Long, 1977; Hoffmann et al., 2008). When stationary, big ears not only function as large acoustic antennae, but by their independent movements, also provide directional information by differentiating and focusing incoming acoustic input. In flight, in contrast, big ears are likely to create even more noise due to their higher air resistance. If we assume a source level according to our data of around 100 dB SPL at 10 cm (rms) and an increase in detection threshold from 0 to 20 dB SPL when flying compared to hanging (conservative estimate given the large ears of *T. cirrhosus*, **Figure 1**), we can estimate detection ranges for insect-sized prey with a target strength of −20 dB (Surlykke et al., 1999) using the simple form of the sonar equation:

$$\mathrm{EL} = \mathrm{SL} - 2\mathrm{TL} + \mathrm{TS}$$

EL = echo threshold level, TL = one way transmission loss (geometric spreading and atmospheric attenuation at 90 kHz, 28°C, 80% relative humidity), TS = target strength (Surlykke and Kalko, 2008). A perching bat would be able to detect insect echoes at a distance of 2.9 m, but only at 1.6 m when flying. The bat can lower its source level by up to 20 dB when hanging without paying with detection range compared to when flying. In fact, at 88 dB SPL, the echolocation source level we mostly recorded on the perch, the detection distance would be ca. 2.1 m, i.e., substantially longer than when emitting 100 dB SPL in flight. Thus, in addition to reducing energy consumption from flight, perching may create even larger acoustic advantages from reduced noise and more precise directional information for big-eared than for other bats.

In conclusion, our results show that *T. cirrhosus* emits a very narrow sonar beam both when hanging and flying. To understand the functional and ecological significance of different hunting modes it is important to integrate all aspects of hunting behavior, not only energy consumption, but also the critical features of echolocation including intensity and directionality. Our study indicates that the high directionality and moderate sound level in phyllostomid bats are adapted to the mode of hunting, i.e., largely motionless prey in dense clutter, and is not governed or affected by additional sensory cues the bats may receive from their quarry (e.g., passive acoustics, olfactory cues, etc.).

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Intensity and directionality of bat echolocation signals

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The paper reviews current knowledge of intensity and directionality of bat echolocation signals. Recent studies have revealed that echolocating bats can be much louder than previously believed. Bats previously dubbed "whispering" can emit calls with source levels up to 110 dB SPL at 10 cm and the louder open space hunting bats have been recorded at above 135 dB SPL. This implies that maximum emitted intensities are generally 30 dB or more above initial estimates. Bats' dynamic control of acoustic features also includes the intensity and directionality of their sonar calls. Aerial hawking bats will increase signal directionality in the field along with intensity thus increasing sonar range. During the last phase of prey pursuit, vespertilionid bats broaden their echolocation beam considerably, probably to counter evasive maneuvers of eared prey. We highlight how multiple call parameters (frequency, duration, intensity, and directionality of echolocation signals) in unison define the search volume probed by bats and in turn how bats perceive their surroundings. Small changes to individual parameters can, in combination, drastically change the bat's perception, facilitating successful navigation and food acquisition across a vast range of ecological niches. To better understand the function of echolocation in the natural habitat it is critical to determine multiple acoustic features of the echolocation calls. The combined (interactive) effects, not only of frequency and time parameters, but also of intensity and directionality, define the bat's view of its acoustic scene.

Keywords: intensity, directionality, beam shape, bat, echolocation, biosonar

INTRODUCTION

The evolutionary success of bats is accredited to their ability, as the only mammals, to fly and navigate in darkness by echolocation, thus filling a niche exploited by few other predators. Over 90% of all bat species use echolocation to localize obstacles in their environment by comparing their own high frequency sound pulses with returning echoes (Griffin, 1958). The ability to localize and identify objects without the use of vision allows bats to forage for airborne nocturnal insects, but also for a diverse range of other food types including motionless perched prey or non-animal food items (Schnitzler and Kalko, 2001; Brinkløv et al., 2011; Geipel et al., 2013).

The agility and precision with which bats navigate and forage in total darkness, is in large part due to the accuracy and flexibility of their echolocation system. The echolocation clicks of the few echolocating Pteropodidae (*Rousettus*) are fundamentally different from the echolocation sounds produced in the larynx that we focus on here, and thus not part of this review. Many studies have shown that bats adapt their echolocation calls to a variety of conditions, changing duration and bandwidth of each call and the rate at which calls are emitted in response to changing perceptual demands (Griffin et al., 1960; Schnitzler and Kalko, 2001). In recent years the intensity and directionality of echolocation signals has received increasing research attention and it is becoming evident that these parameters also play a major role in how bats successfully navigate and forage. To perceive an object in its surroundings, a bat must ensonify the object with enough energy to return an audible echo. Hence, the intensity and duration of the emitted signal act together to determine how far away a bat can echolocate an object. Equally important is signal directionality. Bat echolocation calls are directional, i.e., more call energy is focused in the forward direction than to the sides (Simmons, 1969; Shimozawa et al., 1974; Mogensen and Møhl, 1979; Hartley and Suthers, 1987, 1989; Henze and O'Neill, 1991). An object detectable at 2 m directly in front of the bat may not be detected if it is located at the same distance but off to the side. Consequently, at any given echolocation frequency and duration, it is the combination of signal intensity and signal directionality that defines the search volume, i.e., the volume in space where the bat can detect an object.

The aim of this review is to summarize current knowledge about intensity and directionality of bat echolocation calls, and show how both are adapted to habitat and behavioral context. Finally, we discuss the importance of active motor-control to dynamically adjust both signal intensity and directionality to solve the different tasks faced by echolocating bats.

INTENSITY

Call intensity is a main determinant of echolocation range, i.e., the distance from a bat where objects, such as obstacles and food, reflect echoes intense enough for detection. The more intense the call, the further sound travels from the bat and the larger the echolocation range. Emitted intensities (source level) of bat echolocation signals are referenced to a standard distance of

10 cm from the bat's mouth. Thus, when recording bats at a distance, one must add the transmission loss due to geometric spreading [$20 \times \log_{10}(R)$] and frequency-dependent atmospheric attenuation (ANSI, 1995) over the distance from the bat to the microphone.

When Griffin first investigated how loud bats call, he found that insectivorous bats flying in open space, e.g., aerial hawking vespertilionids, called at around 110 dB SPL (Sound Pressure Level; re. 20 μPa at 0.1 m) and closed-space gleaners operating in or near vegetation, like the phyllostomid *Carollia perspicillata*, called at around 70 dB SPL (Griffin, 1958). Consequently, Griffin divided bats into two groups, the loud insectivorous bats, and the "whispering" gleaning bats. Recordings from the field have since shown that bats are orders of magnitude louder than what Griffin measured, and the border between loud and whispering is much blurrier than initially believed. Open-space insectivorous bats emit calls up to, and beyond, 140 dB SPL (Holderied et al., 2005; Surlykke and Kalko, 2008). Remarkably, even "whispering" bats are capable of emitting calls up to 110 dB SPL (Brinkløv et al., 2009). This means that, while echolocation in air is still a relatively short-range system, its range is considerably larger than first assumed.

The huge difference between the values for signal intensity obtained by Griffin and more recent measurements illustrates the great flexibility of the echolocation system. Bats dynamically adjust signal intensity to changes in their environment and the task at hand, lowering the output as they approach objects such as prey or vegetation. The dynamic range, or the difference between the loudest and the quietest calls emitted by individual bats is in the order of at least 30–40 dB for most species. When object detection occurs at long range or under predictable lab conditions most studies report a reduction in output level of around 6 dB for every halving of distance to the target (Hartley, 1992b; Hiryu et al., 2007, 2008; Brinkløv et al., 2010; Koblitz et al., 2010, 2011; Nørum et al., 2012). If the object reflects impinging sound like a point target, the echo level at the bats ears would increase by 12 dB per halving of distance if the bat emitted a constant source level. Thus, the consequence would be an enormous increase in echo level through a pursuit, e.g., +80 dB from detection at 5 m to capture at 5 cm, likely to overload central auditory processing. The output reduction of 6 dB per halving of distance removes half of the echo increase such that the sound pressure at the bat's ear increases by only 6 dB per halving of distance. Further, psychophysical experiments have shown that sensitivity on the receiver side is not constant, but decreases by the remaining 6 dB for each halving of distance probably due to contraction of the bats middle-ear muscles (Suga and Jen, 1975). Hence, in a predictable situation the combined adjustment of output and input results in echoes perceived at a relatively stable intensity (Henson, 1965; Suga and Jen, 1975; Hartley, 1992a). Data from more unpredictable situations in the natural environment have also indicated a 6 dB reduction in signal output intensity per distance halved. New data, however, show that the reduction in intensity for individual approaches is mostly much steeper in the wild, up to as much as 30 dB per halving of distance, with considerable variation. The relatively shallow slopes reported from other field studies are probably the result of pooling multiple sequences with steep slopes but initiated at different distances (**Figure 1**, Nørum et al., 2012). The results suggest that sudden detection of prey or obstacles at close range may prompt an initial dramatic intensity reduction. Curiously, for bats landing on an extended surface, the reduction in output intensity is likewise within the 6 dB per halving of distance range (Koblitz et al., 2011). If the sensitivity on the receiver side changes as well, this results in a gradual decrease in perceived echo strength as the bat approaches the surface.

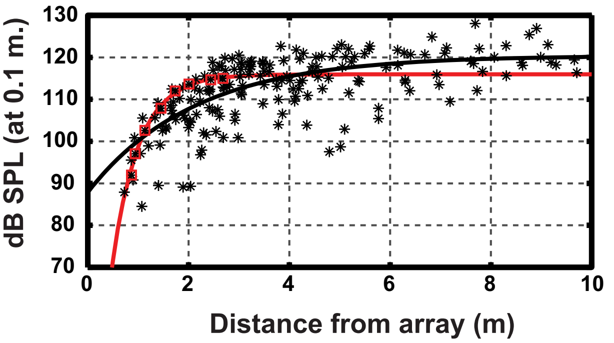

FIGURE 1 | Relationship between source level and distance to the microphone array for *Myotis daubentonii*. Datapoints (*) from 14 approaches. The black line is the least-squares fitted exponential function to the entire data set (for details see Nørum et al., 2012). Red squares highlight a single approach and the red line is the fitted function using only these points, showing a much steeper slope. The figure illustrates that estimating the slope using a large data set, comprising many individual steep slopes with different onsets, can yield an artificially shallow slope as compared to individual approaches. Data points from Nørum et al. (2012).

There may be a less clear-cut separation between loud and whispering bats than previously assumed, but it is still evident that bats flying close to or within dense vegetation are considerably less intense than bats flying in open space. This is true for species that differ in overall habitat use, but also for individual bats switching between habitats with varying degrees of clutter. Under field conditions, the trawling insectivorous phyllostomid *Macrophyllum macrophyllum* lowers mean signal intensity from 111 dB SPL in open space to 105 dB SPL in semi-cluttered space. Signal intensity is further reduced to 100 dB SPL when *M. macrophyllum* navigates a small flight room, demonstrating an obvious dynamic adjustment of output intensity in response to varying degrees of habitat clutter (Brinkløv et al., 2010).

The adjustment of signal intensity in *M. macrophyllum* occurs in parallel with changes in signal duration and peak frequency. Open space calls are not only louder, but also longer and with lower peak frequency than those emitted in semi- or densely cluttered conditions. These changes all contribute to an increase in sonar range in open space. The increased duration increases the signal energy and the lower frequency reduces the effects of atmospheric attenuation. Attenuation of sound in air increases drastically with frequency (Lawrence and Simmons, 1982; ANSI, 1995) which presumably represents a major constraint for echolocating bats resulting in a trade-off between sonar range on one hand and resolution and localization on the other (Kalko and Schnitzler, 1993). The low intensities and high frequencies emitted by most gleaners in clutter likely indicate that sonar range is not an issue. Thus, the low intensities serve to prevent self-deafening and the high frequencies serve to increase resolution and localization (Kalko and Schnitzler, 1993).

The use of high frequencies also increases clutter rejection along the acoustic axis when the prey is closer to the bat than the clutter. This is because the increased atmospheric attenuation at higher frequencies will generate a relatively weaker echo the further away an object is. An increase in emitted frequency from 45 to 90 kHz increases atmospheric attenuation from 1.4 to 4 dB/m (at 25°C and 80% humidity). If clutter is present 0.5 m behind the prey, the prey/clutter echo-ratio will be 2.6 dB higher at 90 kHz than at 45 kHz, thus increasing prey conspicuousness.

An added advantage of using low intensity echolocation is that it may prevent prey from detecting an approaching bat. The sound pressure reaching the prey will always be higher than the echo returning to the bats ears, but eared insects, such as moths, generally have much higher hearing thresholds than bats (Wenstrup, 1984; Schmidt et al., 1992; Esser and Daucher, 1996; Koay et al., 1997; Surlykke et al., 1999). While intensity at the insect increases by $20 \times \log_{10}$ (R) as the bat approaches, the echo the bat receives increases by $40 \times \log_{10}$ (R). Thus, every time the bat halves the distance to the prey, the sound pressure increases with 6 dB at the prey and 12 dB at the bat. By concurrently reducing its output level by 6 dB, the bat maintains a constant sound pressure at the prey, but still increases the returning echoes by 6 dB. This keeps prey out of the loop while increasing echo strength for the approaching bat (Surlykke, 1988). By emitting low intensity calls, the aerial hawking bat, *Barbastellus barbastellus*, can detect its prey before the prey detects the bat, and by reducing its output level during approach it can remain undetected during the pursuit (Goerlitz et al., 2010). The low-intensity calls from *B. barbastellus* do come at a cost; a reduction in output level also reduces the detection distance for the bat, but given that *B. barbastellus* feeds almost exclusively on eared insects, the benefit of not being detected seems to outweigh the cost of operating at short range.

DIRECTIONALITY

A directional echolocation signal provides bats with a number of advantages over an omni-directional signal: (1) inherent directional information; by focusing sound in the forward direction, returning echoes are likely to originate from that direction, simplifying object localization. (2) A reduction in clutter; when less sound is radiated to the back and sides of the bat, less sound is reflected off objects of little or no interest in these directions, reducing the amount of information the bat has to process. (3) An increase in source level (on-axis intensity); by focusing energy in a narrow cone instead of radiating it in all directions. On the other hand, a highly directional sound beam will also restrict the bats "field of view" which may be a disadvantage in certain situations.

Beam shape is a spatial filter that determines what information is available to the bat and what information is filtered out before echoes return. This may be critical in light of the very short time bats have to make decisions. A typical pursuit often takes less than half a second and the time to process information and make decisions on a call-to-call basis is even shorter, perhaps a few tens of milliseconds. A clear advantage of a highly adapted and dynamic emission (and reception) system is a reduction in processing load on the receiving side i.e., smart sensing over smart processing. This may be one of the adaptations that allow for the very fast reaction times in echolocating bats, subsequently leading to high foraging, and, in turn, evolutionary, success.

Beam shape is determined by the size and shape of the sound emitter and the frequency of the emitted signal (Strother and Mogus, 1970; Urick, 1983) such that an increase in size or frequency generates a more directional sound beam (**Figure 2**). Frequency is easily measured, but emitter size and shape is not as apparent when dealing with live animals, let alone bats in flight. For bats emitting sound through the open mouth, gape size presumably dictates directionality. Opening the mouth more while emitting a given frequency will generate a more directional beam and vice versa (Surlykke et al., 2009). For nose emitting bats, beam shape is likely dictated by the distance between the nostrils and the size and shape of the nose-leaf (Hartley and Suthers, 1987).

Beam directionality has so far been measured in 17 bat species from seven different families (**Figure 3**). The methods differ substantially. Many studies were performed on restrained bats, often with calls elicited through stimulation of the brain by implanted electrodes (Shimozawa et al., 1974; Schnitzler and Grinnell, 1977; Mogensen and Møhl, 1979; Hartley and Suthers, 1987, 1989; Henze and O'Neill, 1991; Hiryu et al., 2006). This made it easy

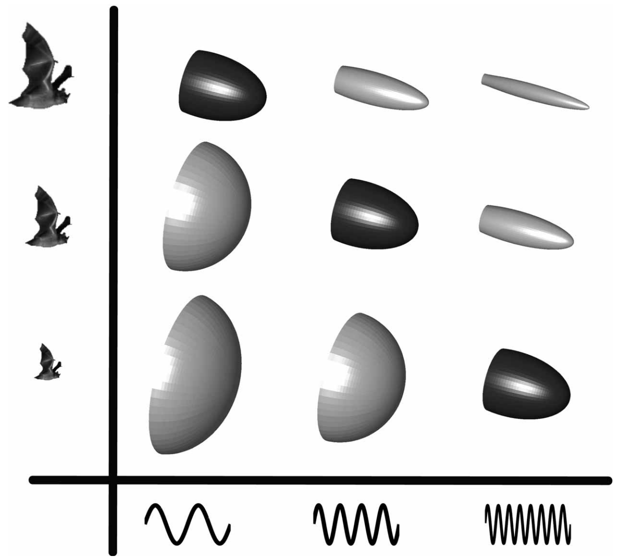

FIGURE 2 | Echolocation beam shape as a function of emitter size and frequency. The beam-shape schematics illustrate how directionality increases as either frequency or emitter size increase. The highlighted diagonal beam patterns illustrate how bats of different sizes can converge on similar beam patterns by adjusting the emitted frequency to their size i.e., small bats emit higher frequencies than large bats. Figure from Jakobsen et al. (2013).

to control the bat's position and acoustic axis, but probably prevented active beam shape control by the bats, and in some cases produced calls very different from those produced by freely behaving bats. A few early and most later studies focus mostly on freely behaving bats in the lab (Griffin, 1958; Simmons, 1969; Ghose and Moss, 2003; Jakobsen and Surlykke, 2010; Jakobsen et al., 2012, 2013) with one report from the field (Surlykke et al., 2009). In spite of the large differences in methodology, a few general trends emerge from the data set. It is clear that all bats recorded emit directional signals and it is also clear that they emit a bilaterally symmetrical sound beam. Most results come from the Vespertilionidae (nine species), where directionality is remarkably uniform across species for bats echolocating under similar conditions, in spite of large differences in bat size and emitted frequency (**Figure 3**). This indicates that directionality may have been one of the major constraints on the evolution of echolocation frequency, forcing small bats to echolocate at higher frequencies to produce a sufficiently directional beam (Jakobsen et al., 2013). Echolocation frequency is also important for echo reflection (Møhl, 1988; Pye, 1993) and ranging accuracy (Stamper et al., 2009) and frequency-dependent directionality may help bats segregate target and clutter echoes (Bates et al., 2011). Thus, echolocation frequency is probably under several simultaneous evolutionary constraints. There is a negative correlation between size and echolocation frequency in most families of bats (Jones, 1999), and it will be interesting to see if this translates into a convergence of beam width for other families than the Vespertilionidae. Data from the emballonurids seem to deviate from this pattern. *Cormura brevirostris* emits a narrower beam in the flight cage than does *Saccopteryx bilineata* (*DI* of 11.5 and 9.3 dB, respectively, **Figure 3**), but given that emission patterns from only two species have been recorded so far (Jakobsen et al., 2012), conclusive evidence is still lacking. In contrast to other bats Phyllostomids do not appear to show correlation between body size and emitted frequency. Curiously, data show that nose-leaf size is not correlated with body size either in phyllostomids (Hartley and Suthers, 1987; Jones, 1999). Even though the nose-leaf is not the emitter *per se*, it has been shown to define the vertical directionality (Hartley et al., 1989; Vanderelst et al., 2012). Thus, if directionality is a driving force for echolocation frequency in phyllostomids as well, we would expect to find that the emitted frequency is correlated to the size of the nose-leaf and the nostril separation but not to body size.

Bats adapt many features of their echolocation calls in response to changes in their surroundings and to behavioral context. Signal bandwidth and peak frequency are increased in many species when they navigate in cluttered space probably to improve resolution and localization accuracy (Kalko and Schnitzler, 1993). Further, an increase in frequency will result in an increase in directionality. However, as directionality depends on both frequency and effective emitter size, combined changes of the two may result in the opposite effect. This seems to be the case for

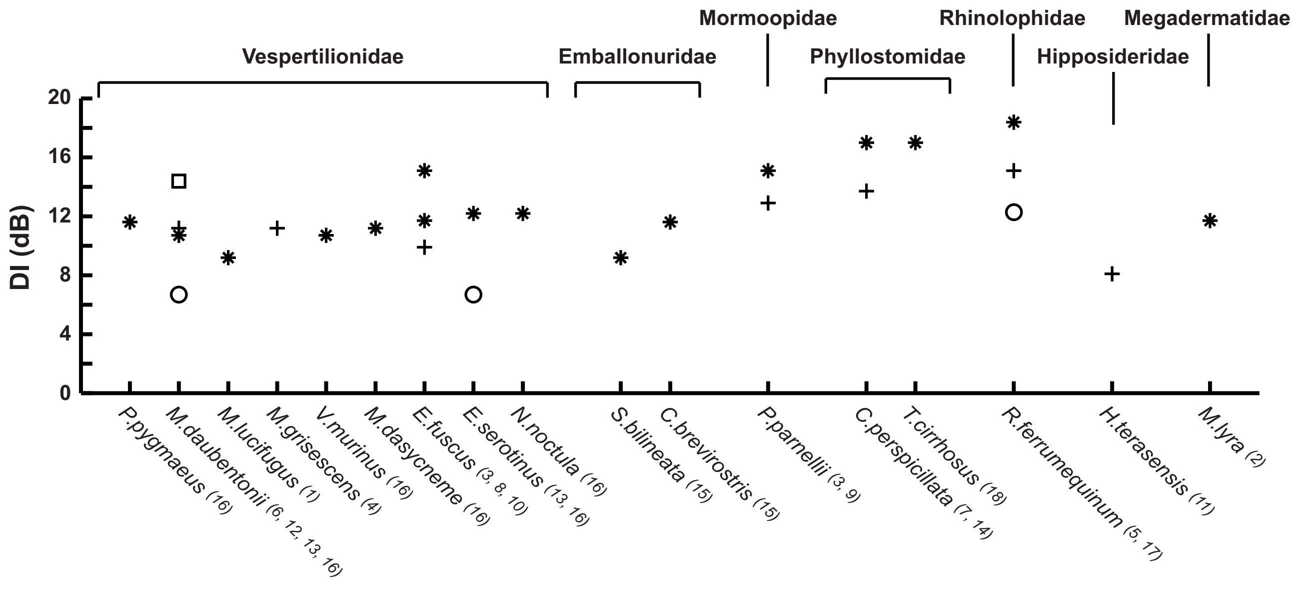

FIGURE 3 | Directivity index (DI) for the 17 bat species measured to date. The DI compares the emitted source level with that of an omni-directional source producing a signal with the same acoustic power. The species are grouped by family and arranged within families by increasing size (forearm measurements). Vespertilionids, Emballonurids, and Mormoopids emit sound through the mouth. Phyllostomids, Rhinolophids, Hipposiderids, and Megadermatids emit sound through the nose. * indicates a recording of a freely flying bat, + indicates that the bat was restrained, ☐ indicates a field recording, and ○ are recordings of the terminal buzz. Measurements from: [1]Griffin, 1958; [2]Möhres and Neuweiler, 1966; [3]Simmons, 1969; [4]Shimozawa et al., 1974; [5]Schnitzler and Grinnell, 1977; [6]Mogensen and Møhl, 1979; [7]Hartley and Suthers, 1987; [8]1989; [9]Henze and O'Neill, 1991; [10]Ghose and Moss, 2003; [11]Hiryu et al., 2006; [12]Surlykke et al., 2009; [13]Jakobsen and Surlykke, 2010; [14]Brinkløv et al., 2011; [15]Jakobsen et al., 2012; [16]Jakobsen et al., 2013; [17]Matsuta et al., 2013; [18]Surlykke et al., submitted. Where measurements were not available from the literature, DI was calculated for a piston source emitting a sound beam with the reported half amplitude angle (the angle where the sound pressure is reduced by 6 dB relative to 0°).

the vespertilionid *Myotis daubentonii*. When navigating in the field, *M. daubentonii* emits calls with lower peak frequency than in the lab (45 vs. 55 kHz), which by itself would produce a *less* directional beam. Yet, the signals are more directional in the field than in the lab, presumably because the bats also increase their emitter size by opening the mouth wider (Surlykke et al., 2009).

Echolocating bats must adjust directionality not only to adapt to the environment, but also in response to rapid changes in the perceived echo-scene, especially when hunting prey doing their best to escape. At least six orders of insects have ultrasound sensitive ears and exhibit "anti-bat tactics" i.e., they perform erratic escape behaviors like power dives and passive falls in response to intense ultrasound (Miller and Surlykke, 2001). The relatively high directionality of echolocation signals will, in close proximity to prey, become a disadvantage to the bat since the prey only has to move a short distance to escape the bat's sound beam. *M. daubentonii* and *Eptesicus serotinus* (Vespertilionidae) in fact broaden their beam in the last part of prey pursuit by lowering call frequency by roughly an octave (Jakobsen and Surlykke, 2010). A similar frequency drop is seen in a large number of insectivorous vespertilionids and is known as Buzz II (**Figure 4**). Buzz II calls have been thought an artifact of the extremely high repetition rate of calls emitted during this stage of pursuit, sometimes exceeding 200 calls/s (Kalko and Schnitzler, 1989; Faure and Barclay, 1994). From a purely physiological perspective, however, this seems unlikely as such fast call rates would result in additive tension build-up in the laryngeal muscles, ultimately increasing, rather than reducing call frequency during the buzz (Ratcliffe et al., 2013). Further, many species of echolocating bats use repetition rates as high as vespertilionids but without the frequency drop (Surlykke et al., 1993; Ibáñez et al., 2002). Thus, we argue that the lower frequency of Buzz II calls is not a non-functional epiphenomenon. Rather, it is an adaptive feature that broadens the echolocation beam considerably in the last phase of pursuit to counter the evasive maneuvers performed by many eared insects when exposed to intense ultrasound (Jakobsen and Surlykke, 2010). This argument is corroborated by recent results from horseshoe bats. During prey pursuit, the Japanese greater horseshoe bat, *Rhinolophus ferrumequinum nippon*, will likewise broaden its echolocation beam, but only when the prey moves (Matsuta et al., 2013). In horseshoe bats beam broadening is, contrary to vespertilionids, not achieved by lowering the call frequency. The mechanism underlying the change in beam shape is still unknown, but it is likely facilitated by manipulating the fine structures of the nose leaf (Feng et al., 2012).

As discussed above, the optimal directionality is likely to differ from situation to situation, but also between bats with different feeding ecology. Gleaning bats, foraging in dense vegetation for inconspicuous stationary food items, presumably benefit more from a narrower beam shape than an open space aerial hawking bat. Directionality data from the frugivorous bat, *C. perspicillata*, and the frog-eating bat, *Trachops cirrhosus*, corroborate this. Both phyllostomid species have echolocation beam widths with *DI* of 17 dB (half amplitude angle of ∼15°) when flying in a flight cage (Brinkløv et al., 2011, Surlykke et al., submitted). This is considerably narrower than the 10–12 dB (half amplitude angle of ∼37°) measured for aerial hawking bats flying in similar conditions (Ghose and Moss, 2003; Jakobsen et al., 2013) (**Figure 3**). So far the only report of active beam shape adjustments in nose-emitting bats come from the horseshoe bats (Matsuta et al., 2013), an adjustment likely facilitated by manipulating the shape of the nose leaf. However, the phyllostomid, *M. macrophyllum*, shifts

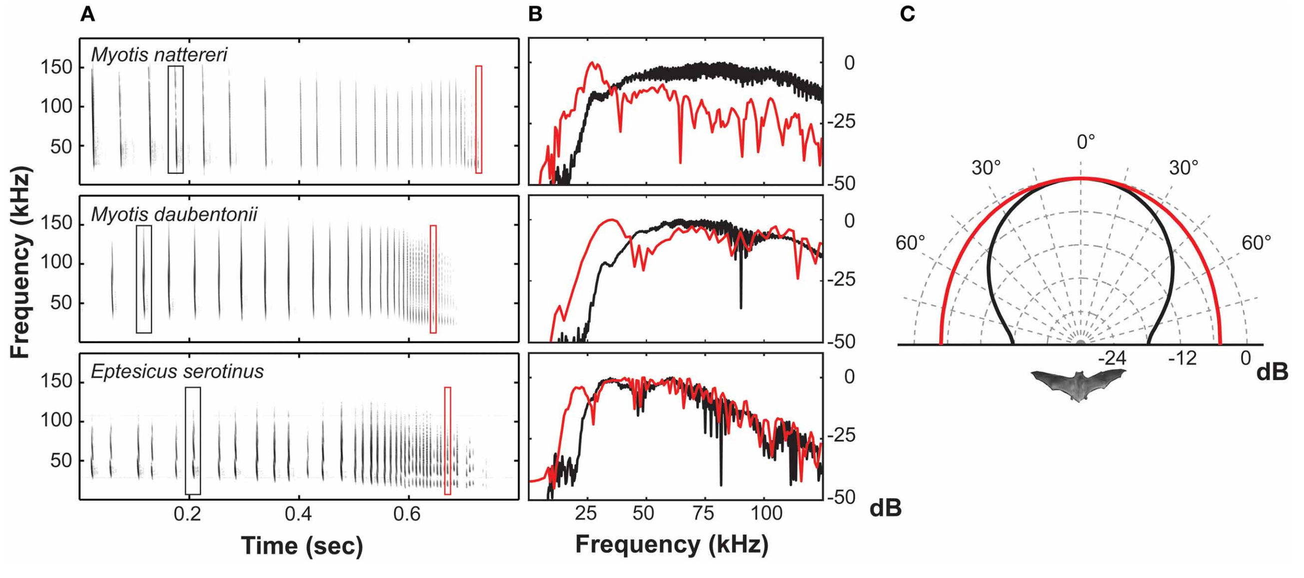

FIGURE 4 | (A) Spectrograms of three prey-capture sequences from vespertilionid bats. **(B)** Power spectra illustrating the frequency drop from the approach phase (black trace) to the terminal phase (red trace). **(C)** Measured directionality for approach signals at 55 kHz (black trace) and for buzz II signals at 27.5 kHz (red trace) for *Myotis daubentonii*.

maximum energy to the second harmonic in the open, but to the third (or fourth) harmonic in cluttered space (Brinkløv et al., 2010), indicating that phyllostomid bats may also alter beam shape by changing emitted frequency.

MODELING EMISSION PATTERNS

Modeling bats as physical sound emitters allows predictions about parameters, which cannot or have not been measured. The simple piston model describes the beam pattern of a rigid circular piston oscillating in an infinite baffle and has been used as a model for the emission pattern of mouth emitting bats (Strother and Mogus, 1970; Mogensen and Møhl, 1979):

$$R_P(\theta) = \left| \frac{2 \times J_1(k \times a \times \sin(\theta))}{k \times a \times \sin(\theta)} \right|$$

$R_p(\theta)$ is the ratio between the on-axis pressure and the pressure at an angle θ, J_1 is a first order Bessel function of the first kind, k, the wavenumber $= 2\pi/\lambda$, λ the wavelength, and a is the radius of the piston. Even though, there are a number of obvious differences between bats and the model, the model performs surprisingly well in predicting the emission pattern from mouth emitting bats (Mogensen and Møhl, 1979; Hartley and Suthers, 1989; Jakobsen and Surlykke, 2010; Jakobsen et al., 2012).

The emission pattern from nose emitting phyllostomid bats has been modeled using a two point-source model, but with limited success in particular for freely flying bats (Strother and Mogus, 1970; Hartley and Suthers, 1987; Brinkløv et al., 2011). A model simulating two small pistons with the same separation as the nostrils appears a much better approximation to the horizontal directionality of phyllostomid bats (Vanderelst et al., 2010), but the vertical pattern has so far not been successfully modeled by simple means (see Zhuang and Müller, 2006, 2007; Vanderelst et al., 2010, 2012 for more advanced procedures).

The directionality and intensity of sound signals are not independent features. Intensity changes with directionality, such that an increase in directionality will lead to a corresponding increase in intensity along the acoustic axis. The directivity index (DI) of the source reflects this relationship. A *DI* of e.g., 18 dB implies that sound intensity along the acoustic axis is 18 dB higher than it would be for an omnidirectional sound source radiating sound with the same acoustic power (**Figure 5**). For the piston model, the DI simply follows from the relation between size and wavelength:

$$DI = 20 \log_{10}(k \times a)$$

where $k = 2\pi/\lambda$, λ is the wavelength, and a is the radius of the piston. Using measured data, the calculation of DI is slightly less simple. It requires an estimation of the sound field behind the bat and assumes that the beam is rotationally symmetric (Møhl et al., 2003).

Hence, by increasing or decreasing the directionality of the signal, the on-axis intensity inherently changes as well. *M. daubentonii* emits a source level of 111 dB SPL in the lab and 119 dB SPL in the field. Since the call is broader in the lab ($DI = 11$ dB) than in the field ($DI = 16$ dB) it follows that the bat only increases its output intensity by 3 dB while the remaining 5 dB are accounted for by the greater directionality in the field (Surlykke et al., 2009).

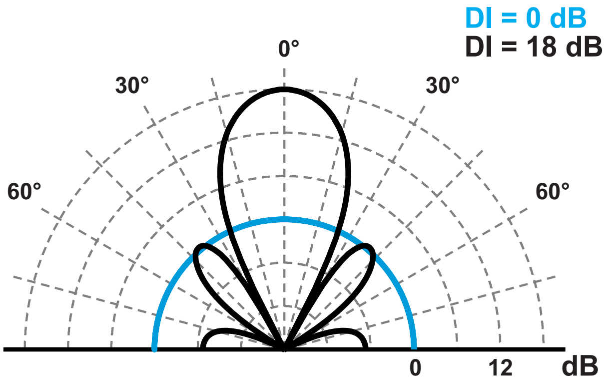

FIGURE 5 | Beam pattern from an omnidirectional sound source (*DI* = 0 dB, cyan trace) and a directional source following the piston model (*DI* = 18, black trace). The sources radiate sound of equal acoustic power.

The increase in on-axis intensity with increasing directionality also means that increasing the signal frequency does not necessarily lead to a reduction in detection distance, in spite of the increased atmospheric attenuation at higher frequencies. This is because an increase in frequency increases the signal directionality and thereby the on-axis sound level. Again the situation is simple for the piston model, where a change in frequency from f_1 to f_2 leads to a change in DI of:

$$\Delta DI = 20 \log_{10}\left(\frac{f_2}{f_1}\right)$$

The total atmospheric attenuation depends on the distance the sound travels, whereas, an increase in on-axis sound level affects the source level and thus echo level irrespective of distance. Thus, at short echolocation ranges bats can increase frequency to achieve a higher directionality without sacrificing sonar range. At longer ranges the increase in atmospheric attenuation outweighs the increase in source level. For example a doubling in frequency from 25 to 50 kHz, increases DI and thus source level by 6 dB while the atmospheric attenuation increases from 0.7 to 1.7 dB/m (at 20°C and 50% humidity). Thus, up to a distance of 3 m (two-way travel distance: 6 m), the increased atmospheric attenuation at 50 kHz does not outweigh the increase in source-level. Due to the non-linear increase in atmospheric attenuation with frequency in air, the distance where these two effects cancel each other out depends on the absolute frequencies (**Figure 6**).

Many factors combine to define the optimal echolocation signal for a given situation. There are clear differences between bats with different feeding ecologies even when they navigate similar scenarios, indicating a critical role of feeding ecology for how evolution has shaped echolocation signals. Many phyllostomids, such as *C. perspicillata*, feed primarily on fruit and must navigate dense vegetation. *Pipistrellus pygmaeus* is an example of a typical vespertilionid bat hunting moving insects in open fields or along forest

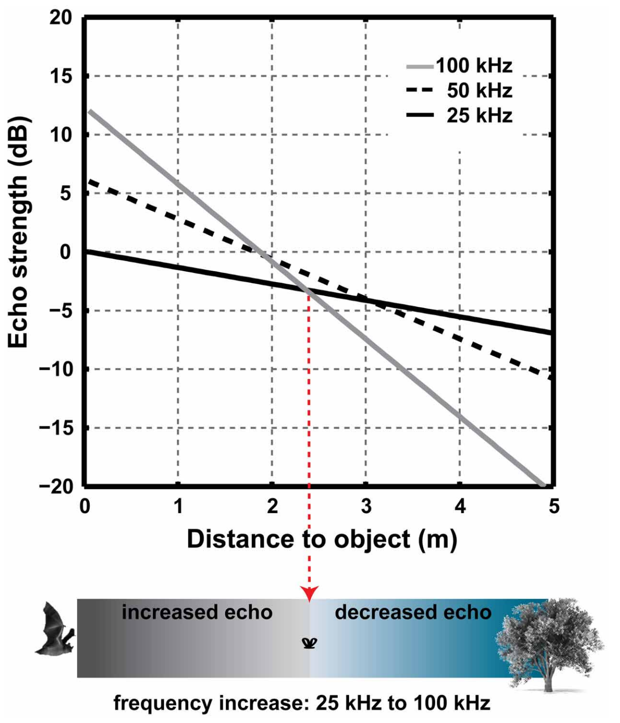

FIGURE 6 | The combined effect of increased DI and atmospheric attenuation over distance when the emitted frequency is increased from 25 to 50 to 100 kHz and emitter size is constant. An increase in frequency will lead to an increase in source level and thus in echo strength at short ranges. Note that calculations do not include spherical spreading loss or scattering from the target.

edges. The requirements to the sonar systems of these two bats are very different and reflected in the combination of emitted intensity, directionality, and frequency, even when the bats are flying under similar conditions. In the lab, *P. pygmaeus* emits calls at 111 dB SPL with a *DI* of 12 at 55 kHz (peak frequency). Under similar conditions, *C. perspicillata* emits 99 dB SPL, with a *DI* of 17 dB and a peak frequency of 90 kHz. **Figure 7** shows the acoustic field of view for the two species and illustrates that the combined effects of intensity, directionality, and frequency generate dramatically different search volume for the two bats. The aerial hawking *P. pygmaeus* uses a sonar signal of much longer range and broader width than the gleaning *C. perspicillata*. *P. pygmaeus* searching for moving insect prey can probably "afford" to scan a relatively large volume of space with each call because its quarry will move in the foreground and thus stand out. *C. perspicillata*, on the other hand, must detect an inconspicuous (motionless) fruit-target in heavy clutter. By reducing the ensonified search volume it probably also focuses its attention on a smaller area and thereby increases the likelihood of detecting desirable objects caught in the sonar beam (Dukas, 2004).

CONCLUSION

The combined research on intensity and directionality of echolocation calls from bats show clear differences between restrained and unrestrained bats, and between bats flying in the lab and in the field. These differences highlight the huge flexibility of the echolocation system and highlight the importance of active motor control for perception through echolocation. At the same time, they point to the importance of recording naturally behaving bats in the wild.

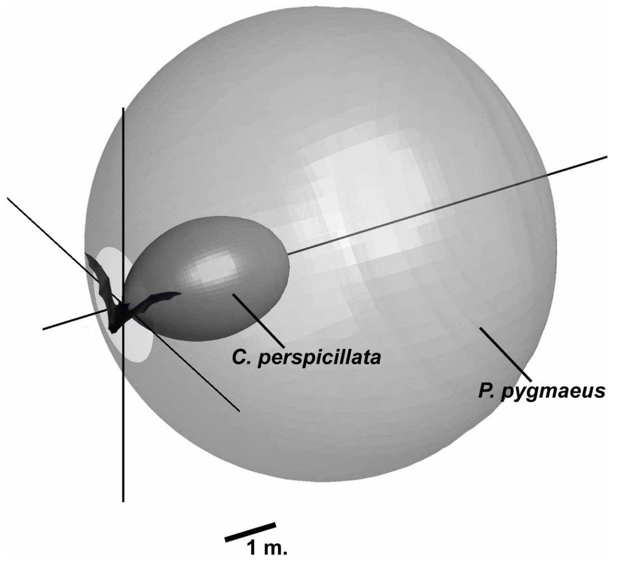

FIGURE 7 | Search volume for *P. pygmaeus* and *C. perspicillata* navigating an indoor flight room. The shape illustrates the volume in space where a smooth large surface that absorbs 20 dB of the incoming sound is detectable. Calculations are based on measured parameters (*P. pygmaeus*: 111 dB SPL, 55 kHz, *DI* = 12 dB, *C. perspicillata*: 99 dB SPL, 90 kHz, *DI* = 17). For both bats, the hearing threshold is assumed to be noise limited to 20 dB SPL. The calculations do not account for directional properties on the receiving side.

The volume of space a bat probes with its echolocation beam is a product of the emitted frequency, intensity, directionality, and call duration. The combined effect of adjustments to these components can result in dramatic changes in the overall search volume. The dynamic control of all acoustic features probably plays a key role in the flexibility and adaptability of bat echolocation and is thus a major contributor to their extreme evolutionary success across a vast range of habitats worldwide. This emphasizes the importance of determining all acoustic features, not just frequency and time parameters, to understand the function of echolocation and its adaptation through evolution to habitats and behavioral contexts.

While our knowledge of both intensity and directionality has increased substantially over recent years, it is clear that we are still barely scratching the surface. Presently, we have directionality measurements from only 17 out of more than 1000 species of echolocating bats. So what appears a general rule today may yet prove to be the exception.

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Effects of sound intensity on temporal properties of inhibition in the pallid bat auditory cortex

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Auditory neurons in bats that use frequency modulated (FM) sweeps for echolocation are selective for the behaviorally-relevant rates and direction of frequency change. Such selectivity arises through spectrotemporal interactions between excitatory and inhibitory components of the receptive field. In the pallid bat auditory system, the relationship between FM sweep direction/rate selectivity and spectral and temporal properties of sideband inhibition have been characterized. Of note is the temporal asymmetry in sideband inhibition, with low-frequency inhibition (LFI) exhibiting faster arrival times compared to high-frequency inhibition (HFI). Using the two-tone inhibition over time (TTI) stimulus paradigm, this study investigated the interactions between two sound parameters in shaping sideband inhibition: intensity and time. Specifically, the impact of changing relative intensities of the excitatory and inhibitory tones on arrival time of inhibition was studied. Using this stimulation paradigm, single unit data from the auditory cortex of pentobarbital-anesthetized cortex show that the threshold for LFI is on average ~8 dB lower than HFI. For equal intensity tones near threshold, LFI is stronger than HFI. When the inhibitory tone intensity is increased further from threshold, the strength asymmetry decreased. The temporal asymmetry in LFI *vs.* HFI arrival time is strongest when the excitatory and inhibitory tones are of equal intensities or if excitatory tone is louder. As inhibitory tone intensity is increased, temporal asymmetry decreased suggesting that the relative magnitude of excitatory and inhibitory inputs shape arrival time of inhibition and FM sweep rate and direction selectivity. Given that most FM bats use downward sweeps as echolocation calls, a similar asymmetry in threshold and strength of LFI *vs.* HFI may be a general adaptation to enhance direction selectivity while maintaining sweep-rate selective responses to downward sweeps.

Keywords: pallid bat, echolocation, FM sweeps, sideband inhibition, spectrotemporal

INTRODUCTION

Bats of the suborder, microchiroptera, can be broadly classified as constant frequency-frequency modulation (CF-FM) or frequency modulation (FM) bats based on their echolocation calls (Jones and Teeling, 2006). The diversity of echolocation call structure within these broad classes provides the opportunity to explore the evolution of diverse spectral and temporal neural processing strategies using behaviorally-relevant sounds. Studies of auditory neurons in CF-FM and FM bats reveal selective responses to features present in the species-specific echolocation calls (Suga et al., 1987; Suga, 1989; Dear et al., 1993; Wenstrup et al., 2012). In terms of FM sweeps, neural selectivity for the rate and direction of change in frequency have been well characterized (Casseday and Covey, 1992; Gordon and O'Neill, 1998; O'Neill and Brimijoin, 2002; Gittelman et al., 2009; Fuzessery et al., 2011; Washington and Kanwal, 2012).

Beginning with studies by Suga (1965), one conceptual framework to study mechanisms underlying FM sweep rate and direction selectivity is based on asymmetries in sideband inhibition. Auditory neurons, like those in the visual and somatosensory systems, exhibit excitatory and inhibitory components in the receptive field (Calford and Semple, 1995; Brosch and Schreiner, 1997; Gordon and O'Neill, 1998; Sutter et al., 1999; Faure et al., 2003; Wehr and Zador, 2003; Razak and Fuzessery, 2006; Wu et al., 2008; Sadagopan and Wang, 2010; Kuo and Wu, 2012). The inhibitory sideband refers to frequencies of sounds that do not elicit an excitatory response when presented alone, but can suppress spontaneous or excitatory sound-evoked responses. Such inhibitory frequencies are present below the low-frequency edge (low-frequency inhibition, LFI) and/or above the high-frequency edge (high-frequency inhibition, HFI) of the excitatory tuning curve (henceforth, "tuning curve"). In its simplest form, the asymmetry hypothesis suggests that absent inhibition on one side of the tuning curve will cause a neuron to be sweep direction selective.

More recent studies have provided refinement to this hypothesis as well as identified additional mechanisms such as facilitation and duration tuning to explain FM sweep selectivity across different levels of the auditory system and across species (Gordon and O'Neill, 1998; Fuzessery et al., 2006; Razak and Fuzessery, 2006, 2008; Gittelman et al., 2009; Sadagopan and Wang, 2010; Trujillo et al., 2013). It is now established that it is not simply the presence or absence of inhibition on either side of the excitatory tuning curve that shapes FM sweep direction/rate selectivity. More complex interactions between the relative bandwidth, timing and strength of inhibitory and excitatory inputs are involved.

The pallid bat (*Antrozous pallidus*) has served as a useful model in these investigations because of a high percentage of neurons selective for FM sweeps used in echolocation (Fuzessery et al., 2006, 2011; Razak and Fuzessery, 2006). The pallid bat echolocates using downward FM sweeps [60→30 kHz, 2–5 ms duration (Barber et al., 2003)]. Between 65–75% of neurons tuned between 25–70 kHz in the inferior colliculus (IC) and auditory cortex are selective for the downward direction and the range of sweep rates present in the echolocation call (Fuzessery, 1994; Razak and Fuzessery, 2002). The two-tone stimulation paradigm has been used to study underlying mechanisms. In this method, two tones are presented with different delays to characterize spectral and temporal interactions within the receptive field in neurons with known FM rate and direction selectivity (Gordon and O'Neill, 1998; Fuzessery et al., 2006; Razak and Fuzessery, 2008, 2006). These studies showed that most neurons have sideband inhibition on both sides of the tuning curve, but show temporal asymmetries such that HFI arrives later than excitation and LFI arrives earlier than excitation. Upward sweeps will first pass through the LFI which will arrive at the neuron before and during excitation to squelch responses. Downward sweeps with fast sweep rates reach the excitatory frequencies before the delayed HFI arrives and elicit a neural response. For slow downward sweeps, the delayed HFI has sufficient time to arrive at the neuron with or before the excitation and reduces responses. Thus, early LFI and delayed HFI shape direction and rate selectivity for downward FM sweeps, respectively. Removal of LFI from the sweep or reducing inhibition with GABAa receptor antagonists reduces direction selectivity (Razak and Fuzessery, 2009; Williams and Fuzessery, 2011). Likewise, removing HFI from the sweep or iontophoresis of GABAa receptor antagonists reduces rate selectivity.

In previous studies, sideband inhibition was determined using two tones that were presented at the same intensity. Recent studies suggest that the temporal interactions between excitatory and inhibitory inputs are modulated by the relative strength of inhibition and excitation (Wu et al., 2006; Gittelman et al., 2009; Razak, 2012). Temporal asymmetries in the pallid bat cortex may therefore arise from differences in the strength of HFI and LFI. The main goal of the present study was to characterize sideband inhibition in the auditory cortex by varying both relative intensity and time delays between tones in the two-tone paradigm. This paradigm allows a quantification of the intensity-arrival time relationship between excitatory and inhibitory frequencies. The data show that: (1) the threshold of LFI is lower than the threshold of HFI and, (2) if the relative intensity of the inhibitory tone is increased, the arrival time decreases for both HFI and LFI, and temporal asymmetry decreases.

MATERIALS AND METHODS

Pallid bats were netted in Arizona, California and Texas and housed in a 11 × 14 ft room. The bats were able to fly in this room and were provided crickets/mealworms and water *ad libitum*. The room was maintained on a reversed 12:12 light cycle. All procedures followed the animal welfare guidelines required by the National Institutes of Health and the Institutional Animal Care and Use Committee.

SURGICAL PROCEDURES

Recordings were obtained from the right auditory cortex of bats (both males and females, $n = 9$ bats) anesthetized with isoflurane or methoxyflurane inhalation, followed by an i.p. injection of urethane (0.7 mg/g) or pentobarbital sodium (30 μg/g). A previous study comparing urethane and barbiturate anesthetics showed no differences in FM sweep selectivity or arrival time/bandwidth of sideband inhibition (Razak and Fuzessery, 2009) Therefore, the data obtained using the different anesthetics were combined here. To expose the auditory cortex, the head was held in a bite bar, a midline incision was made in the scalp, and the muscles over the dorsal surface of the skull were reflected to the sides. The front of the skull was scraped clean and a layer of glass microbeads applied, followed by a layer of dental cement. The bat was then placed in a Plexiglas holder. A cylindrical aluminum head pin was inserted through a cross-bar over the bat's head and cemented to the previously prepared region of the skull. This pin served to hold the head secure during the recording session. The cross-bar holding the head pin was secured behind the bat, leaving no interference between the speaker and the ear. The location of A1 was determined relative to the rostrocaudal extent of the midsagittal sinus, the distance laterally from the midsagittal sinus, and the location of a prominent lateral blood vessel that lies parallel to the midsagittal sinus. The size of the exposure was usually ~2 mm^2. Exposed muscle was covered with petroleum jelly, and exposed brain surface was covered with silicone oil to prevent desiccation.

RECORDING PROCEDURES

Experiments were conducted in a warm (~80°F), sound-proof chamber lined with anechoic foam (Gretch-Ken Industries, Oregon). Bats were kept anesthetized throughout the course of the experiments with additional urethane or pentobarbital sodium (one-third of pre-surgical dose) injections. Acoustic stimulation and data acquisition were driven by custom software and Microstar DSP board based hardware. Programmable attenuators (PA5, Tucker-Davis Technologies, Florida) allowed control of sound intensities before amplification by an integrated amplifier (Yamaha AX430). Stimuli were delivered either using an LCY-K100 ribbon tweeter (Madisound, Wisconsin) placed 8 in from the left ear at 45° to the long-axis of the bat's body or presented as contralateral ear closed-field stimuli through the ribbon tweeters fitted with funnels. Each neuron reported in this study was tested with one or the other method (free-field or closed-field). Preliminary data from neurons in which the closed-field and free-field data were compared indicated that the minimum thresholds (MTs) were ~5 dB higher for the free-field presentation. Because all neurons in this study were tested with excitatory tones presented at 10–20 dB above threshold regardless of the presentation method used, it is unlikely that the results were due to the presentation method. Most FM sweep selective neurons tuned between 30–60 kHz in the pallid bat auditory cortex are also binaurally insensitive (EO/O type neurons) when tested with interaural intensity differences (Razak and Fuzessery, 2002). Therefore, IID sensitivity is unlikely to create differences between free-field and closed-field data. The frequency response curve of the delivery systems, measured with a 1/4-in microphone (Bruel and Kjaer, Denmark), was flat within ±5 dB for frequencies from 6–50 kHz. The roll-off from 50–80 kHz was gradual at a rate ~20 dB/octave.

Data shown are from extracellular single-unit recordings identified based on window discriminator threshold-crossing and consistency of action potential amplitude and waveform displayed on an oscilloscope. Recordings were obtained using glass electrodes (1M NaCl, 2–10 MΩ impedance) at depths between 200 and 600 μm. Penetrations were made orthogonal to the surface of the cortex. Action potentials were amplified by a Dagan extracellular preamplifier (2400A) and a spike signal enhancer (FHC, Maine) and band-pass filtered (0.3–3 kHz, Krohn-Hite, MA). Waveforms and peri-stimulus time histograms were stored. Responses were quantified as the total number (20 stimulus repetitions, 1 Hz repetition rate) of action potentials occurring within 200 ms of stimulus onset. Adjustments for spontaneous activity were not necessary because there was no spontaneous activity in these recordings.

The focus of this study was on the high-frequency FM sweep-selective region of the pallid bat A1 (Razak and Fuzessery, 2002). This region is likely to be involved in echolocation behavior. The FM sweep-selective region contains neurons tuned between 25–70 kHz and is located rostral and medial to the lower frequency neurons (tuning 5–35 kHz) that are noise-selective (Razak and Fuzessery, 2002, 2006). The FM sweep-selective neurons respond better to downward sweeps than to noise or upward sweeps with energy in the same spectral band. Using tones, noise, upward, and downward sweeps as search stimuli, neurons with characteristic frequency (CF) >25 kHz, and with stronger response to downward FM sweeps than noise and upward FM sweeps were isolated. The following response properties were then determined:

EXCITATORY FREQUENCY TUNING CURVE

Pure tones (25–70 kHz, 5 ms duration, 1 ms rise/fall times, 1 Hz repetition rate) were used to determine the CF and MT for tones. CF was defined as the frequency that elicited action potentials to at least five successive stimulus repetitions at the lowest intensity. The intensity was then increased in 5 or 10 dB steps to record the frequency-intensity combinations that produced excitatory responses (tuning curve). A 1 kHz resolution was used to determine excitatory tuning curves. Because, the excitatory tuning curves in the echolocation region of the pallid bat are typically broader than 5 kHz between 10–30 dB above threshold, this resolution was deemed sufficient to characterize the tuning curve (Razak and Fuzessery, 2007).

TWO TONE INHIBITION OVER TIME TUNING CURVES

To determine the arrival time of inhibition, a two tone inhibition over time (TTI) method was used (Calford and Semple, 1995; Brosch and Schreiner, 1997; Gordon and O'Neill, 1998; Fuzessery et al., 2006; Razak and Fuzessery, 2006). Two tones were presented with different delays between them (**Figure 1A**). The frequency of one tone was at the CF (excitatory tone) and was presented at an intensity of 10–20 dB above threshold and duration of 5–10 ms. The second tone was presented at the same intensity and duration of 5–10 ms. The frequency of the second tone was varied between 25–70 kHz and its onset time was varied with respect to

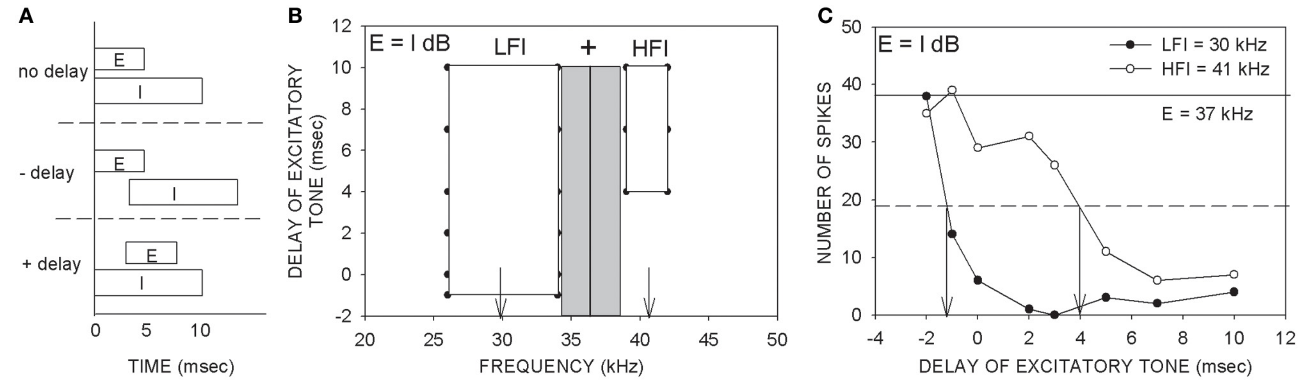

FIGURE 1 | Description of the two-tone inhibition method. (A) An excitatory (E) tone at CF was paired with a putative inhibitory (I) tone with varying delays between the two tones. Positive delays indicate that the excitatory tone was delayed with respect to the inhibitory tone. Negative delays indicate earlier onset of the excitatory tone. **(B)** An example two-tone inhibition plot obtained with two tones of equal intensity (E = I dB). The gray rectangle indicates the excitatory tuning curve at the tested intensity. The vertical line indicates the CF used as the excitatory tone. The frequency of the putative inhibitory tone was varied from 25–70 kHz to determine the delay-frequency combinations that caused at least 80% reduction of response to the CF tone presented alone. The white rectangles represent the frequency-delay combinations that produced 80% inhibition. This neuron showed both low- and high-frequency inhibition (LFI, HFI). The vertical arrows indicate the LFI and HFI tones that were used to generate the plot shown in **(C)**. **(C)** An example two-tone inhibition plot in which response magnitudes at different delays between the tones were quantified. The intensities of the two tones were the same (E = I dB). The excitatory tone used was 37 kHz. The 'number of spikes' on the ordinate is in response to 20 repetitions of each stimulus. The solid horizontal line is the control response (response to CF alone). The dashed horizontal line is at 50% of control response. The LFI tone (30 kHz) produces at least 50% inhibition at a delay ~ −1 ms. The HFI tone (41 kHz) produces 50% inhibition only when the excitatory tone was delayed at least 4 ms. These data indicate that the LFI was relatively fast compared to HFI. The main goal of the present study was to obtain plots as shown in **(C)**, but by varying the relative intensities between the two tones and quantifying how arrival times of LFI and HFI change.

that of the excitatory tone. The inhibitory sideband on the low-frequency side is ~10 kHz wide (Razak and Fuzessery, 2006). On the high-frequency side, the bandwidth of inhibition is ~2–4 kHz. Therefore, a resolution of 0.5 kHz was used to search for the high-frequency sideband, and a resolution of 2 kHz was used for the low-frequency sideband.

The delay-frequency combinations of the two tones that resulted in inhibition of response to the excitatory tone for at least four out of five (80% inhibition) consecutive presentations served to map out the spectrum of inhibitory frequencies (**Figure 1B**, white rectangles). On the ordinate of the TTI plot in **Figure 1B**, negative delays denote that the onset of the excitatory tone occurred before that of the inhibitory tone. Positive delays indicate that the onset of the excitatory tone occurred after that of the inhibitory tone. The example neuron in **Figure 1** exhibited both LFI and HFI. The timing of LFI and HFI was characterized in more detail (**Figure 1C**) by choosing an inhibitory tone at the center of the inhibitory sidebands (downward arrows in **Figure 1B**) and pairing it with the CF tone at various delays. In the description of TTI plots and data below, "control" response indicates response of the neuron to the excitatory tone presented alone. "Arrival time of inhibition" refers to the smallest delay between the two tones at which the response of the neuron was reduced by 50% of response to the control. It is important to note that arrival time of inhibition is a measure of when inhibition arrives at a neuron relative to excitation. In the example neuron shown in **Figure 1C**, the arrival times of LFI and HFI were −1 ms and +4 ms, respectively. Negative arrival times mean inhibition occurred even when the inhibitory tone was delayed relative to the excitatory tone. Therefore, negative arrival times are interpreted as fast arriving inhibition. Positive arrival times mean inhibition occurred only when the inhibitory tone was advanced relative to excitatory tone. Positive arrival times are interpreted as slow inhibition. The example in **Figure 1** is typical of the pallid bat auditory cortex with slow HFI and fast LFI (Razak and Fuzessery, 2006).

TTI AT DIFFERENT RELATIVE INTENSITIES

In previous studies of the pallid bat auditory system, the TTI curves were obtained with the two tones at the same intensity as described above (e.g., **Figure 1**). These studies suggested that the differences in arrival time between LFI and HFI is a form of asymmetry that explain direction and rate selectivity for downward sweeps in the pallid bat auditory cortex and IC (Fuzessery et al., 2006; Razak and Fuzessery, 2006). However, it has been suggested that timing differences between the high- and low-frequency sidebands may be less important in shaping direction selectivity compared to how relative timing and magnitude of excitatory and inhibitory conductance interact with each other (Gittelman et al., 2009; Gittelman and Pollak, 2011). Modulation of magnitude of inhibitory/excitatory conductance may generate timing differences relevant to FM sweep selectivity. One way to test this hypothesis using extracellular recordings is by characterizing temporal interactions between the excitatory and inhibitory inputs change when the relative intensities of the two tones are varied.

Therefore, TTI tuning curves were determined at different relative intensities between the excitatory and the inhibitory tones. The excitatory tone was presented with an intensity 10–20 dB above threshold. The inhibitory tone was presented at different delays and intensities relative to the excitatory tone. The intensity of the inhibitory tone varied from 20 dB below to 20 dB above the excitatory tone intensity in steps of 5 dB. "Relative threshold of inhibition" refers to the lowest intensity of the inhibitory tone relative to the excitatory tone at which the neuron was inhibited by 50% of control response. Because the intensity of the inhibitory tone was changed in 5 dB steps, the resolution of the threshold of inhibition measure is 5 dB. Arrival time of both LFI and HFI was determined at different intensity combinations.

RESULTS

The goal of this study was to characterize changes in arrival time of inhibition when the intensities of the excitatory and inhibitory tones were changed relative to each other. This was accomplished in 33 FM sweep-selective neurons with CF between 30 and 51 kHz. In 15/33 neurons, stimulus was presented using the free-field speaker. In the remaining neurons, contralateral ear stimulation was used with a funnel inserted in the ear. Because no differences were found due to the method employed, the data are presented together. **Figure 2** shows a neuron in which arrival times of both LFI and HFI were determined at multiple relative intensities. The CF (42 kHz) was used as the excitatory tone. The LFI tone used was 34 kHz (**Figure 2A**), and the HFI tone was 47 kHz (**Figure 2B**). When the LFI tone was presented at an intensity 10 dB lower than the excitatory tone (I-E = −10 dB), the response of the neuron decreased relative to the control response, but did not meet the 50% criterion to determine arrival time. When the intensity of the LFI tone was increased by 5 dB, while maintaining the excitatory tone intensity (I-E = −5 dB), the response of the neuron decreased below 50% of control response when the delay was ~1.5 ms. This intensity difference (I-E = −5 dB) was noted as the relative threshold of inhibition. At equal tone intensities, LFI arrival time was −0.5 ms. There was no reduction in the arrival time with a further increase in intensity of the inhibitory tone indicating a saturation of change in LFI arrival time. For the HFI, inhibition that met the 50% criterion was seen only when the two tones were of the same intensity (I-E = 0 dB), indicating a higher relative threshold for HFI than LFI. With a further 5 dB increase in intensity of HFI (I-E = 5 dB), the arrival time decreased to −1.5 ms. Indeed, at I-E = 5 dB, the arrival time of HFI was slightly faster than the arrival time of LFI. This example neuron lends support to the hypothesis the threshold of LFI was lower than the threshold of HFI, and that the arrival times of both LFI and HFI became faster with increasing relative intensity of inhibitory tone to a point of reduced temporal asymmetry.

Figure 3 shows two additional neurons in which arrival times progressively decreased with increasing intensity of the inhibitory tone. In the neuron shown in **Figure 3A**, 50% inhibition was seen even when the LFI tone was 20 dB lower in intensity than the excitatory tone. With further increase in the inhibitory tone intensity, the arrival time of LFI decreased **Figure 3B** depicts the relationship between relative intensity and arrival time of LFI. For the

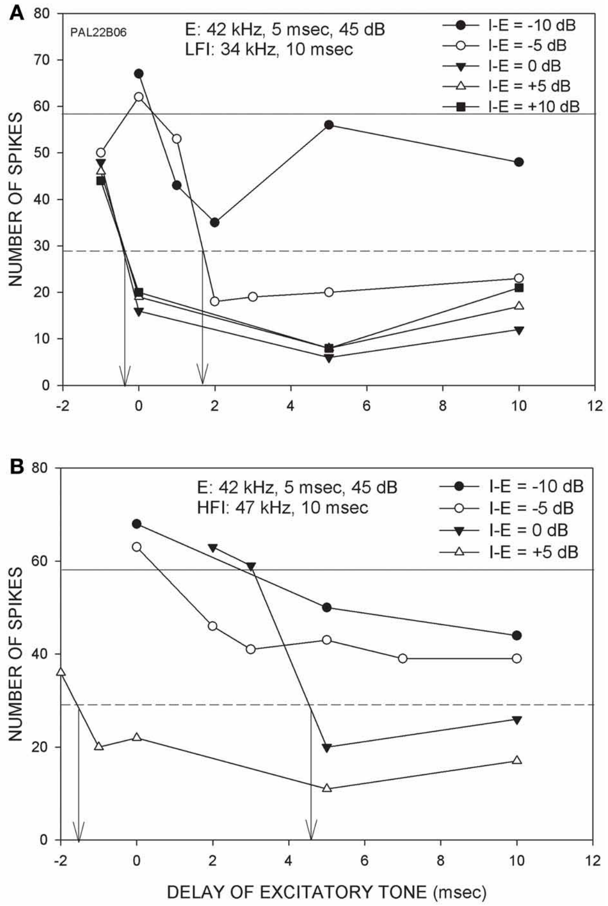

FIGURE 2 | Responses from a neuron (CF = 42 kHz) showing the effects of changing the intensity of inhibitory tone relative to the excitatory tone. (A) The excitatory tone (E) was set at CF. The LFI was centered at 34 kHz. The intensity of E was fixed while the intensity of LFI tone was varied from 10 dB below (I-E = −10 dB) to 10 dB above (I-E = +10 dB) the E tone intensity. Solid horizontal line indicates response to E alone (control response). The dashed line marks 50% of control response. The vertical arrows mark the arrival time of LFI defined as the delay at which two-tone response decreased to 50% of control response. **(B)** In the same neuron, the effect of changing HFI intensity was also measured. For both LFI and HFI, the arrival time becomes faster with increasing relative intensity of inhibitory tone.

neuron shown in **Figures 3C,D**, the HFI tone produced 50% inhibition only when the intensity of the two tones was equal. With further increase in the intensity of HFI, the arrival time decreased (**Figures 3C**,**D**).

Across the population ($n = 33$, **Figure 4A**), the relative threshold of HFI was significantly higher than that of LFI (Mann-Whitney Rank Sum Test, $p < 0.001$). On average, the HFI tone had to be similar in intensity to the excitatory tone to produce the criterion level inhibition. On average, LFI tone intensity produced criterion level inhibition even when its intensity was 10 dB lower than the excitatory tone. **Figure 4B** shows the population data for change in LFI and HFI arrival time with increasing intensity of the inhibitory tone. In general, the arrival time of HFI was slower than the arrival time of LFI. A two-way Anova and Tukey post-hoc pairwise tests comparing LFI and HFI arrival time showed significant differences ($p < 0.05$) at I-E values of −10, −5, 0, and +5 dB (asterisks in **Figure 4B**). However, this temporal asymmetry decreased as the intensity of the inhibitory tone was increased (arrival times not different at +10, +15, and +20 dB, Tukey post-hoc pairwise comparison, $p > 0.05$). When the two tones were of the same intensity, the arrival time of LFI was ~0 ms and the arrival time of HFI was ~4 ms, confirming previously published temporal asymmetries when the tones were of equal intensity (Razak and Fuzessery, 2006).

DISCUSSION

The receptive field of auditory neurons includes both excitatory and inhibitory components (Arthur et al., 1971; Calford and Semple, 1995; Brosch and Schreiner, 1997; Gordon and O'Neill, 1998; Sutter et al., 1999; Faure et al., 2003; Wehr and Zador, 2003; Razak and Fuzessery, 2006; Wu et al., 2008; Sadagopan and Wang, 2010). The main goal of this study was to characterize the intensity dependence of temporal interactions between excitatory and inhibitory frequencies in auditory cortical neurons tuned in the echolocation range in the pallid bat. There were two main findings in this study (schematized in **Figures 5A–D**). First, the relative threshold of inhibition was lower for LFI than HFI (**Figures 4A**, **5A,D**). On average, a LFI tone produced criterion inhibition even when its intensity was ~8–10 dB lower than the excitatory tone. A HFI tone produced criterion inhibition only when its intensity was the same or higher than the excitatory tone. Second, the arrival time of both LFI and HFI decreased when the intensity of the inhibitory tones was progressively increased relative to the excitatory tone (**Figures 4B**, **5A–C**). With further increase in inhibitory tone intensity, the LFI and HFI arrival times reach a saturation level such that the temporal asymmetry decreases (**Figures 4B**, **5C**). Thus, whether temporal asymmetry in sideband inhibition is present or not depends on the intensity relationship between the excitatory and inhibitory tones.

The echolocation call selective region of the pallid bat auditory cortex thus appears to be organized such that each neuron receives a stronger and lower threshold LFI input compared to the HFI input (**Figure 5D**). When tested with FM sweeps in which the LFI, HFI and excitatory frequencies have the same intensity, the evoked LFI will be stronger than the HFI. This difference in strength will translate into a difference in arrival time such that LFI arrives early while HFI has a longer latency compared to excitatory input. The temporal properties of inhibition are involved in shaping both FM rate and direction selectivity (Razak and Fuzessery, 2006; Razak, 2012). The early LFI will reduce responses to upward sweeps of any sweep rate while the slow HFI only reduces responses to downward sweeps with slow rates. This mechanism enhances direction selectivity while preserving responses to the fast downward sweeps used in echolocation.

These data suggest that the information that individual neurons provide is dynamic and depends on the echolocation context. During natural echolocation behaviors, the sweep selectivity of individual neurons will depend on the intensity distribution of various spectral components in the echolocation call. The

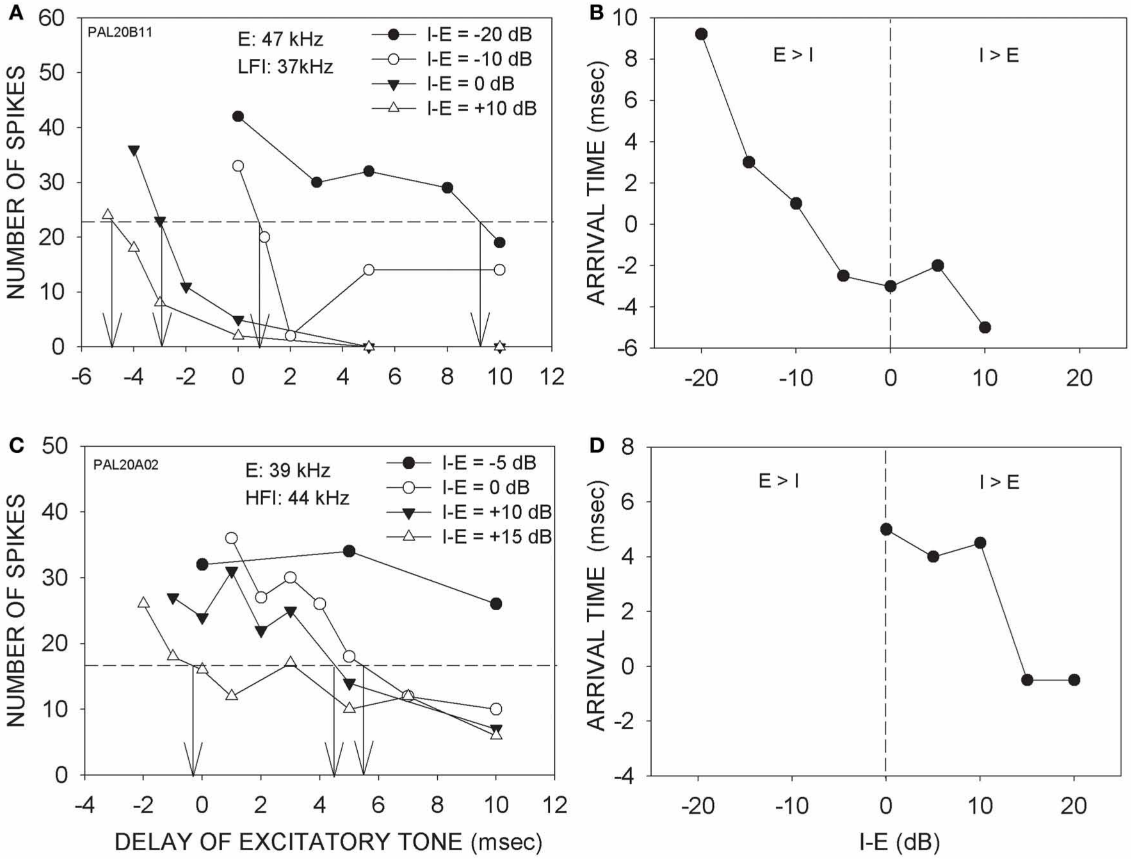

FIGURE 3 | (A) Effects of changing relative intensity of inhibitory tone on LFI arrival time. Notations are as in **Figure 3**. **(B)** The panel shows the intensity-arrival time relationship for the neuron in **(A)**. Vertical dashed line indicates equal intensity of the two tones. **(C)** Effects of changing relative intensity of inhibitory tone on HFI arrival time. **(D)** The intensity-arrival time relationship for the neuron in **(B)**.

intensity distribution of frequencies in the echo will, in turn, depend on many factors including the intensities in the call, directionality of call and ear, degree of environmental attenuation of different frequencies, and the distance to reflecting targets. For example, other factors being equal, a closer target may reflect a call with stronger energy in a neuron's HFI compared to a target that is further away. This will cause the neuron to be more selective for the faster sweep rates present in the echolocation call and selectively enhance responses to an echo relative to other sounds when the target is close-by. The other sounds include slower sweeps (e.g., communication calls) of either direction that overlap in spectrum (Brown, 1976; Kanwal et al., 1994; Bohn et al., 2008, 2009). As downward FM sweeps are commonly used signals to echolocate, a similar model may explain sweep direction and rate selectivity across FM bats. Low frequencies are attenuated less than high frequencies by the environment making it likely that low frequencies have relatively higher intensities than the high frequencies in returning echoes. The frequency-dependent environmental attenuation will add to the threshold differences between LFI and HFI noted here to generate strong downward sweep response bias. These neural and environmental factors may combine to partly explain the preponderance of downward sweep echolocation call usage by FM bats. In gleaners such as the pallid bat that depend on passive hearing for prey localization, the enhanced sweep selectivity provided by the model proposed here may also act to physiologically enhance the segregation of the parallel pathways used for echolocation and passive hearing (Barber et al., 2003; Razak et al., 2007). In fact, the larger percentage of direction selective neurons in the pallid bat auditory system compared even to other bats suggests that the strong LFI favoring asymmetry (stronger, faster, and lower threshold LFI) may serve pathway segregation more than FM processing.

MECHANISMS FOR SPECIES-SPECIFIC FM SWEEP DIRECTION/RATE SELECTIVITY

The pallid bat auditory system contains neurons with asymmetry that favors the LFI (**Figure 5D**). Manipulation (ontogenetically and phylogenetically) of two parameters in the model (**Figure 5D**) can lead to differences in FM sweep rate and direction selectivity. The first is *relative synaptic strength* of excitatory and inhibitory inputs. This can be thought of as variations along the ordinate of the tuning curve. The second is *asymmetry*, defined as the differences in properties between high- and low-frequency sidebands. This can be thought of as variations

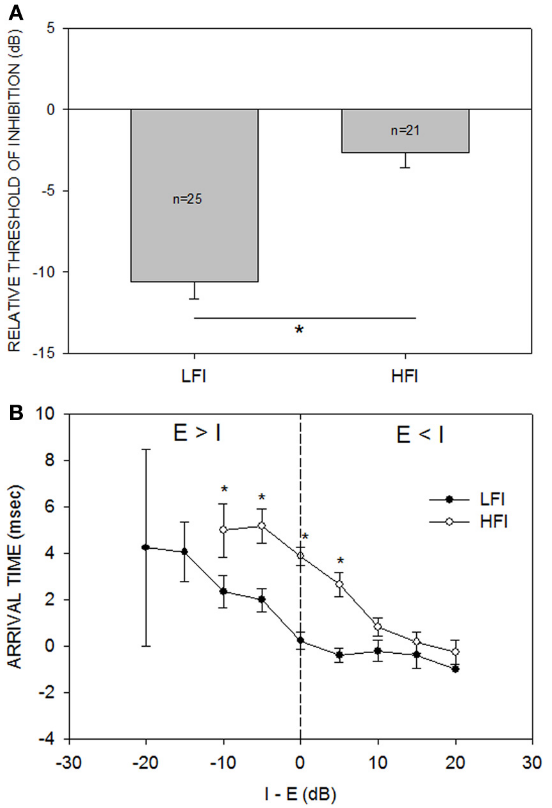

FIGURE 4 | (A) The mean (±s.e.) relative threshold of inhibition of LFI was lower than that of HFI. Relative threshold of inhibition was defined as the smallest intensity difference between the inhibitory and excitatory tone that results in the neural response decreasing by 50% of response to excitatory tone alone (criterion inhibition). When tested with LFI tone, criterion inhibition was observed, on average, even when the inhibitory tone was 10 dB less intense than the excitatory tone. When tested with HFI, the two tones had to be of similar intensities, on average, to cause criterion inhibition. *$p < 0.001$. **(B)** The mean (±s.e.) arrival time of LFI and HFI decreased as the intensity of the inhibitory tone was increased relative to the excitatory tone. "I-E" on the abscissa indicates the intensity difference between the inhibitory and excitatory tone. "Arrival time" on the ordinate indicates the shortest delay at which response to the two tones decreased to 50% of response to excitatory tone alone. The vertical dashed line marks the equal intensity point. There are no data points for HFI at −15 and −20 dB because HFI was not apparent when inhibitory tone was 15 or 20 dB less intense than the excitatory tone. The s.e.m for LFI at the −20 dB point is large because only two neurons showed criterion inhibition at this intensity difference.

along the abscissa of the tuning curve. For example, if synaptic strength of LFI is stronger than excitatory inputs, then LFI will prevent responses to upward sweeps at any sweep rate (**Figures 5D,E,G,H**). The response of the neuron to downward sweeps will be sweep rate selective. The fastest rate that elicits a response will be determined by the difference in the strength of excitatory and HFI inputs. For example, a neuron with weak HFI will still generate rate selective response to downward sweeps, but the neuron will respond to a broader range of rates (**Figures 5E,H**). Likewise, changing the symmetry relationships between the inhibitory and excitatory components along the abscissa of **Figure 5D** will result in different levels of direction selectivity. Species in which direction selectivity is poor, neurons are predicted to have symmetric inhibition around the tuning curve (schematized in **Figures 5F,I**). Asymmetry favoring HFI will result in upward selectivity. A thorough characterization of spectral, temporal and intensity relationships between excitatory and inhibitory components of the receptive field is therefore required to understand the contribution of sideband inhibition to neural selectivity for FM sweeps.

MECHANISMS UNDERLYING INTENSITY-LATENCY RELATIONSHIP OF INHIBITORY INPUT

The two-tone inhibition paradigm has been used to study the interactions between excitatory and inhibitory components at different levels of the auditory pathway and across species. Most of these studies focused on the frequency-time (Calford and Semple, 1995; Brosch and Schreiner, 1997; Gordon and O'Neill, 1998) or frequency-intensity (Sutter et al., 1999) interactions, with few studies emphasizing intensity-time relationships (Arthur et al., 1971; Scholl et al., 2008; Sadagopan and Wang, 2010). In the rat auditory cortex, Scholl et al. (2008) tested the effect of changing relative levels of the two tones and found that the interactions were mostly suppressive, and did not shift to facilitation with intensity. The pallid bat cortex data are consistent with this finding in that no evidence for intensity-dependent switch from inhibition to facilitation was found. Together with the present study, these data indicate that interactions between at least three sound parameters: frequency, intensity, and time need to be characterized to describe inhibition in the receptive field.

The arrival time of inhibition determined using the two-tone inhibition paradigm is a measure of latency of inhibitory input relative to the excitatory input. It is established that the latency of excitatory tone responses decrease with increasing intensities above the MT (Klug et al., 2000). Latency saturates at a minimum value at a supra-threshold intensity and typically shows little change with further increase in intensity. Data from the present study suggest that the latency of inhibitory input also undergo similar intensity-dependent changes. With increasing intensity of the inhibitory tone, the arrival time of inhibition decreases systematically up to a point of saturation. It should be noted that arrival time of inhibition relative to excitation was measured here. However, the intensity of the excitatory tone was fixed suggesting that the change in arrival time was specific to inhibitory input. Latency of excitatory response can also show a non-monotonic relationship with intensity such that the latency reaches a minimum and then increases again with increasing intensity [paradoxical latency shift, (Galazyuk and Feng, 2001)]. No evidence for such a non-monotonic relationship for the arrival time of inhibition with intensity was found in the pallid bat cortex.

Although multiple mechanisms contribute to FM sweep rate/direction selectivity, asymmetric sideband inhibition is the dominant mechanism in the auditory cortex of the pallid bat (Fuzessery et al., 2011). Similar findings in rodent, primate and carnivore auditory systems indicate that these mechanisms are general principles of spectrotemporal processing and not just adaptations in an auditory specialist (Shamma et al., 1993;

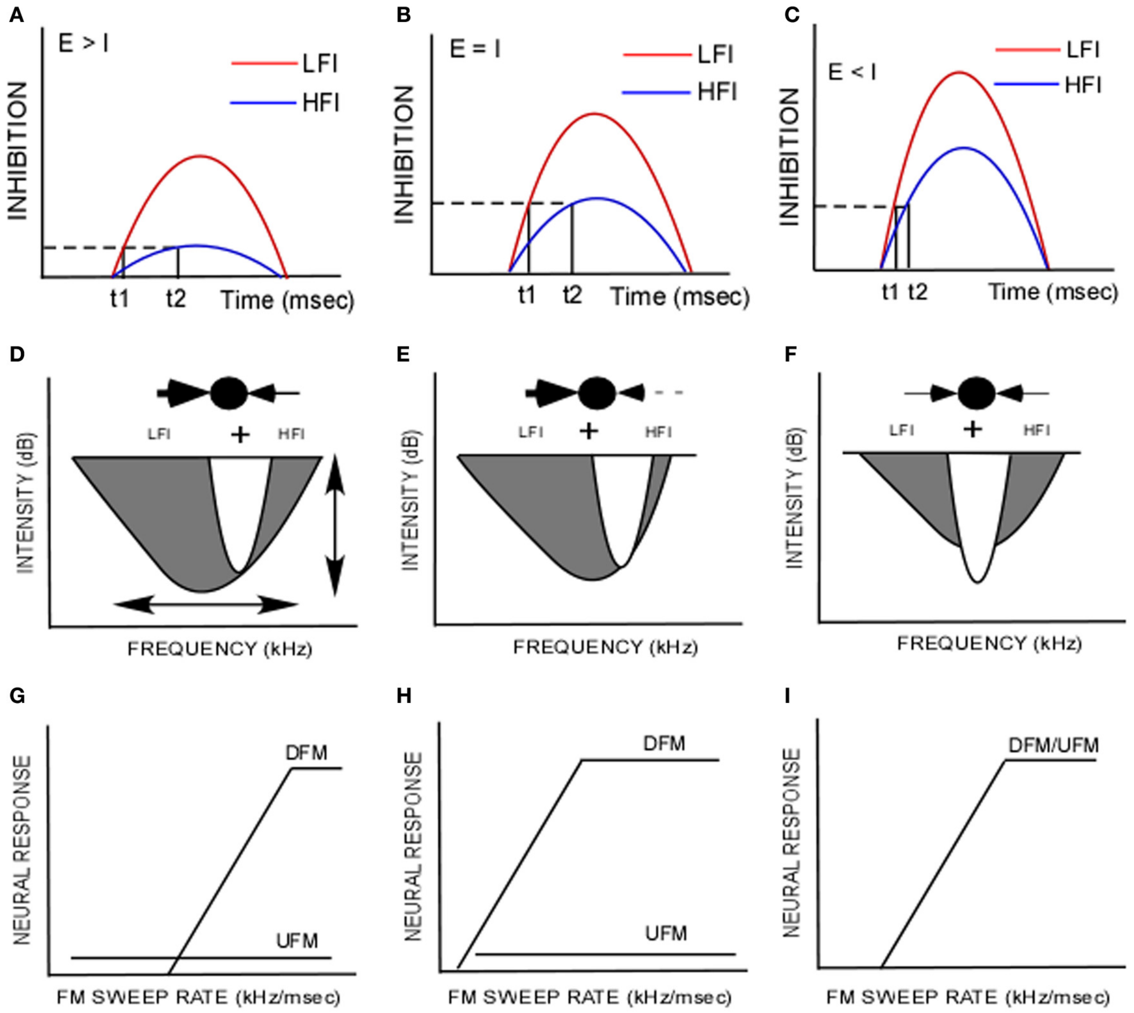

FIGURE 5 | (A–C) The difference in arrival time of low-frequency inhibition (LFI, t1, red) and high-frequency inhibition (HFI, t2, blue) is related to the differences in threshold and amplitude of inhibitory input. The dashed line indicates a criterion level of inhibition. The solid vertical lines indicate the time at which the set level of inhibition is reached. **(A)** When the excitatory tone (E) is of greater intensity than the inhibitory tone (I), it is hypothesized that the HFI does not reach criterion level but LFI does, indicating a lower threshold for LFI than HFI. **(B)** When E = I, it is hypothesized that both LFI and HFI grow in strength, but LFI is stronger. It reaches the criterion inhibition sooner than HFI creating the temporal asymmetry relevant to FM sweep rate and direction selectivity as shown in Razak and Fuzessery (2006). **(C)** When E < I, both LFI and HFI grow in amplitude to a point of saturation resulting in reduced temporal asymmetry. **(D,E)** Schematic of inhibitory and excitatory components of the frequency receptive field. In the "ball and arrows" drawing, LFI is shown to the left and the HFI is shown to the right. **(G–I)** Idealized FM rate selectivity functions for upward and downward sweeps (UFM, DFM) for neurons with corresponding receptive fields shown in **(D–F)**. These schematics illustrate how species-specific FM sweep selectivity may be obtained by modifying two properties in a network, "synaptic strength" and "symmetry." **(D)** A schematic of the strong asymmetry favoring the LFI in the pallid bat auditory cortex. This model illustrates the data from this paper that most neurons receive lower threshold LFI than HFI. The broader bandwidth of LFI is based on data in Razak and Fuzessery (2006). This gives rise to strong responses to downward sweeps in a rate-selective manner and weak/no response to upward sweeps. The vertical arrow in **(D)** indicates that relative synaptic strengths of HFI and excitatory input can be modified to obtain the tuning curve in **(E)**. The neuron is still strongly direction selective **(H)**, but is less selective for sweep rate compared to the neuron in **(G)**. The horizontal arrow in **(D)** indicates that the symmetry of LFI and HFI can be varied relative to the excitatory inputs. For example, if LFI and HFI are symmetrical in strength, bandwidth and timing, similar responses to upward and downward FM sweeps in a rate selective manner will result **(I)**.

Zhang et al., 2003; Sadagopan and Wang, 2010; Ye et al., 2010; Kuo and Wu, 2012; Trujillo et al., 2013). In the pallid bat, spectrotemporal properties of sideband inhibition predict FM rate and direction selectivity. Exclusion of sideband frequencies from the sweep reduces/eliminates selectivity. Iontophoresis of antagonists of inhibitory neurotransmitters reduces/eliminates selectivity by altering sideband inhibition. These studies suggested that the sequence of excitatory and inhibitory inputs arriving at a neuron in response to FM sweeps influences selectivity for sweep direction and rate. Based on *in vivo* whole cell recording and modeling of IC responses, Gittelman and Pollak (2011) suggested that timing differences *per se* may be less important in shaping direction selectivity compared to interactions between relative timing and magnitude of excitatory and inhibitory conductance. Modulation of magnitude of inhibitory/excitatory conductance may generate timing differences relevant to FM direction selectivity (**Figures 5A–C**). This hypothesis is supported by findings in the rat auditory cortex where the relative timing of excitatory and inhibitory inputs depends on sound intensity (Wu et al., 2006) and in cat cortex in which latency of hyperpolarization in layer three pyramidal neurons decreases with sound intensity (Ojima and Murakami, 2002). The intensity-arrival time relationships in the cortex (current study), as well as the changes in arrival time of inhibition with inhibitory neurotransmitter receptor antagonists (Razak and Fuzessery, 2009; Williams and Fuzessery, 2011) are consistent with a relationship between strength and timing of inhibition. Threshold and strength of inhibition may therefore be substrates for modulation of timing of sideband inhibition implicated in FM sweep selectivity.

The decrease in latency of inhibitory input with increasing tone intensity may reflect faster excitatory inputs onto inhibitory neurons. In the rat auditory cortex, inhibitory input latencies advanced faster compared to excitatory latency when tested with tones at increasing intensities (Wu et al., 2006). Thus, the latency-intensity relationship may be stronger at the excitatory input to inhibitory neurons compared to excitatory inputs to excitatory neurons. In an integrate and fire model, the time taken for the membrane potential to reach threshold is determined by the maximum amplitude and rising slope of the post-synaptic potential (Wu et al., 2006). If the rising slope is steeper with sound intensity for the inhibitory conductance compared to excitatory input, the integration time will also be shorter for inhibition manifesting as faster latencies. The excitatory thalamocortical inputs to inhibitory neurons in the cortex are stronger than the inputs to excitatory neurons (Cruikshank et al., 2007). Thalamocortical excitatory currents rise faster in inhibitory interneurons than in excitatory neurons providing a basis for the faster advance of inhibitory latencies with increasing intensities compared to excitatory inputs.

It is noteworthy that in the rat cortex, the neurons in which inhibitory latencies advanced faster than excitatory latencies were non-monotonically tuned for sound intensity (intensity tuned neurons). In the pallid bat auditory cortex, the vast majority of the neurons in the echolocation region are also intensity tuned (Measor and Razak, unpublished observations). Thus, the intensity-latency relationship shown in the present study may not only shape FM sweep rate/direction selectivity, but also lead to intensity tuning for the echolocation calls. This hypothesis is currently being investigated.

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Evolution of the heteroharmonic strategy for target-range computation in the echolocation of Mormoopidae

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Echolocating bats use the time elapsed from biosonar pulse emission to the arrival of echo (defined as echo-delay) to assess target-distance. Target-distance is represented in the brain by delay-tuned neurons that are classified as either "heteroharmonic" or "homoharmormic." Heteroharmonic neurons respond more strongly to pulse-echo pairs in which the timing of the pulse is given by the fundamental biosonar harmonic while the timing of echoes is provided by one (or several) of the higher order harmonics. On the other hand, homoharmonic neurons are tuned to the echo delay between similar harmonics in the emitted pulse and echo. It is generally accepted that heteroharmonic computations are advantageous over homoharmonic computations; i.e., heteroharmonic neurons receive information from call and echo in different frequency-bands which helps to avoid jamming between pulse and echo signals. Heteroharmonic neurons have been found in two species of the family Mormoopidae (*Pteronotus parnellii* and *Pteronotus quadridens*) and in *Rhinolophus rouxi*. Recently, it was proposed that heteroharmonic target-range computations are a primitive feature of the genus *Pteronotus* that was preserved in the evolution of the genus. Here, we review recent findings on the evolution of echolocation in Mormoopidae, and try to link those findings to the evolution of the heteroharmonic computation strategy (HtHCS). We stress the hypothesis that the ability to perform heteroharmonic computations evolved separately from the ability of using long constant-frequency echolocation calls, high duty cycle echolocation, and Doppler Shift Compensation. Also, we present the idea that heteroharmonic computations might have been of advantage for categorizing prey size, hunting eared insects, and living in large conspecific colonies. We make five testable predictions that might help future investigations to clarify the evolution of the heteroharmonic echolocation in Mormoopidae and other families.

Keywords: target-range, echolocation, heteroharmonic computation, Mormoopidae, call-echo delay

INTRODUCTION

Echolocation allows bats to create perceptual images of complex night environments (Griffin, 1959; Moss and Surlykke, 2010). A key piece of information obtained during echolocation is the space-depth of surrounding objects that constitute possible targets (Simmons, 1973, 2012; Wenstrup and Suthers, 1984). Target distance is assessed from the time delay between the outgoing call and the returning echo (Simmons, 1971; Simmons et al., 1979). The central auditory system of echolocating bats contains specialized neurons that respond to particular call-echo delays (Feng et al., 1978; O'Neill and Suga, 1979). The combined activity of populations of delay-tuned neurons presumably determines the bat's ability for target-range computation (Suga, 1990; Simmons, 2012).

The neural processing of target-distance has been studied in six bat species from four different families: Mormoopidae (*Pteronotus parnellii*; O'Neill and Suga, 1979; Suga et al., 1979 and *Pteronotus quadridens*, Hechavarría et al., 2013); Rhinolophidae (*Rhinolophus rouxi*; Schuller et al., 1991); Vespertilionidae (*Myotis lucifugus*; Sullivan, 1982; Wong and Shannon, 1988 and *Eptesicus fuscus*; Feng et al., 1978; Dear et al., 1993); and Phyllostomidae (*Carollia perspicillata*; Hagemann et al., 2010, 2011). Two different neuronal strategies for target-range computation have been identified. In bats that broadcast frequency-modulated (FM) calls, delay-tuned neurons respond to similar harmonics in the calls and echoes, thus employing a homoharmonic computation strategy (HmHCS) (Feng et al., 1978; Sullivan, 1982; Dear et al., 1993; Hagemann et al., 2010). In two bat species from the family Mormoopidae (*P. parnellii* and *P. quadridens*) and one species from the family Rhinolophidae (*R. rouxi*) delay-tuned neurons are activated by the combination of the FM component of the fundamental harmonic in the call and one of the higher harmonic

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Abbreviations: HtHCS, heteroharmonic computation strategy; HmHCS, homoharmonic computation strategy; HDC, high duty-cycle; LDC, low duty-cycle; CF, constant frequency; FM, frequency modulation; DSC, Doppler shift compensation.

FM components in the echo (O'Neill and Suga, 1979; Suga et al., 1979; Schuller et al., 1991).

The "heteroharmonic computation strategy" (HtHCS) was first described in *P. parnellii* (Suga et al., 1978) and *R. rouxi* (Schuller et al., 1991). These two bat species use echolocation calls that combine long constant-frequency (CF) and FM components. For this reason it was long believed that HtHCS was an exclusive feature of the so called long CF-bats (Schuller et al., 1991; Wenstrup and Portfors, 2011). Recently, Hechavarría et al. (2013) reported that HtHCS is also a feature of neurons in the auditory cortex of the mormoopid *P. quadridens*, a species that uses short CF (sCF)-FM echolocation (Macías and Mora, 2003; Macías et al., 2006). That *P. quadridens* is able to use HtHCS echolocation is interesting not only from a functional point of view but also from an evolutionary angle, since (to our knowledge) Mormoopidae is the only family of bats including both CF-FM and sCF-FM species.

The evolution of echolocation has received much attention in the last decade. Recent molecular phylogenies (Eick et al., 2005; Teeling et al., 2005) have shaped new perspectives on the evolution of bat echolocation behavior (Jones and Teeling, 2006). Signal design (Jones and Holderied, 2007), duty cycle (Fenton et al., 2012), call frequency (Stoffberg et al., 2011), and Doppler shift compensation (Schnitzler and Denzinger, 2011) have been reviewed in the light of new phylogenetic insights.

In this review, we explore the evolution of the HtHCS in bat species from the family Mormoopidae. There are several recent findings that motivated this work. (1) HtHCS was found in *P. quadridens* (Hechavarría et al., 2013). (2) The CF-bat *P. parnellii* holds a basal position in the lineage of the genus *Pteronotus* (Van den Bussche and Weyandt, 2003; Dávalos, 2006). (3) The auditory cortex of newborn bats that do not yet echolocate is equipped with a set of fully functional delay-tuned neurons (Kössl et al., 2012) which suggests that target-range computation strategies could be genetically pre-determined. (4) A scheme for the evolution of "Doppler shift compensation" by bats of the family Mormoopidae was proposed (Smotherman and Guillen-Servent, 2008). (5) New call designs, activity patterns and diets were described in Caribbean mormoopids (Mora et al., 2011; Goerlitz et al., 2012; Mancina et al., 2012; Rolfe and Kurta, 2012).

We discuss how brain adaptations, distinctive characteristics of calls- and echoes- and phylogenetic relationships in mormoopids could have led to the acquisition of the heteroharmonic target-range computation strategy in this family. We argue that the HtHCS provides mormoopids with behavioral and ecological advantages for categorizing prey-size, hunting eared insects, and living in large colonies. By conducting the analysis in the light of recent molecular phylogenies, we are able to explore the evolutionary relationships between HtHCS and CF-specializations. We present the hypothesis that in Mormoopidae, HtHCS echolocation evolved independently from long-CF echolocation, high duty cycle (HDC) echolocation and Doppler Shift Compensation. We make five specific, testable predictions that might help future investigations to decipher the evolution of the heteroharmonic echolocation in Mormoopidae and other families.

DELAY TUNING IN AUDITORY NEURONS OF DIFFERENT BAT SPECIES

The most commonly used approach to determine whether a neuron is tuned to echo-delay or not consists in presenting the animal with artificial (or natural) pulse-echo pairs with different delays. The response of the neurons is measured as the number of spikes fired by the neuron in response to each echo-delay. If the echo-level is also changed during the recording, then the neuronal response is represented in the two dimensional space of echo-delay and echo-level in the form of a delay response area (DRA). Delay tuned neurons respond only (or maximally) to a few combinations of echo-delay and echo-level (see examples DRAs in **Figure 1**).

Different methods have been used to study the harmonic sensitivity of delay-tuned neurons in different bat species. By deleting components of the echolocation call and echo, Suga and co-workers (Suga et al., 1983) demonstrated that in the cortex of *P. parnellii*, the maximum response of delay-tuned neurons occurs when the fundamental FM-harmonic in the biosonar pulse (FM1) is followed by one of the upper FM-harmonics in the echo (i.e., FM2, FM3, or FM4) with a certain delay. Delay-tuned neurons are classified according to their best harmonic combination, i.e., the combination of pulse and echo harmonic that elicits the largest response. In *P. parnellii*, neurons tuned to combinations of FM1 and FM2, FM1-FM3, and FM1-FM4 have been found (Suga et al., 1983; Hagemann et al., 2011). Heteroharmonic neurons can be found in newborn *P. parnellii* long before they start to echolocate (Kössl et al., 2012). The latter could indicate that this neuronal ability is imprinted in the genome of the species and therefore it could have been subjected to evolutionary pressures.

In *P. quadridens* the frequency profile of cortical delay-tuned neurons was studied by presenting the bat with combinations of different harmonic components that included FM1/FM2, FM1/FM3, FM1/FM1, and FM2/FM2 (Hechavarría et al., 2013). The frequency profile of delay-tuned neurons in *P. quadridens* is quite similar to the frequency profile of delay-tuned neurons of *P. parnellii*. Example heteroharmonic neurons of *P. quadridens* are shown in **Figure 1A**. The delay-tuned neurons of *P. quadridens* fire only (or more strongly) in response to heteroharmonic pulse echo-pair combinations, i.e., FM1/FM2 and FM1/FM3. It has been suggested that neurons tuned to different harmonic combinations could provide information about targets with different acoustic properties i.e., preys of different sizes (**Figure 1B**).

Although *P. parnellii and P. quadridens* use comparable heteroharmonic computations, they differ in the cortical organization of neurons according to their best harmonic combination (**Figure 2**). In *P. parnellii*, delay-tuned neurons are clustered together forming three distinct cortical areas defined as the FM–FM, dorsal fringe and ventral fringe areas. Within the FM–FM and dorsal fringe areas, there is a "harmonic organization" of neurons, i.e., neurons with different best harmonic combinations occur in distinct cortical subdivisions (Suga and O'Neill, 1979). The most ventral subdivision is dominated by neurons tuned to FM1/FM2, the middle subdivision is dominated by neurons tuned to FM1/FM4 and the most dorsal subdivision is dominated by neurons tuned to FM1/FM3 (O'Neill and Suga,

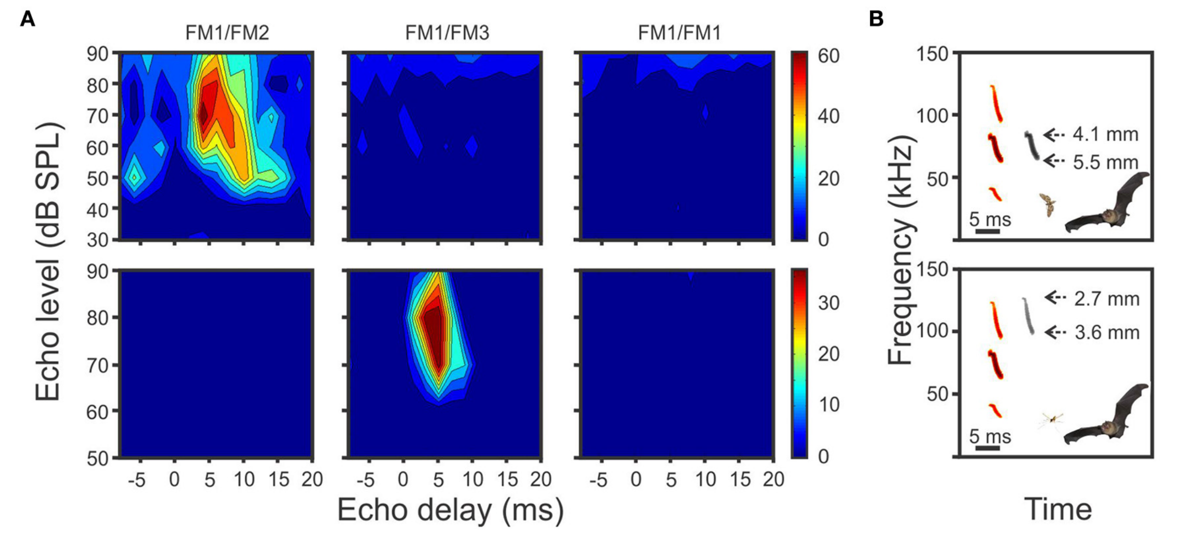

FIGURE 1 | (A) Example delay response areas (DRAs) of two units from *P. quadridens*. The units were tuned to different harmonic combinations. Responses of the same unit were aligned horizontally. **(B)** The range of theoretical insect sizes generated from echoes of each biosonar harmonic. Insect size was calculated according to the maximum and minimum frequencies in each harmonic after Macías et al. (2006). It is suggested that each neuron could play a role in prey categorization according to size.

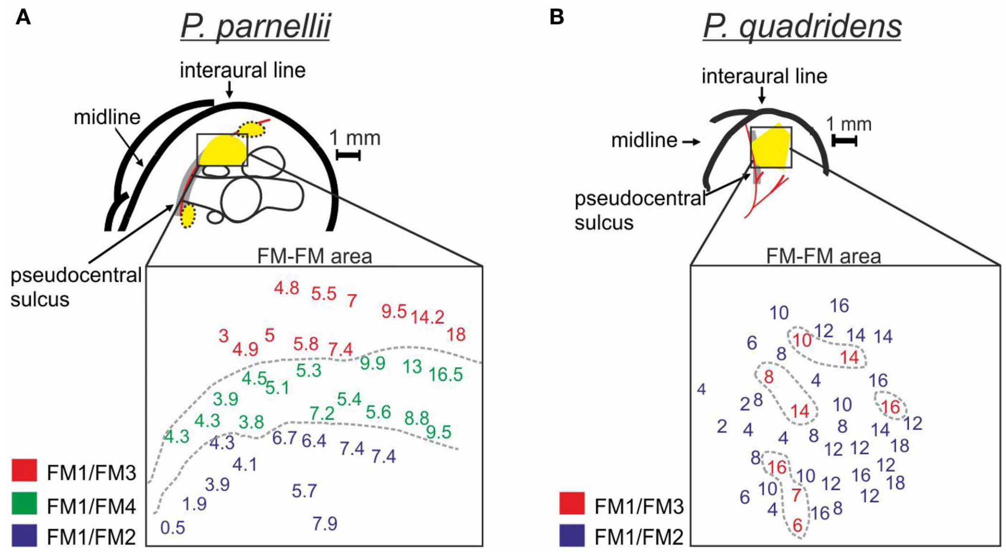

FIGURE 2 | Topographic organization of the FM/FM area of (A) *P. parnellii* and (B) *P. quadridens*. In each species, schematic representations of the brain are given. In the schematic brain representations, prominent landmarks and blood vessels are indicated. The yellow areas indicate cortical regions dominated by delay-tuned neurons. Note that in the dorsal auditory cortex of both species, close to the pseudocentral sulcus, there are large areas dedicated to the processing of call-echo delay. In each species, a detailed map of the FM/FM area is given. The data from *P. parnellii* is from one specimen [modified from Hagemann et al. (2011)]. The data from *P. quadridens* was pooled from 3 specimens [see Hechavarría et al. (2013) for methods for reconstruction of cortical maps]. In FM/FM area maps, numbers positioned at the coordinates of each neuron indicate characteristic delays. Numbers were color-coded to indicate the best harmonic combination of each neuron. Note that in *P. parnellii* neurons processing different harmonic combinations form different clusters in the cortical surface. However, in *P. quadridens*, neurons processing FM1/FM2 and FM1/FM3 are intermixed. In both species neurons processing shorter delays are located rostrally and those processing longer delays are located more caudally.

1982) (**Figure 2A**). In the boundaries between subdivisions, there are "multiple-combination sensitive neurons" that respond maximally when the echo contains combinations of 2nd, 3rd, and 4th biosonar harmonics (Misawa and Suga, 2001). The cortex of *P. quadridens* is different from the cortex of *P. parnellii* in the sense that it is not "harmonically" organized (Hechavarría et al., 2013). In *P. quadridens* only the FM–FM area has been studied and within this area neurons tuned to FM1/FM3 are interspersed with neurons tuned to FM1/FM2 (**Figure 2B**). One organizational principle shared by the cortices of *P. parnellii* and *P. quadridens* is the "chronotopic" organization of neurons. In these two species, neurons tuned to short echo-delays are located rostrally, while neurons tuned to longer echo-delays are located more caudally (Suga and O'Neill, 1979; O'Neill and Suga, 1982; Schuller et al., 1991; Hagemann et al., 2011; Hechavarría et al., 2013).

Phylogeny studies have shown that *P. parnellii* and *P. quadridens* stem from the most basal and most recent branches in the *Pteronotus* lineage, respectively (Van den Bussche and Weyandt, 2003; Dávalos, 2006). Because of the latter, and the fact both *P. parnellii* and *P. quadridens* possess comparable heteroharmonic neurons, Hechavarría et al. (2013) suggested that the HTCS could be a generalized feature of the genus *Pteronotus* that was preserved during the evolution. The same was suggested for the chronotopic organization of the cortex that is found in both species. On the other hand, harmonically organized chronotopic axes either evolved only in *P. parnellii* or were lost during the evolution of *P. quadridens* (Hechavarría et al., 2013).

Besides *P. parnellii* and *P. quadridens*, heteroharmonic neurons have been found in *R. rouxi* (Schuller et al., 1991). Only neurons tuned to FM1-FM2 were found in this species. Like in *P. parnellii* and *P. quadridens*, in *R. rouxi* there is a clear chronotopic organization of delay tuned neurons. The genus *Rhinolophus* is not closely phylogenetically related to the genus *Pteronotus* (Jones and Teeling, 2006). In fact rhinolophid bats seem to be more phylogenetically related to the megabats than to the remaining microbats (Teeling et al., 2005). The latter suggests that any specialization shared by *Pteronotus* and *Rhinolophus* could be the product of parallel evolution.

Delay-tuning has been studied in other three bat species besides the two *Pteronotus* and *R. rouxi*. In *M. lucifugus, E. fuscus,* and *C. perspicillata* delay tuning seems to be "homoharmonic," i.e., delay-tuned neurons of these three species respond strongly to pulse-echo combinations of the same harmonic (Sullivan, 1982; Dear et al., 1993; Hagemann et al., 2010). *M. lucifugus* uses a simple FM-pulse for echolocation without prominent harmonics (Griffin, 1962) and therefore it is not surprising that this species uses homoharmonic computations. *E. fuscus* and *C. perspicillata* use biosonar calls that contain at least two harmonics (Thies et al., 1998; Monroy et al., 2011) although call structure can change drastically depending on the behavioral task and the reflective properties of the environment. Yet the delay-tuned of these two species respond strongly to homoharmonic pulse-echo pairs (Dear et al., 1993; Hagemann et al., 2010). Among the homoharmonic species studied so far, only *C. perspicillata* is reported to have a chronotopically organized representation of delay-tuned neurons (Hagemann et al., 2010).

BRAIN ADAPTATIONS FOR HETEROHARMONIC COMPUTATIONS

The mechanisms for the central implementation of delay tuning have been intensively investigated in *P. parnellii* and excellent reviews are available (Wenstrup and Portfors, 2011; Wenstrup et al., 2012). Heteroharmonic delay-tuning is implemented in the auditory midbrain (Wenstrup et al., 2012). Heteroharmonic neurons integrate information from the fundamental biosonar harmonic that provides information about the timing of the pulse and one or several of the upper harmonics in the echo (Portfors and Wenstrup, 1999). Delay-tuned neurons perform as coincidence detectors, i.e., they respond only when there is a temporal coincidence of subthreshold excitations triggered by call and echo. It has been demonstrated that inhibition plays an instrumental role in delaying the response to the call so that it can be aligned in time with the response to the echo. If call-triggered inhibition similarly plays an instrumental role in the implementation of homoharmonic delay tuning is still unknown.

Integrating information from multiple biosonar harmonics is generally accepted as a building block for the implementation of heteroharmonic delay tuning. However, integrating multiple frequency bands (otherwise known as combination sensitivity) is not an exclusive feature of heteroharmonic neurons tuned to echo-delay. For example, combination sensitive responses have been found in mice, birds, monkeys and homoharmonic bat species, among others (Margoliash and Fortune, 1992; Dear et al., 1993; Rauschecker et al., 1995; Hernández et al., 2005; Portfors and Felix, 2005; Felix and Portfors, 2007; Hagemann et al., 2010). The currently available data suggests that combination sensitivity is a generalized principle of the mammalian auditory system that was further used by heteroharmonic bats for the implementation of a specialized strategy for target-distance computation.

CALL DESIGN AND TARGET RANGE

The examination of call design could provide a better understanding of the evolution of the HtHCS for target-range computation in bats and specifically in the family Mormoopidae. Bats use a highly diverse repertoire of call designs. Biosonar call diversity is observed both across (e.g., Schnitzler et al., 2003) and within species (e.g., Mora et al., 2011). One approach for categorizing bat calls distinguishes short FM from long CF calls. Typically, bats that broadcast pure-FM calls listen for echoes before emitting the next call to avoid temporal overlapping of call and echo. This calling strategy maintains a low duty cycle (LDC) (i.e., the proportion of time occupied by biosonar calls during an echolocation sequence is <25%). On the other hand, bats that use long CF echolocation calls separate call and echo in the frequency domain (because of the Doppler shifted echo). CF-bats are able to broadcast calls and receive echoes at the same time and therefore they can use HDC echolocation, with duty cycle values above 25%. Call design is tightly linked to duty cycle. Most echolocating bats use LDC echolocation (Fenton et al., 2012). HDC echolocation is a feature of only a few bats species (i.e., species from the families Rhinolophidae and Hipposideridae, and *P. parnellii* from Mormoopidae). Only the family Mormoopidae includes both LDC and HDC species. Although useful as a first approach, classifying bats into FM-LDC and CF-HDC according to their calling strategy is not fine-grained enough to explore the evolution of target-range computation in mormoopids.

Call design is polymorphic within the family Mormoopidae. FM calls are emitted by the two species of the genus *Mormoops*, long CF-FM calls are emitted by *P. parnellii* and sCF-FM and FM-sCF calls are emitted by the other five species of the genus *Pteronotus*: *P. personatus*, *P. davyi*, *P. gymnonotus*, *P. macleayi*, and *P. quadridens* (Fenton, 1994; O'Farrell and Miller, 1997; Ibañez et al., 1999, 2000; Kössl et al., 1999; Macías and Mora, 2003; Macías et al., 2006; Smotherman and Guillen-Servent, 2008; Mora and Macías, 2011) (**Figure 3A**). For ranging, the following parameters of signal design are expected to be of special importance: (1) the number of harmonics, (2) the frequency overlap of harmonics, (3) the bandwidth of the FM component,

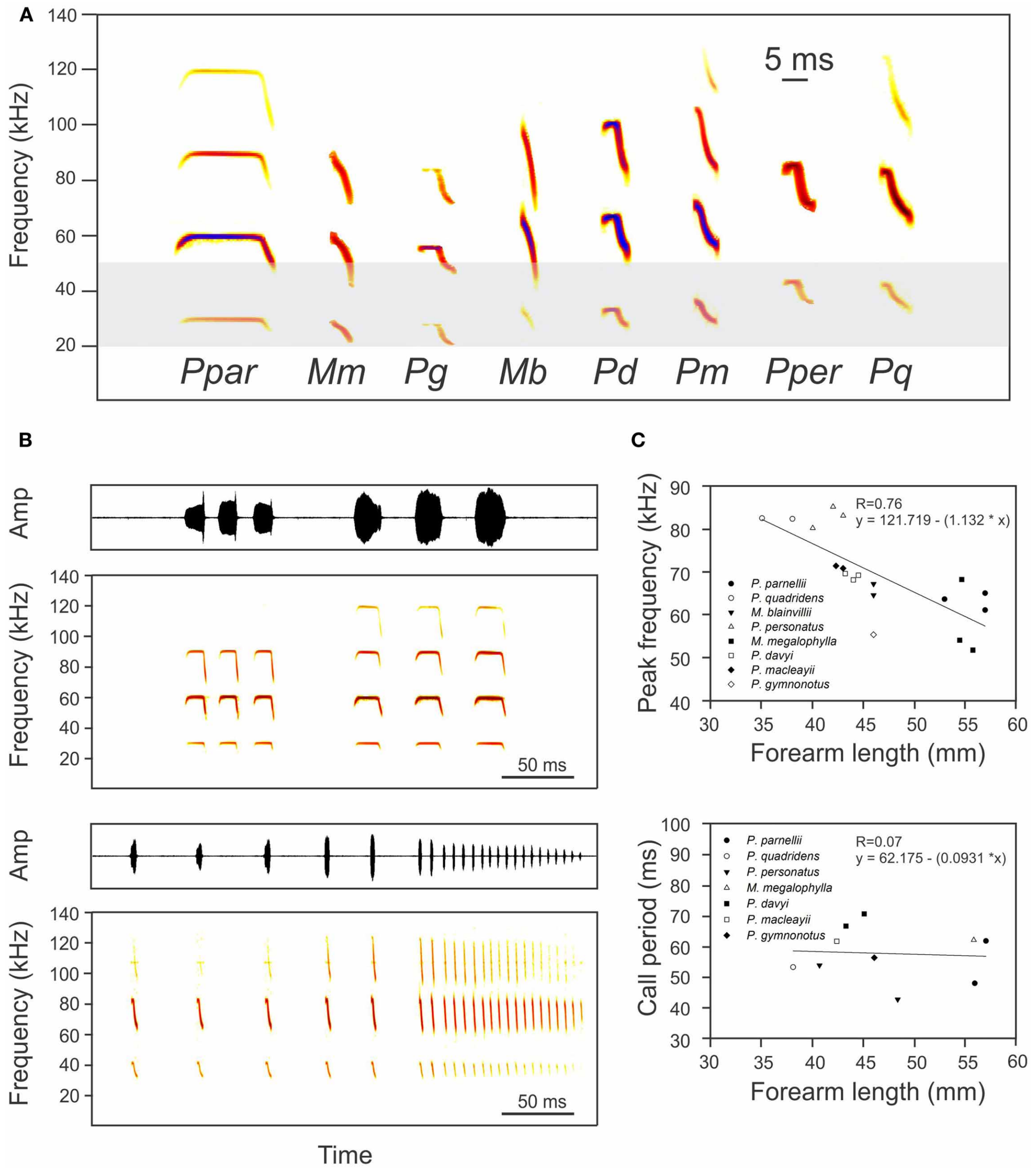

FIGURE 3 | (A) Spectrograms of typical search calls of the eight bat species of the family Mormoopidae (Ppar: *Pteronotus parnellii*, Mm, *Mormoops megalophylla*; Pg, *Pteronotus gymnonotus*; Mb, *Mormoops blainvillei*; Pd, *Pteronotus davyi*; Pm, *Pteronotus macleayii*; Pper, *Pteronotus personatus*; Pq, *Pteronotus quadridens*). The light-gray area represents the frequency range of best audition in eared moths, after Fullard (1988). **(B)** Typical echolocation sequence (oscillogram and spectrogram) emitted by *P. parnellii* (up) and *P. quadridens* (down) during foraging. Note that call harmonics never overlap. **(C)** Relationships between peak frequency (up) and call period (down) and forearm length for the eight species of mormoopid bats. Lower frequency calls are emitted by larger bats. Call period is independent of body size and signal design. Data was taken from: Silva-Taboada (1979), Herd (1983), Adams (1989), Rodríguez-Durán and Kunz (1992), Rezsutek and Cameron (1993), Lancaster and Kalko (1996), O'Farrell and Miller (1997), Ibañez et al. (1999), Ibañez et al. (2000), Macías et al. (2006), Smotherman and Guillen-Servent (2008), MacSwiney et al. (2008), de la Torre and Medellin (2010), and Mancina et al. (2012).

(4) the duration and curvature of the FM component, and (5) the frequency range and intensity of each FM-component.

Obviously, a heteroharmonic mechanism for target-range computation can only operate on call-echo pairs with at least two harmonics. The two mormoopids in which the HtHCS has been reported [i.e., (*P. parnellii* and *P. quadridens*)] broadcast calls with three or more harmonics, as do the remaining *Pteronotus* and *Mormoops* species (**Figure 3A**). Also, the echolocation calls of *R. rouxi* (the third species in which HtHCS has been described) contain two harmonics (Neuweiler et al., 1987). *E. fuscus* and *M. lucifugus* (two homoharmonic species) use echolocation calls with one or two harmonics (Moss et al., 1997; Surlykke and Moss, 2000). Besides Mormoopidae, other families that use multiharmonic echolocation calls (i.e., three or more harmonics) are Megadermatidae, Nycteridae, and Phyllostomidae (Jones and Teeling, 2006; Jones and Holderied, 2007). It is known that at least one phyllostomid species (*Carollia perspicillata*) uses the HmHCS (Hagemann et al., 2010). Therefore, broadcasting multiple harmonics does not seem to be sufficient for using the heteroharmonic target-distance computations.

In the three species known to use HtHCS, there is no overlapping between the harmonics of the FM-component of the calls (**Figures 3A,B**). Therefore the ability of calling (and hearing) in spectrally independent bands could be a prerequisite for using HtHCS. Supporting this idea is the fact that *C. perspicillata* (which uses HmHCS) uses multiharmonic calls with harmonic overlapping (Thies et al., 1998). The echolocation calls from all mormoopid species show non-overlapping harmonics. Non overlapping harmonics are also observed in the biosonar calls of rhinolophids and hipposiderids.

To be able to keep harmonics fully separated in the frequency domain, bats need to limit the bandwidth of their FM calls or components. Increasing bandwidth is appropriate to develop a detailed acoustic snapshot of the surrounding and to separate prey from background clutter (Simmons and Stein, 1980; Siemers and Schnitzler, 2004). It is known that most bat species are capable of adjusting call bandwidth according to echolocation task. In Rhinopomatidae (e.g., Habersetzer, 1981), Vespertilionidae (e.g., Kalko and Schnitzler, 1993), Molossidae (e.g., Mora et al., 2011), Emballonuridae (e.g., Kalko, 1995) and Phyllostomidae (e.g., Mora and Macías, 2007) for example, several species can adjust the bandwidth of their calls to broadcast from quasi-constant frequency calls (BW < 4 kHz) to wideband FM calls (BW > 15 kHz) by adjusting the frequency band of their FM components. Mormoopids are different; they keep the bandwidth of their calls remarkably constant (**Figure 3B**) (but see *Mormoops*: Macías et al., 2006; Smotherman and Guillen-Servent, 2008), thus avoiding harmonic overlap (Macías and Mora, 2003; Macías et al., 2006; Mora and Macías, 2011).

Not only the bandwidth of the FM-sweep but also its duration and curvature may affect the estimation of target-range and influence the performance of the computation process. Both from the "distance of focus" theory (Boonman et al., 2003; Holderied et al., 2006) and from behavioral (Simmons, 1973) and neurophysiological data (Jen and Wu, 2008), there is evidence showing that short calls and echoes are more appropriate for an accurate estimation of short target-distances, which might decrease collision risks and increase the probability of a successful capture. For a bat flying faster than 3.4 m/s (1% of the speed of sound), Doppler effects will lead to a distortion of the perceived range due to compression of echo delay time and elevation of echo frequency. However, the accuracy of short target-distance estimation increases if short hyperbolic FM calls are used, and also if strong harmonics are added (Boonman et al., 2003; Simmons et al., 2004). To the best of our knowledge mormoopid calls have not yet been used to investigate how signal design could affect the measurement of echo-delay at different flight speeds as it has been done in other species (Simmons, 1973; Altes, 1980; Boonman et al., 2003). Nonetheless the visual inspection of mormoopid calls suggests a call structure suited to minimize errors in measuring distance caused by Doppler Effect, mainly if the FM-component is taken into account (**Figure 3B**). The CF-component of variable length in the mormoopid calls will effectively widen the envelope of the cross-correlation function, causing Doppler tolerance to decrease (Simmons, 1973). However, in a filter bank model Doppler tolerance will not decrease dramatically by adding a CF-component to the wideband FM-component, since this affects only a portion of the receiver channels (Boonman et al., 2003). In other words, the CF-component, thought to be used in the estimation of relative velocity and the recognition of fluttering insects (Schuller, 1984; Suga, 1990; review: Schnitzler and Denzinger, 2011), and the FM-component, used to measure target-distance (Simmons, 1973; Saillant et al., 1993), must be analyzed independently.

An additional ranging error is expected while flying since bats approach the target as the reflected echoes travel to the bat ears. Since this error causes an underestimation of target range while the Doppler-related error causes an overestimation of range, they cancel each other at a certain target distance (defined as the distance of focus; Boonman et al., 2003). By adjusting the design of the FM-sweep during flight in a range dependent way, bats can avoid these sources of error so that nearby objects are localized accurately, a behavior termed focusing (Boonman et al., 2003; Holderied et al., 2006). Future studies will show if mormoopids employ acoustic focusing and if they are able to adapt the duration, bandwidth, and curvature of FM biosonar elements to cancel out flight-speed-related ranging errors as a function of target-distance. If that is the case, delay-tuned neurons might show sharper delay tuning curves the shorter the "distance of focus" of the FM call-echo pairs used as acoustic stimuli in the neurophysiology experiment, improving the positive correlation between the best delay and the width of the delay tuning curves (see **Figure 1**) already observed in the auditory cortex (Suga and Horikawa, 1986; Hagemann et al., 2010; Hechavarría et al., 2013). Whether heteroharmonic bats correlate the returning echo with the actual outgoing call, or whether "hard-wired" replicas of the bat's characteristic signals are contained in the auditory system remains a very interesting question to be solved.

There is evidence that call design in mormoopids is linked to the HtHCS. Duty cycle, however, is not. In other words, call design may need to fulfill certain requirements for the bat to operate the HtHCS, but the HtHCS can operate both in LDC and HDC echolocation. For more than three decades (O'Neill and

Suga, 1979) the heteroharmonic target-range computation was known only for two CF-HDC bats. However, the sCF-FM-LDC *P. quadridens* also computes call-echo delay heteroharmonically (Hechavarría et al., 2013). The relatively high proportion of the "on time" of the call is achieved in HDC rhinolophids and hipposiderids by increasing the duration of the call relative to the call period (i.e., the time between the onset of successive calls) (Fenton et al., 2012). However, call period in most insectivorous bat species studied to date, is determined by the species wingbeat period (Speakman and Racey, 1991). If the same applies to the CF-HDC mormoopid *P. parnellii* then call period would remain at values equivalent to those in the other species of the family, as it is shown in **Figure 3C**. What is of relevance for target-range computation is the time interval between the emissions of two consecutive FM-components. Therefore, it is not surprising that the general rules that govern the temporal parameters of the calling strategy in FM-HmHCS bats also govern those of CF-HtHCS bats if only the FM-components are taken into account, i.e., as bats get closer to targets, they shorten the call's duration (or that of the FM-component) and the interval between calls (or between FM-components) thus increasing ranging performance (Boonman et al., 2003) and the accuracy in the estimation of the target's angular position (Suga, 1990). In consequence, once CF-HDC bats detect, lock and start tracking fluttering insects, the CF-component will shorten principally to accommodate the temporal changes of the FM-component that will rule the distance-to-target dependent temporal adaptations of the bat calling behavior.

In conclusion, it seems likely that any bat making use of the HtHCS will broadcast FM calls (or calls with FM components) with two or more harmonics without frequency overlap. In addition, it is of advantage if the duration and curvature of the FM components are adjusted for acoustic focusing as a function of distance to target, in correlation with neuronal adaptations for the processing of call design as a complement of the target-range computation strategy. Also it could be predicted that bats that use HtHCS are capable of a precise control of call frequency and intensity. The latter will be explored in the following section.

FREQUENCY AND INTENSITY OF CALL AND ECHO

Echolocating bats dynamically change the acoustic parameters of calls (i.e., frequency, intensity, temporal parameters) to cope with their environment and perceptual task. A closer view at the common principles used by HtHCS bats to exploit frequency and intensity of calls- and echoes- may help to assess the evolution of their target-range computation strategy. This section focuses on the analysis of frequency and intensity because both parameters are closely related in the heteroharmonic target-range computation strategy. The frequency spectrum of each call is determined by the amount of energy or sound intensity distributed between harmonics, and frequency and intensity are the two main parameters used to characterize the receptive field of delay-tuned neurons (see section Delay Tuning in Auditory Neurons of Different Bat Species).

The most obvious difference between HtHCS and HmHCS bats is in the frequency content of interest for assessing the timing of calls and echoes. Species that compute target-range homoharmonically broadcast and listen in the same frequency band since relevant wavelengths in calls and echoes are the same (Simmons, 2012). In contrast, heteroharmonic bats always pay attention to the fundamental harmonic in the call but to the higher order harmonics in the echoes. All HtHCS bats focus energy in higher harmonics but assign very little (as little as 1% of the total energy) to the fundamental harmonic (**Figure 3A**). The bat will still hear the faint fundamental harmonic of its call due to the small distance between mouth and ear, and the relatively weak attenuation of low frequencies (Lawrence and Simmons, 1982). However, conspecifics will mainly hear the higher harmonics. Attenuating the fundamental harmonic in HtHCS bats could minimize call-echo interference in bat colonies with hundreds or thousands of individuals since FM-components of higher harmonics by themselves cannot excite FM–FM neurons (Suga, 1990). The high frequency FM-components of the echoes will only elicit auditory responses in delay-tuned neurons if the calling bat have previously emitted and listened to its own fundamental harmonic. In the Caribbean islands, mormoopid bats are dominant in cave ecosystems where they enjoy the advantages of living in large colonies (Silva-Taboada, 1979; Goerlitz et al., 2012; Lima and O'Keefe, 2013). However, it is worth mentioning that the largest bat colonies known to mankind are of presumed homoharmonic species i.e., *Tadarida brasiliensis* (Betke et al., 2008; Hristov et al., 2010). Future research is needed to unveil how HmHCS bats deal with target-range computation in environments with so much overlapping frequency interference.

Rather fixed frequency-limits of FM-components also distinguish HtHCS from HmHCS bats. Mormoopids, but also rhinolophids and hipposiderids, keep the maximum frequency of their FM-components at the value of their CF-components (**Figures 3A,B**). In mormoopids flying in open spaces, even the minimal frequency of each FM-component seems to be restricted by the addition of a lower sCF-component to the call (O'Farrell and Miller, 1997; Mora and Macías, 2011). In contrast, frequency limits of individual harmonics are less fixed in HmHCS bats that vary either the maximal and/or the minimal frequencies of the emitted calls to adjust bandwidth (Kalko and Schnitzler, 1993; Surlykke and Moss, 2000; Mora et al., 2005).

The most widely used hypothesis to explain dominant call frequencies in bats is the *allometry hypothesis* (Jones, 1996, 1999). Due to the physics of sound, the structures associated with sound production generate lower-frequency sounds as size increases (Pye, 1979), and therefore it is predicted that larger bats emit at lower frequencies. In Mormoopidae, call frequency scales negatively with body size (i.e., forearm length) (**Figure 3C**). Since the *allometry hypothesis* explains call frequencies in several other bat families including the presumed HtHCS Rhinolophidae and Hipposideridae (Heller and Helversen, 1989; Jones, 1999), this hypothesis is not of much value to explore the evolution of the heteroharmonic strategy.

The *allotonic frequency hypothesis* suggests that relatively high or low echolocation frequencies are the result of selection to become less audible to eared insects, especially moths (Fullard, 1987). Tympanate moths have maximum hearing-sensitivity between 20 and 50 kHz (Fullard, 1988) which coincides with the frequency range echolocation calls of most bat species (Fenton et al., 1998). The fundamental biosonar harmonic of each mormoopid species contains frequencies syntonic (i.e., between 20 and 50 kHz) with moth hearing, but due to its relatively low intensity it may be barely detectable by the prey, thus offering a good example of harmonic-dependent stealth echolocation (Goerlitz et al., 2010). If multiharmonic echolocation evolved in mormoopids to allow these bats to exploit the soft nutritious moths as a food resource, then it would ideally combine faint first syntonic harmonics with loud high-frequency allotonic harmonics. Such a call would be optimally designed to overcome prey hearing (**Figure 3A**). In fact, several studies have shown that moth constitutes a major prey item in the diet of many Caribbean mormoopids (Silva-Taboada, 1979; Rolfe and Kurta, 2012). Therefore, hearing-mediated detection of bats by moths could have operated as an important evolutionary force for the acquisition of the heteroharmonic target-range computation strategy in Neotropical mormoopids.

The frequencies used by HtHCS mormoopids may be also explained by the *prey detection hypothesis* (Houston et al., 2004) which relates the strength of an echo with the wavelength of the call and the dimensions of the prey. The non-overlapping harmonics of the call theoretically allow mormoopids to exploit a broad range of prey sizes. For example, it is generally accepted that insects generate relatively strong echoes from biosonar wavelengths that match the dimensions of their prominent scattering points (i.e., head and wings). If the latter is true, *P. quadridens* could target a variety of insects with size differences of about 3 mm according to echoes from the minimum frequencies of the second harmonic (61.22 kHz, wavelength 5.5 mm) and from the maximum frequencies of the third harmonic (124.00 kHz, wavelength 2.7 mm). Distinction of insect size will be favored by individual auditory neurons responding to either the echoes from the second or the third harmonics (see **Figures 1A,B**). Smaller preys could be detected by adding a fourth harmonic to the call, which will significantly increase strength of echoes generated in smaller insects (Houston et al., 2004). Frequency-dependent atmospheric attenuation, however, would be a serious limitation in the use of high-order harmonics, but negligible at short range where it has been found that mormoopid bats incorporate a third and even fourth harmonic to their vocalizations (Macías and Mora, 2003; Mora and Macías, 2011). We argue that bats using HtHCS get a bonus in the categorization of insect size by focusing acoustic energy in discrete harmonic bands which in addition safes energy.

Two other hypotheses have been used to explain the frequency composition of biosonar calls: the *foraging habitat hypothesis* (Jones and Barlow, 2004) and the *acoustic communication hypothesis* or *acoustic resource partitioning hypothesis* (Duellman and Pyles, 1983; Heller and Helversen, 1989). According to the foraging habitat hypothesis, bats species that forage in more-cluttered habitats should use calls of higher frequencies than species foraging in less cluttered/more-open habitats (Stoffberg et al., 2011). Due to the multiharmonic structure of mormoopid calls this hypothesis is of limited value for explaining the emission of high frequencies in relation to clutter; i.e., high frequency demands are solved in HtHCS species by adding more harmonics. However, it is important to note that *P. parnellii* (a species that forages in highly-cluttered environments), uses one of the lowest frequencies within the genus *Pteronotus* (**Figure 3A**). The *acoustic communication hypothesis* predicts that different frequencies could evolve under selection pressures imposed during social interactions (Heller and Helversen, 1989; Thabah et al., 2006). In our opinion, this hypothesis does not add new insights to the evolution of the target-range mechanism in Mormoopidae.

Doppler shift compensation is not linked to the HtHCS. DSC involves lowering the frequency of the next echolocation call to compensate for the flight-induced increase in the frequency of echoes from a previous emission (Schnitzler and Denzinger, 2011). By compensating for Doppler effects, bats ensure that the CF-component of the echoes remains within the range of frequencies to which their auditory system is most sensitive, i.e., the "auditory fovea" (Neuweiler, 1990, 2003). The frequency value for which the auditory fovea shows its highest sensitivity is defined as the resting frequency (Suga and Jen, 1976; Smotherman and Guillen-Servent, 2008). The CF-FM *P. parnellii* compensates for Doppler shifted echoes (Henson et al., 1980) but the sCF-FM *P. quadridens* does not (Mora and Macías, 2011). However, these two species measure target-distance using the HtHCS. It is tempting to predict that DSC will have an influence on the target-range computation of mormoopids. For example, in neurophysiological experiments with species showing DSC such as *P. parnellii* and *P. personatus*, the best responses of delay-tuned neurons might occur when the bat is presented with call-echo combinations in which the call's fundamental harmonic (FM1) is lowered in frequency and the echo's higher harmonics (FMx) is set at the species resting frequency. In contrast, the non-compensating smaller mormoopids should show best responses of delay-tuned neurons for combinations of the call's resting FM1 and the echoes' shifted FMx.

Changes in the amplitude of call and echo are also relevant for the target-range computation mechanism. During flight, both HmHCS and HtHCS echolocating bats decrease the intensity of their emitted pulses when approaching a prey item or an obstacle (Kobler et al., 1985; Boonman and Jones, 2002; Hiryu et al., 2007, 2008). Call intensity is adjusted in relation to the distance to target while maintaining echo intensity within an optimal sensitivity range. This intensity compensation will surely affect the shape of the response areas of delay-tuned neurons (DRAs, see section Delay Tuning in Auditory Neurons of Different Bat Species), that so far have been obtained by keeping constant the level of the call while changing the level of the echo (Suga, 1990; Hagemann et al., 2010; Kössl et al., 2012; Hechavarría et al., 2013). It is expected that in both HtHCS and HmHCS bats, lower call intensities will shift best call-echo delays to shorter values. Previous results from neurophysiology experiments in the HmHCS bat *E. fuscus* are in agreement with this prediction (Jen and Wu, 2008).

Intensity compensation has been mainly analyzed for whole calls and echoes, regardless of the species that is studied (Hiryu et al., 2007, 2008; Surlykke and Kalko, 2008). However, in bats using the HtHCS, the intensity compensation and its effect on target-range computation, need to be analyzed on a harmonic level. The changes of call/echo intensity in the fundamental harmonic may not be the same in the second or higher harmonics. Combining the acoustic/neuronal rules that seem to describe the

HtHCS with those of intensity compensation, two main predictions arise: (1) that the intensity of the fundamental harmonic of the calls will remain stable while the intensity of the echo will increase as bats approach targets; and (2) that the intensity of the call's second or higher harmonics will decrease while the amplitude of the correspondent echoes will remain stable as bats approach targets.

HETEROHARMONIC ECHOLOCATION IN THE PHYLOGENY OF MORMOOPIDAE

To summarize the ideas discussed in the present review four important echolocation traits for mormoopid bats were mapped on a phylogenetic tree (**Figure 4**) (adapted from molecular data from Van den Bussche and Weyandt, 2003 and Dávalos, 2006). Mormoopid echolocation is characterized by quite diverse call designs and biosonar strategies that outside Mormoopidae distinguish different bat families. This diversity offers the opportunity to revise each species echolocation with the intention of tracking the ancestral condition and the evolutionary paths of each sonar trait. In some cases where there is not sufficient supporting data available, the two categories, "probably present" or "probably absent," are used to be able to speculate on phylogenetic trends. Future research is needed to fill the gaps in knowledge and to evaluate the present speculations.

The first look to the echolocation in Mormoopidae (**Figure 4**) confirms non-overlapping harmonics in the calls of each species of *Pteronotus*. Non-overlapping harmonics have been identified in this review (see sections Brain Adaptations for Heteroharmonic Computations and Call Design and Target Range) as a promising feature that would support the HtHCS for target-range computation. In addition, both the most basal and one of the most recently evolved species of *Pteronotus* computes call-echo delay heteroharmonically (Suga, 1990; Hechavarría et al., 2013). As mentioned in the preceding text (section Introduction), it is likely that every other *Pteronotus* species will also use the HtHCS for ranging. In addition, we speculate that both *Mormoops* species will also compute target-range heteroharmonically. Not only are the echolocation calls of *Mormoops* of a multiharmonic structure but also they contain a prominent second harmonic and a faint first harmonic (O'Farrell and Miller, 1997; Macías et al., 2006), features that as dicussed here could be related to the HtHCS. Indirect support for a HtHCS comes from the observation that *Mormoops* lives in large colonies and is a specialized moth predator (Silva-Taboada, 1979; Goerlitz et al., 2012; Rolfe and Kurta, 2012), behaviors that might profit from using heteroharmonic computations (Suga, 1990). Arguing against the possibility of finding a heteroharmonic strategy in *Mormoops* is the fact that in this genus, the echolocation calls broadcasted while approaching a target show some degree of frequency overlapping (Macías et al., 2006; Smotherman and Guillen-Servent, 2008). Frequency overlapping is not observed in the echolocation calls of *Pteronotus* species (Macías and Mora, 2003; Macías et al., 2006; Smotherman and Guillen-Servent, 2008; Mora and Macías, 2011). However, if the HtHCS is finally demonstrated in *Mormoops*, it will support the theory that the common ancestor of *Pteronotus* and *Mormoops* already featured this echolocation trait.

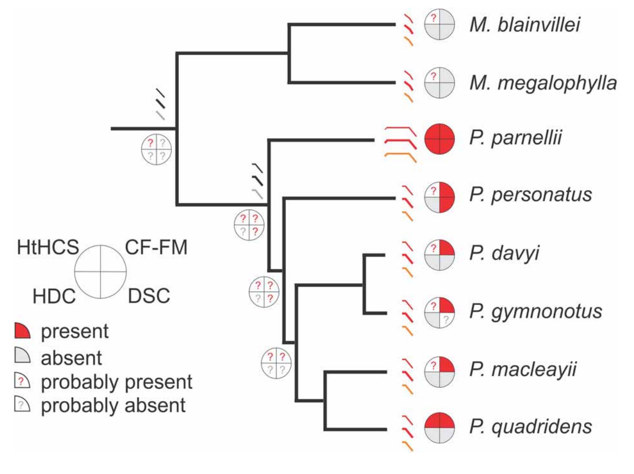

FIGURE 4 | Echolocation traits of mormoopid bats mapped onto the molecular phylogenetic tree of the family, after Van den Bussche and Weyandt (2003) and Dávalos (2006). Schematics of echolocation calls from extant species (in red) and suggested common ancestors (in gray) have been represented. The heteroharmonic computation strategy is expected to be found in each species of the family, in contrast to the other three traits that characterize only some of the extant species. The evolutionary position of *P. parnellii* points to the long-CF calls and HDC as evolutionary singularities.

The other three mormoopid echolocation traits, i.e., CF-FM calls, DSC and HDC are restricted to some mormoopid species (**Figure 4**). CF-FM calls (regardless of the duration of the CF component) are typical of *Pteronotus* and not of *Mormoops*, but using a long CF-component is a unique characteristic of *P. parnellii*. We therefore propose that the common ancestor of *Pteronotus* featured CF-FM calls. In this context, the long CF calls of the mustached bat, that allowed the species to echolocate at HDCs, are better explained as an evolutionary singularity probably produced by genetic change that introduced specialized modifications in cochlear development leading to an exceptionally sharp tuning to the CF call component [see discussion in Vater (1999); Kössl et al. (1999)]. DSC could have also characterized the *Pteronotus* ancestor since both *P. parnellii* and *P. personatus* compensate for flight-induced frequency shifts (Smotherman and Guillen-Servent, 2008; review: Schnitzler and Denzinger, 2011). DSC in *P. parnellii* has been interpreted as instrumental to assure the processing of CF echoes carrying information about fluttering insects by an exceptionally sharply tuned auditory fovea (reviews: Neuweiler, 1990, 2003). There are no previous studies on the auditory system of *P. personatus*, but at least a disproportionate representation of neurons processing the resting sCF-component frequency and an enhanced sensitivity to this frequency range is to be expected. If it is assumed that the common ancestor of the genus *Pteronotus* already possessed DSC, it would be quite challenging to explain the loss of DSC in the most recent *Pteronotus* species. The most parsimonious hypothesis would be that the foraging strategy adopted by the smaller *Pteronotus* relies upon a more broadly tuned auditory system (Kössl et al., 1999) and like most FM bats, they can tolerate modest Doppler effects (Boonman et al., 2003). A detailed analysis of the possible evolutionary scenario for the acquisition of DSC is beyond the scope of this work, but excellent reviews on this echolocation attribute are available (Schnitzler and Denzinger, 2011; Fenton et al., 2012).

Phyllostomidae is a sister family of Mormoopidae (Teeling et al., 2003; Eick et al., 2005). Because mormoopids are heteroharmonic (Hechavarría et al., 2013) and phyllostomids homoharmonic (Hagemann et al., 2010, 2011), it is difficult to infer the ranging strategy of the common ancestor of the two families. No indications for homoharmonic echolocation are apparent within the family Mormoopidea. Therefore if the ancestor of Mormoopidae was homoharmonic, this strategy was completely replaced by the heteroharmonic strategy during the evolution of the family. On the other hand, if the ancestor of Mormoopidae used HtHCS, some evidence could still be found within the many species of Phyllostomidae. Since *C. perspicillata* uses the HmHCS, and this species is relatively recent in the phylogeny of phyllostomids (Rojas et al., 2011), one should look into more ancient taxa to try to find any indication of HtHCS. *Macrotus*, the most basal genus of Phyllostomidae, could by the right taxon to find out whether the HtHCS was lost before the first phyllostomids appeared or during their evolutionary history. The two extant species of *Macrotus* (*Macrotus californicus* and *Macrotus waterhousii*) are gleaning bats that emit multiharmonic calls with faint fundamental harmonics, but showing frequency overlap (Murray et al., 2009).

Outside the New World, the same features characterizing the echolocation of Mormoopidae are found in species of the families Rhinolophidae and Hipposideridae. CF-FM, DSC and HDC in those bat families and in Mormoopidae are frequently taken as good examples of convergent evolution to emphasize how perceptual challenges imposed by the environment can override phylogenetic constraints (Jones and Teeling, 2006; Jones and Holderied, 2007). Rhinolophid bats make use of the HtHCS for ranging, long CF-FM calls, high duty-cycle and Doppler shift compensation (review: Schnitzler and Denzinger, 2011). Hipposiderids show similar echolocation traits but with shorter CF calls, lower duty cycles and a less advanced DSC. Their calls show the same signal structure suggested here to be necessary to perform the HtHCS.

CONCLUSIONS AND FUTURE DIRECTIONS

This review presents the hypothesis that the HtHCS for target-range estimation assisted the ancestors of mormoopid bats in categorizing target size, hunting for eared prey and inhabiting caves in large numbers. We suggest that the implementation of the HtHCS evolved in parallel to the ability of using CF calls, HDC echolocation, and DSC behavior. The detailed analysis of echolocation signal design and its task-dependent adaptations in acoustic parameters, on top of recent gene-based phylogenies obtained for the species in the family Mormoopidae, allow the identification of common principles in the evolution of target-range computation in mormoopids and other heteroharmonic bats. The following predictions might help to define some of the evolutionary building blocks for this echolocation strategy.

1. *Each species of the genera Pteronotus and Mormoops is predicted to perform HtHCS*. This is supported by the findings that within Mormoopidae, the most ancient and the most recent lineages show HtHCS and within Moormopidae call designs are similar across species.
2. *Mormoopids should be able of dynamic harmonic hopping, i.e., individuals can shift energy between the high order harmonics*. In theory, the HtHCS supports harmonic hopping to minimize high interference (i.e., from conspecifics) or to aid in the discrimination of different target sizes.
3. *If the ability of HtHCS computation characterized the ancestors of Noctilionoidea it may have prevailed at least in descendent species with limited frequency overlap between harmonics*. The genera *Noctilio* and *Macrotus* are appropriate candidates to test this hypothesis.
4. *Intensity compensation is harmonic-dependent in mormoopids and other bats with HtHCS*. Calls and echoes represent different harmonic interests for the heteroharmonic echolocator and therefore the rules describing the dynamic adjustment of call/echo intensity will distinguish one harmonic from the other.
5. *If the echolocation calls of mormoopids evolved to hunt eared prey, they will be relatively inaudible to moths if compared to calls from HtHCS bats of comparable size*.

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Echolocation of static and moving objects in two-dimensional space using bat-like frequency-modulation sound

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Bats use frequency-modulated echolocation to identify and capture moving objects in real three-dimensional space. The big brown bat, *Eptesicus fuscus*, emits linear period modulation sound, and is capable of locating static objects with a range accuracy of less than 1 μs. A previously introduced model can estimate ranges of multiple, static objects using linear frequency modulation (LFM) sound and Gaussian chirplets with a carrier frequency compatible with bat emission sweep rates. The delay time for a single object was estimated with an accuracy of about 1.3 μs by measuring the echo at a low signal-to-noise ratio. This model could estimate the location of each moving object in two-dimensional space. In this study, the linear period modulation sounds, mimicking the emitting pulse of big brown bats, were introduced as the emitted signals. Echoes were measured from moving objects at two receiving points by intermittently emitting these sounds. It was clarified that this model could localize moving objects in two-dimensional space by accurately estimating the object ranges.

Keywords: bat, echolocation, model, localization, linear period modulation

INTRODUCTION

Bats emit high-frequency sound waves, allowing them to track and catch flying insects (Griffin, 1958; Simmons et al., 1995). Bats perceive the location of moving objects in three-dimensional (3D) space using frequency modulation. Experimental evidence indicates that bats are capable of locating static objects at high signal-to-noise ratios (SNRs) achieving sub-microsecond accuracy (Simmons, 1979; Menne et al., 1989; Moss and Schnitzler, 1989; Simmons et al., 1990). In echolocation, many kinds of bats, including *Eptesicus fuscus* and *Noctilio leporinus*, emit linear period modulation (LPM) sound, the instantaneous period of which increases linearly with time. It was clarified that the LPM signal is useful for the range estimation of moving objects because of its Doppler tolerance using matched filters (Altes and Titlebaum, 1970; Altes and Skinner, 1977). Several previously proposed models estimate the delay times of multiple objects from an echo spectrogram, which is computed by IIR filters or short-time Fourier transform, which corresponds to convolution of the constant-frequency (CF) carrier wave at each frequency (Saillant et al., 1993; Matsuo et al., 2001; Neretti et al., 2003). However, it is difficult to accurately determine the delay time for each object using the peak time, because the integration time of the cochlear filters is long. An echolocation model was proposed to estimate the delay times of multiple objects from the time–frequency pattern using linear frequency modulation (LFM) sound (Matsuo and Yano, 2004; Matsuo et al., 2004; Matsuo, 2011, 2013). In this model, the time–frequency pattern is computed through the convolution of Gaussian chirplet filters for which the carrier frequency agrees with the sweep rate of emission (Matsuo and Yano, 2004; Matsuo et al., 2004; Matsuo, 2011, 2013). It was demonstrated that this proposed model could estimate the range of the moving object or accurately localize the moving object in two-dimensional (2D) space using the interaural range difference (IRD), computed as the difference between the object's range at two receiving points. In addition, Gaussian chirplet filters have been proposed for LPM sounds (Guarato et al., 2011). The present study examines whether this model can localize moving objects in 2D space from echoes, which are measured from static and moving objects at two receiving points by intermittently emitting LPM sounds, corresponding to the emitting pulse of big brown bats.

METHODS

Acoustic data were recorded in a soundproof chamber (length × width × height = 2.8 m × 1.7 m × 1.8 m). The measuring system, including one loudspeaker, two microphones, and objects to be detected were located on an optical base (Chuo Precision Industrial, TT-D6090), as shown in **Figure 1**. The loudspeaker and microphones were placed at a height of 70 cm, and the distance between them was 4 cm. The origin was defined as the center of the speaker's surface. The reflecting objects used were erect poles (radius of 8 mm) set on a rotating table controlled by a computer via an electric rotary actuator (Taiyo, ESR1).

The emitted signal was generated by a computer (National Instruments, PXI-8106), digital-to-analog (DA) converted (PXI-5412), amplified (TDT, ED1), and emitted by the loudspeaker (TDT, ES1). The sampling frequency of the DA converter was 1 MHz, and the resolution was 16 bits. The echoes reflected by the objects were recorded using a 1/8-inch condenser microphone (Brüel & Kjær, 4138), amplified (Brüel & Kjær, NEXUS

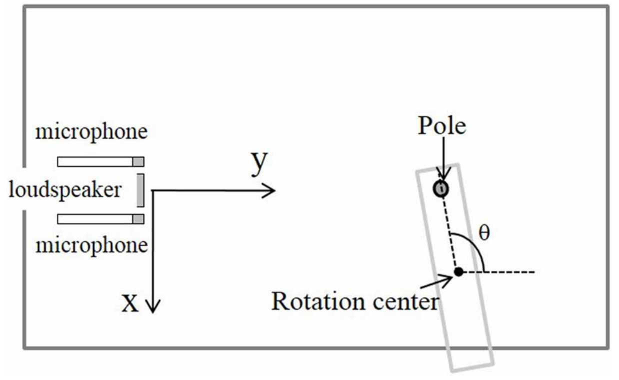

FIGURE 1 | Measuring system (top view) with a pole set on a rotating table.

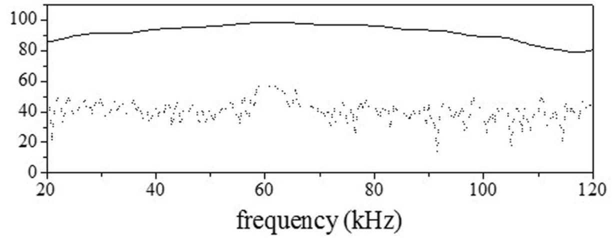

FIGURE 2 | Characteristics of the loudspeaker. The solid curve shows the amplitude spectrum computed from the measured waveform with the loudspeaker facing the microphone. The dotted curve shows the noise level.

2670, 2690), bandpass-filtered (NF, CF-4BL, CF-4BH), and analog-to-digital (AD) converted (NI, PXI-6133). The sampling frequency of the AD converter was 400 kHz, and the resolution was 14 bits. The temperature was measured in the chamber to compute the sound velocity. To estimate the characteristics of the measuring system, LFM sound, sweeping from 135 to 5 kHz over 2 ms, was used and the waveform was measured when the loudspeaker and microphone were positioned face-to-face. **Figure 2** shows the spectrum computed by taking the Fourier transform of the measured waveform. At a distance of 40 cm, the maximum and average values for the amplitude spectrum in the range of 30–100 kHz were 98.6 and 89.3 decibels sound pressure level (dB SPL), respectively, and the half-power (3-dB) bandwidth was 32 kHz (Matsuo, 2013).

In this paper, the bat-like LPM sound signal was synthesized by referring to the sound emitted by a big brown bat, *Eptesicus fuscus*, during approach of an object. The sound duration was almost 1.9 ms and the LPM signal started at 53 kHz and swept down to 25 kHz. **Figure 3A** shows the emitted waveform. The echoes were measured from the rotating pole for two situations. The first measurement was of the echo from the object moving back and forth, and the second was of the echo from the object moving from side to side. In addition, to clarify the effect of the Doppler shift on the accuracy, echoes from the static object were measured.

MODEL

TRANSFORMATION OF THE WAVEFORM INTO A SPECTROGRAM USING CHIRPLET FILTERS

The waveforms of the object echoes were entered into the echolocation model discussed in the Introduction. They were transformed into spectrograms in a manner that simulated the process in the mammalian cochlea. The temporal changes in the interference pattern were extracted using Gaussian chirplet filters with a carrier frequency consistent with the sweep rate of emission (Matsuo and Yano, 2004; Matsuo et al., 2004; Guarato et al., 2011). The temporal characteristics of the filter can be described by

$$F(f_j, t) = \exp\left(-\frac{t^2}{\alpha_j}\right)\exp\left(2\pi j\frac{\ln(kt + l)}{k}\right) \qquad (1)$$

Here f_j (kHz) is the center frequency for the jth bandpass filter, t is time (s), and α_j is a parameter that describes the width of the window function:

$$\alpha_j = \frac{w/2}{\ln(0.7)},$$

$$w = bw\left(\frac{f_1}{f_j}\right),$$

where f_1 is the start frequency of the signal and bw is the filter's base bandwidth fixed as 160 μs. Constants k and l are defined by

$$k = \frac{f_{\text{sta}} - f_{\text{end}}}{\text{dur} \cdot f_{\text{sta}} \cdot f_{\text{end}}},$$

$$l = \frac{1 - k \cdot t_{\text{sta}} \cdot f_{\text{sta}}}{f_{\text{sta}}},$$

where dur is the duration of the signal, f_{sta} (53 kHz) and f_{end} (25 kHz) are the starting and end frequencies of the signal, and t_{sta} is the start time of the signal. The bandpass filter bank comprised 24 filters with center frequencies ranging 27–50 kHz, positioned at regular intervals. The quality factor at 10 dB (Q10 dB) values ranges from 1.7 at 27 kHz to 3.1 at 50 kHz.

The waveforms for both the emitted waves and the echoes were transformed into a spectrogram $P(f, t)$ through convolution with the filters as shown in Equation 1. **Figure 3B** shows the outputs of the cochlear filters for the emitted waveform. **Figure 3C** shows the temporal pattern corresponding to the spectrogram $P(f, t)$ of the emission for one filter (with a center frequency of 30 kHz). The shapes of the temporal patterns corresponding to the spectrogram $P(f, t)$ for all filters were the same because the window lengths were set dependent on center frequencies.

To demonstrate the output from the cochlear filters, we considered the situation of a static object with position (x, y) of (0 mm, 450 mm) and range of 901.8 mm. **Figure 4A** shows the measured waveforms including the object's echo as well as the sound transmitted from the loudspeaker. The spectrogram $P(f, t)$, which was computed from the outputs of the Gaussian chirplets, was transformed into a range-frequency pattern $S_{\text{echo}}(f, \tau)$ with 10-μs intervals by compensating for

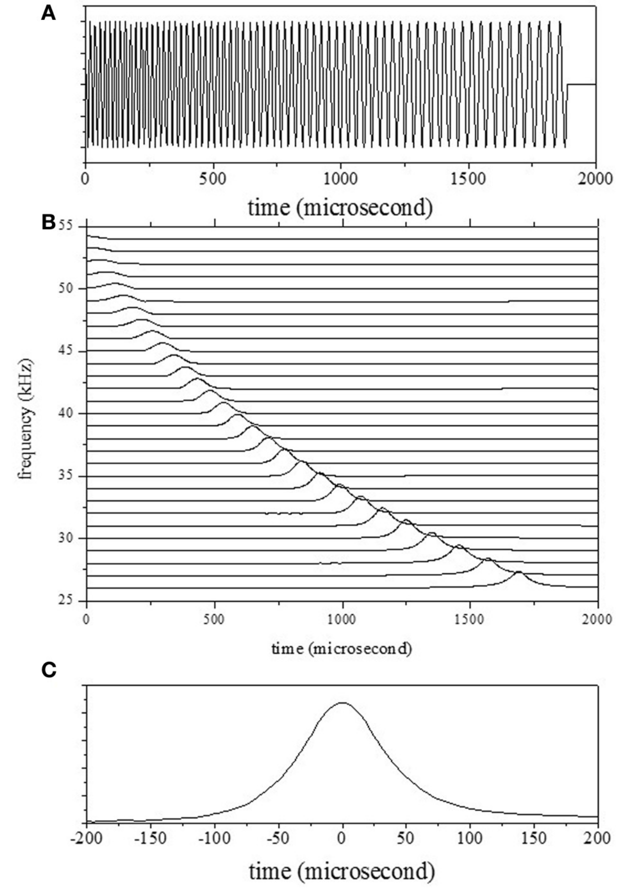

FIGURE 3 | (A) Emission waveform. **(B)** Emission spectrogram, computed by convolution of the Gaussian chirplets. **(C)** The temporal emission pattern W_{emi} corresponds to the spectrogram for one bandpass filter (with a center frequency of 30 kHz).

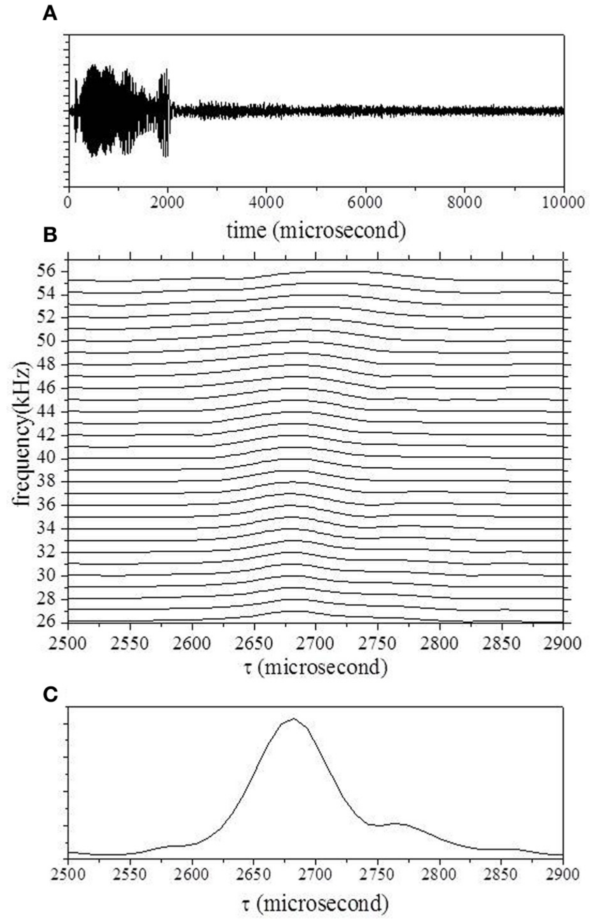

FIGURE 4 | (A) Echo waveform. **(B)** Range–frequency pattern computed from the spectrogram, through convolution of the Gaussian chirplets. **(C)** Averaged pattern computed by integration of bandpass filter channels.

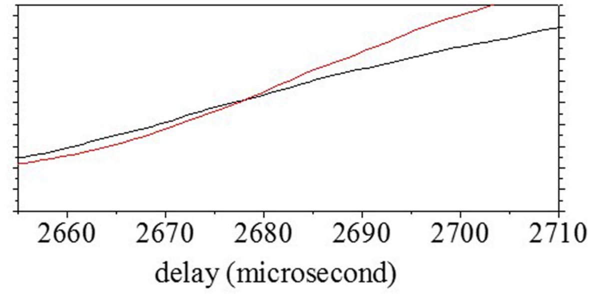

FIGURE 5 | Determination of T_1. The solid curve shows candidates for the reflected intensity distribution estimated from S_{echo} at onset delay time τ_{on}. The dotted curve shows candidates for the reflected intensity distribution estimated from S_{echo} at 10 μs after τ_{on}. T_1 was determined by estimating the correspondence between the reflectivity of candidates at each delay timepoint.

the sweep rate, as shown in **Figure 4B**. The compensation time is denoted τ, and it is implied that the range corresponds to the delay time since the start time of emission was zero.

DETERMINATION OF THE OBJECT'S RANGE AND LOCATION IN 2D SPACE

The delay time for one object, T_1, was estimated from the range–frequency pattern around the onset (Matsuo et al., 2004; Matsuo, 2011, 2013). First, the averaged pattern was computed by the integration of bandpass filter channels. The delay times for the onset and offset were determined using a threshold corresponding to almost four times the noise level (Matsuo, 2011, 2013). T_1 and the corresponding reflectivity, r_1, were uniquely determined from the averages of the two spectra at the onset delay time τ_{on} and 10 μs later. **Figure 4C** shows the averaged pattern, which was computed from the range–frequency pattern shown in **Figure 4B**. In this case, the delay time τ of the onset was estimated using a threshold of 2630 μs. The black curve in **Figure 5** shows candidates for T_1 according to the reflected intensity distribution estimated from the average of S_{echo} at the onset delay τ_{on} (2630 μs). The red curve shows candidates estimated from the average of S_{echo} 10 μs after τ_{on} (2640 μs). The delay time for T_1 was determined to be 2678 μs, corresponding to 908.6 mm, by comparing the correspondence between the reflected intensities of the two candidates.

The location of the object in 2D space was determined by the difference between the object's ranges at the two microphones. The object's position (x, y) is represented by polar coordinates (r, θ):

$$x = r\sin(\theta),$$

$$y = r\cos(\theta),$$

where r is the distance between the speaker and the object, and θ the direction of the object with respect to the horizontal axis. The distance r between the speaker and object was obtained from the mean of the ranges at the two microphones. If $r >> d$, corresponding to the distance between two microphones, the direction of the object, θ, was computed from this difference, Δr, using the approximation

$$\theta = \sin^{-1}\left(\frac{\Delta r}{d}\right)$$

The object was continuously tracked by estimating its position at each timepoint.

RESULTS

LOCALIZATION OF ONE POLE MOVING BACK AND FORTH

To evaluate the effect of the Doppler shift on the localization accuracy, the echoes from one object moving back and forth were measured and analyzed. The center of rotation was fixed at (250 mm, 450 mm) and the radius of rotation was 250 mm. The circles in **Figure 6A** show the estimated range along the time axis. The object's range could be estimated accurately using the temporal changes of echo spectra at the onset time. **Figure 6B** shows the IRD at each timepoint when the pole was moving back to forth. As shown in **Figure 6C**, the errors of the IRD were less than 4 mm. The circles and curves in **Figure 6D** show the estimated location and position of the object in 2D space. The locations of one pole could be estimated using the object's ranges for two microphones.

LOCALIZATION OF ONE POLE MOVING FROM SIDE TO SIDE

To evaluate the model's performance for different movements, the echoes from one pole moving from side to side were measured

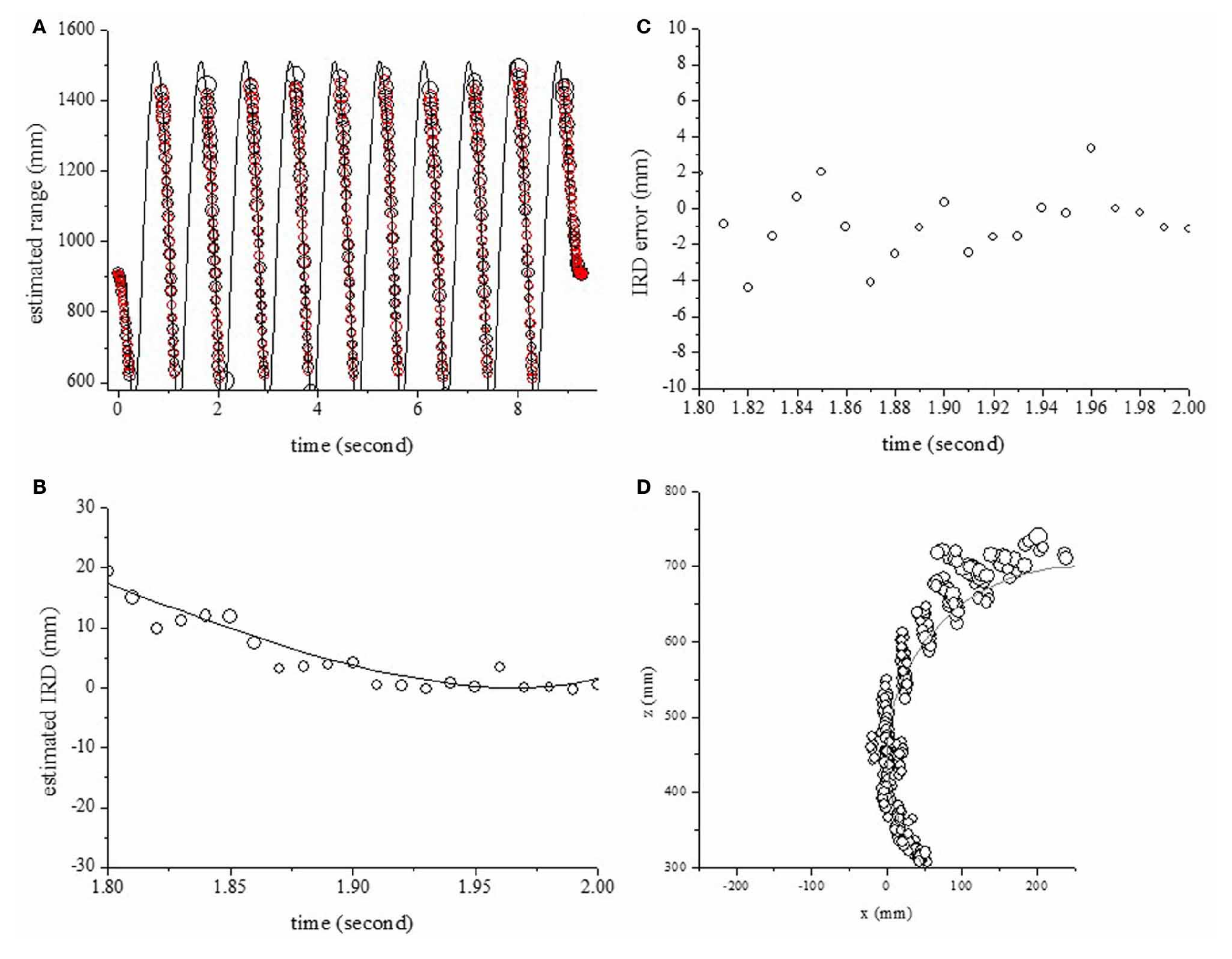

FIGURE 6 | Outputs for one object, moving back and forth with a rotation radius of 250 mm. (A) Estimated and actual ranges. **(B)** Estimated IRDs. **(C)** Estimation errors of IRDs. **(D)** Estimated and actual positions in 2D space.

and analyzed. First, the center of rotation was fixed at (0 mm, 575 mm) and the radius of rotation was 125 mm. The circles and curves in **Figure 7A** show the estimated range and the object's actual range along the time axis. The object's range could be estimated using the temporal changes of echo spectra at the onset time. The circles and curves in **Figure 7B** show the estimated location and object's position in 2D space, respectively. One pole could be localized except for side positions.

In the second measurement scenario, the center of rotation was fixed at (0 mm, 700 mm) and the radius of rotation was 250 mm. The circles and curves in **Figure 8A** show the estimated range and the object's actual range along the time axis. The object's range could be estimated using the temporal changes of echo spectra at the onset time. The circles and curves in **Figure 8B** show the estimated location and object's position in 2D space, respectively. One pole could be localized except for side positions.

DISCUSSION AND CONCLUSION

Bats can locate and discriminate between individual objects even when the objects are moving (Griffin, 1958; Webster and Griffin, 1962; Griffin et al., 1965; Simmons et al., 1995). In a previous study, echoes were measured from a moving object while emitting (LFM) sound intermittently. The object's range and location in 2D space was estimated by extracting the temporal changes of echo spectra. In this paper, bat-like LPM sound was used to localize a moving object. It was demonstrated that this model could extend the localization of the moving object from echoes using the LPM signal. For this model, the errors in the IRD were less than 4 mm, corresponding to 12 μs, as shown in **Figure 6**, while the errors in the IRD using the LFM signal (Matsuo, 2013) were less than 2 mm, corresponding to 6 μs. The range accuracy was dependent on the signal-to-noise ratio (SNR) and the frequency bandwidth (Burdic, 1968; Menne and Hackbarth, 1986; Simmons et al., 2004; Boonman and Ostwald, 2007). The frequency bandwidths were 23 kHz in this model using the LPM signal, and 70 kHz in the previous model using the LFM signal. It is thought that the difference of errors is due to differences in frequency bandwidths of the emitted sound.

Bat can perceive the object in 3D space by localizing object's distance and direction. Directional information by real bats has previously been investigated by measuring the head-related transfer function (Wotton et al., 1995; Aytekin et al., 2004;

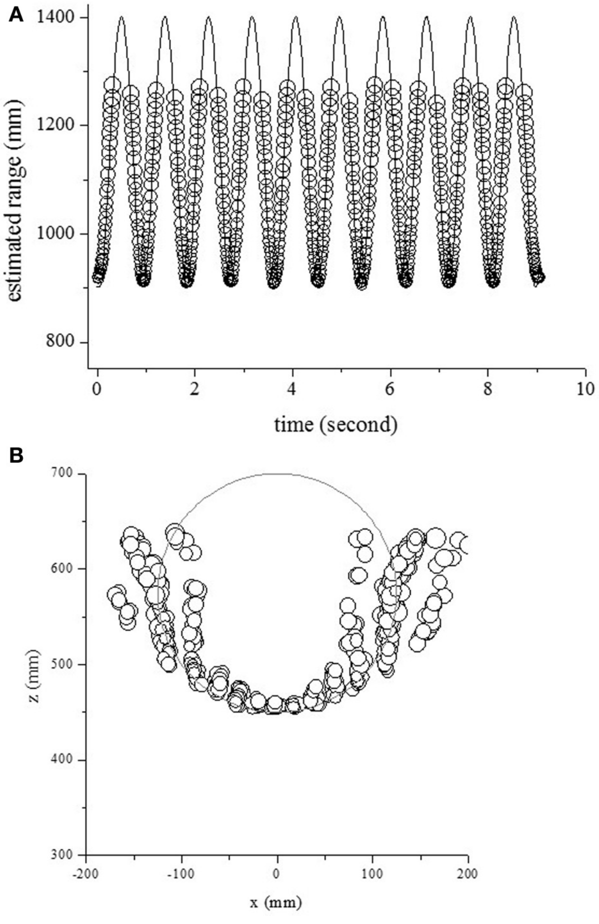

FIGURE 7 | Outputs for one object, moving side to side with a rotation radius of 125 mm. (A) Estimated and actual ranges. **(B)** Estimated and actual positions in 2D space.

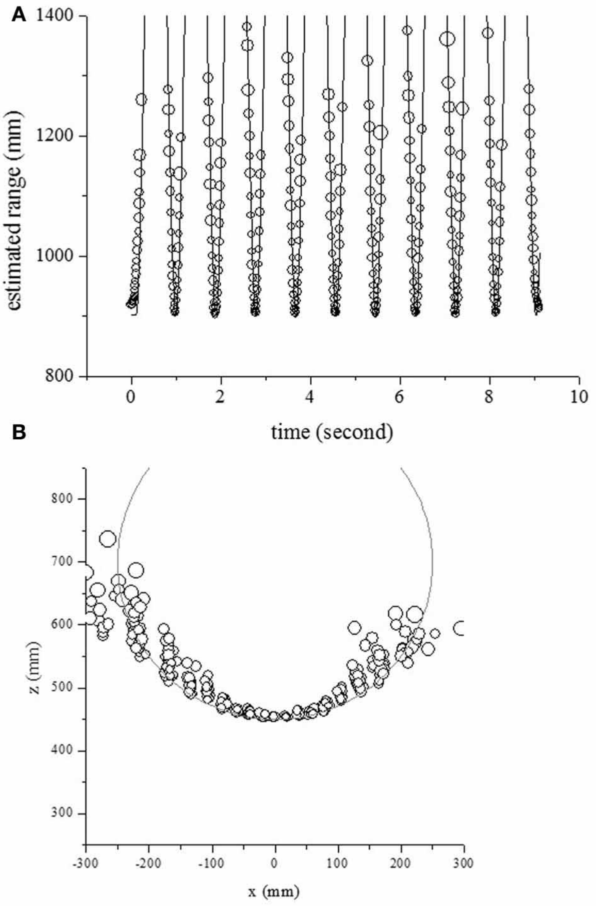

FIGURE 8 | Outputs for one object, moving from side to side with a rotation radius of 250 mm. (A) Estimated and actual ranges. **(B)** Estimated and actual positions in 2D space.

Mey et al., 2008). Therefore, it is necessary to extend to localize objects in 3D space using the IRD in combination with the interaural level difference and the transfer function.

In this paper, only the first harmonics of the LPM signal were used. *Eptesicus fuscus* emits ultrasonic frequency modulation sounds containing two prominent downward-sweeping harmonics. In behavioral studies, echo-delay perception was disrupted by small temporal misalignments of echo harmonics (Bates and Simmons, 2011; Bates et al., 2011). Thus, the temporal cues for two harmonics are important to echolocation in nature. In future work, it will be necessary to extend this model to describe these results using harmonic sound signals.

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Interspecific acoustic recognition in two European bat communities

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Echolocating bats emit echolocation calls for spatial orientation and foraging. These calls are often species-specific and are emitted at high intensity and repetition rate. Therefore, these calls could potentially function in intra- and/or inter-specific bat communication. For example, bats in the field approach playbacks of conspecific feeding buzzes, probably because feeding buzzes indicate an available foraging patch. In captivity, some species of bats recognize and distinguish the echolocation calls of different sympatric species. However, it is still unknown if and how acoustic species-recognition mediates interspecific interactions in the field. Here we aim to understand eavesdropping on bat echolocation calls within and across species boundaries in wild bats. We presented playbacks of conspecific and heterospecific search calls and feeding buzzes to four bat species with different foraging ecologies. The bats were generally more attracted by feeding buzzes than search calls and more by the calls of conspecifics than their heterospecifics. Furthermore, bats showed differential reaction to the calls of the heterospecifics. In particular, *Myotis capaccinii* reacted equally to the feeding buzzes of conspecifics and to ecologically more similar heterospecifics. Our results confirm eavesdropping on feeding buzzes at the intraspecific level in wild bats and provide the first experimental quantification of potential eavesdropping in European bats at the interspecific level. Our data support the hypothesis that bat echolocation calls have a communicative potential that allows interspecific, and potentially intraspecific, eavesdropping in the wild.

Keywords: acoustic communication, eavesdropping, echolocation, feeding buzz, interspecific communication, intraspecific communication, search calls

INTRODUCTION

Many animals are able to recognize members of their own species (conspecifics) and/or to discriminate between members of their own and different species (heterospecific; Gerhardt and Huber, 2002). Some of them react with species-specific behavioral responses depending on the signal or cue of the heterospecific or conspecific (Seyfarth et al., 1980; Manser, 2001; Schuchmann and Siemers, 2010). Recognizing species identity is required in many contexts, for example during mate recognition or predator avoidance. Anurans, for instance, employ acoustic signals intraspecifically for mate recognition (Ryan and Rand, 1993; Gerhardt and Huber, 2002), while vervet monkeys and meerkats distinguish visually between various (heterospecific) predators and react with predator-specific referential alarm calls (terrestrial, ground or aerial predator; Seyfarth et al., 1980; Manser, 2001). Furthermore, the recognition of heterospecifics can be ecologically advantageous if species share similar ecological requirements, e.g., in their diet, habitats or roosting requirements. Potential benefits include the formation of inter-specific foraging associations to improve feeding efficiency (Monkkonen et al., 1996), the eavesdropping on the activity of other individuals to gain information about available food (Übernickel et al., 2012) or shelter (Ruczynski et al., 2007).

Acoustic cues and signals play an important role for species recognition in many animals, including anurans, birds, insects and mammals (e.g., Ryan and Rand, 1993; Bradbury and Vehrencamp, 1998). Beyond species-specific information used for species recognition, acoustic cues and signals can carry several other information about the individual, for example about its morphology (e.g., large body size is related to low call frequency in frogs; Gerhardt and Huber, 2002), its behavior (e.g., foraging or not; Schnitzler and Kalko, 2001; Jones and Siemers, 2011) or certain external situations (e.g., presence of predator; Seyfarth et al., 1980; Manser, 2001). Acoustic stimuli thus provide a variety of information about an individual over some distance to other individuals in the vicinity.

Echolocating bats are particularly interesting for studying acoustic information transfer because they employ two different types of calls: social calls and echolocation calls. Social calls are used for social interactions between individuals (Barclay et al., 1979), while in contrast, ultrasonic echolocation calls are emitted by the bat for its own orientation, navigation and also for foraging in many species (Fenton, 1984; Schnitzler and Kalko, 2001; Neuweiler, 2003; Schnitzler et al., 2003). Echolocation calls are often species-specific, each species having a unique spectro-temporal structure (Barclay, 1999; Siemers et al., 2001; Obrist et al., 2004; Siemers and Schnitzler, 2004). In addition, this spectro-temporal structure is flexibly adapted to the habitat and behavioral task (Schnitzler and Kalko, 2001; Jones and Siemers, 2011). Particularly during foraging, the echolocation call sequence undergoes strong changes in its acoustic spectro-temporal structure (Kalko, 1995; Bradbury and Vehrencamp, 1998; Schnitzler and Kalko, 2001; Siemers, 2006). The search phase is characterized by calls emitted at a regular repetition rate. Upon prey detection, calls become shorter, more broadband and are emitted with an increasing repetition rate (approach phase) until the feeding buzz of up to 200 calls per second just before the capture (Siemers, 2006; **Figure 1**). Since echolocation calls belong to the loudest animal vocalizations (Holderied and von Helversen, 2003; Surlykke and Kalko, 2008), they are also audible to other bats, prey and predators over considerable distances of tens to a hundred or more meters, depending on species (Jones and Siemers, 2011). Echolocation calls are therefore an inevitably distributed source of information for other bats in the vicinity (Jones and Siemers, 2011), which may potentially eavesdrop on this available information about species identity and foraging activity.

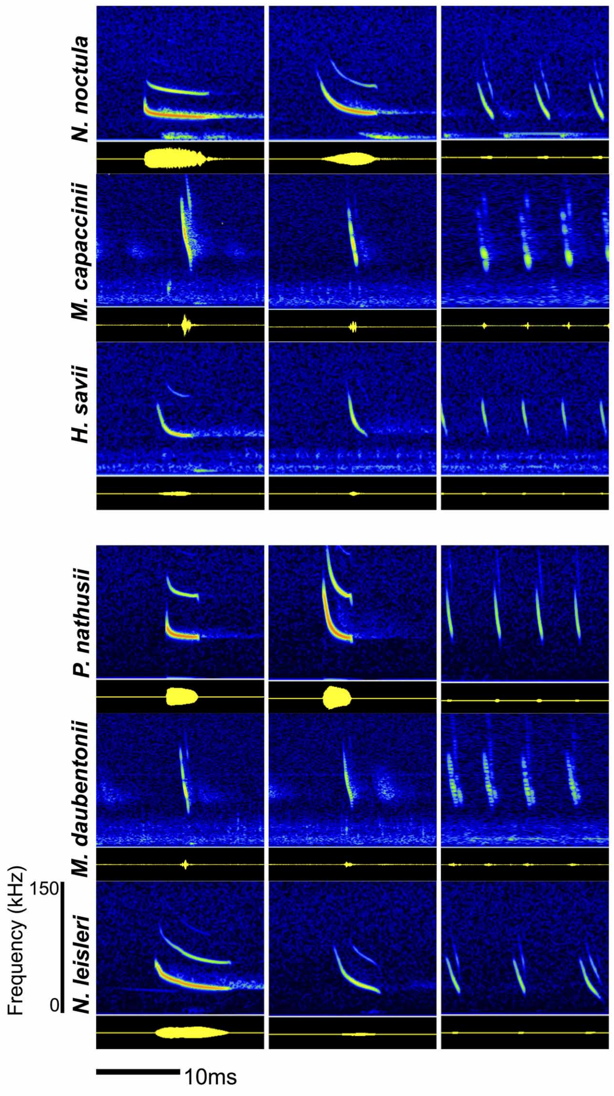

FIGURE 1 | Spectograms (top panel, amplitude color coded) and oscillograms (below, yellow on black) of the echolocation calls of all six recorded bat species. The echolocation calls show species-specific differences and a characteristic change over the course of a feeding event (search phase, approach phase, feeding buzz).

The putative communicative function of echolocation calls has received considerable attention. Within their own species (intraspecifically), some species recognize sex (Kazial and Masters, 2004) and individual identity (Kazial et al., 2008; Yovel et al., 2009) of a conspecific based on echolocation calls and can show sex-specific behavioral responses in the field (Knörnschild et al., 2012). In a foraging context, playback experiments in the field showed that foraging bats approached conspecific feeding buzzes, probably using these signals as an indicator of food availability (Barclay, 1982; Fenton, 2003; Gillam, 2007; Dechmann et al., 2009). In contrast to intraspecific communication, interspecific communication, i.e., the communication between different species, has received little attention, particularly in the field. Two studies showed that bats in captivity are able to differentiate the echolocation calls of conspecifics from those of heterospecifics (Voigt-Heucke et al., 2010) and even differentiate between the echolocation calls of multiple heterospecifics (Schuchmann and Siemers, 2010). To the best of our knowledge, only one study to date has shown interspecific eavesdropping in wild and freely behaving bats, testing a species-rich neotropical bat community (Übernickel et al., 2012). The study tested two trawling bats *Noctilio leporinus* and *N. albiventris* that are sister species with similar echolocation call structure (yet differing in call frequency) and foraging ecologies. Both reacted to the buzz calls and, partially, to the search calls of the other. However, they did not react to any calls of *Saccopteryx bilineata*, a species with different call design and foraging ecology (open-space forager). In contrast, *S. bilineata* did not react to any calls of conspecifics or acoustically or ecologically similar heterospecifics. The results of Übernickel et al. (2012) suggest a relation between reaction strength and acoustic similarity that in turn is related to ecological similarity. Additionally, many other factors, including diet, prey density and distribution, typical foraging behavior, social structure or phylogeny, are likely to contribute, suggesting that reaction strength can vary strongly between different species (Ord and Stamps, 2009), requiring additional studies with different species.

Here, we investigated eavesdropping on the echolocation calls of bats within and across species boundaries. Using a Palearctic community of insectivorous bats, we tested for effects of species identity and call-type on the behavior of four different bat species during foraging. Our general hypothesis postulates that foraging bats evaluate the profitability of foraging patches based on the echo-acoustic information of other bats present in the hunting ground. Since profitable foraging patches can be indicated by foraging-specific calls (feeding buzzes) of species with similar foraging ecology, we predicted that the bats' reactions depend on the call-type and species identity of the calling species. We conducted the study in Germany and Bulgaria, testing in each country one bat species foraging in open-space (i.e., hunting prey in the open air) and one trawling bat species (i.e., taking prey from water surfaces). We presented playbacks of conspecific and different heterospecific species having the same and different foraging ecologies to test for the influence of call-type and foraging ecology. First, we predicted that bats would react more to feeding buzz echolocation calls than to search phase echolocation calls, as only the former indicate a potential food source. Second, we predicted that bats would react more strongly to the echolocation calls (both search calls and feeding buzzes) of conspecifics than to those of heterospecifics. Third, we predicted that bats would react more to the echolocation calls of heterospecific species with a similar feeding ecology than to heterospecifics with a dissimilar feeding ecology.

METHODS

STUDY SITES

We conducted fieldwork in Northern Bulgaria, within ca. 80 km around the village of Tabachka, and in South-East Germany, within ca. 35 km around the city of Munich. We selected a total of 16 sites on open meadows and next to lakes (i.e., areas used by bats as hunting grounds) for call recordings and playback experiments. The sites were covered by low vegetation such as grass, bushes and, in some cases, a few trees. We recorded echolocation calls at four sites (two lakes and two meadows) in Bulgaria and at five sites (four lakes and one meadow) in Germany. All playback experiments were conducted next to lakes at six sites in Bulgaria (including the two lake sites also used for call recordings) and at seven sites in Germany (including the four lake sites also used for call recordings). The distance between recording sites was minimally 23 km and maximally 100 km in Bulgaria and 8–59 km in Germany. For playback sites, distances were 30–123 km in Bulgaria and 7–57 km in Germany. Since none of the bats were marked individually, we cannot ensure that each recording was from a different individual or that each playback was presented to a different individual. However, at all sites we observed at least three and as many as six individuals per night. To avoid presenting individuals their own call recordings, we presented at each playback site only calls that had been recorded at a different site.

CALL RECORDING

We recorded echolocation call sequences of six Vespertillionid bat species for subsequent playbacks (**Figure 1**): two open-space foragers and one trawling bat in each country. In Bulgaria, we recorded calls from *Nyctalus noctula* (open-space), *Hypsugo savii* (open-space) and *Myotis capaccinii* (trawling). In Germany, we recorded *Nyctalus leisleri* (open-space), *Pipistrellus nathusii* (open-space) and *Myotis daubentonii* (trawling; **Figure 1**). Recordings were conducted during 2 weeks of May 2011 in Germany and 2 weeks of June 2011 in Bulgaria during the first 2 h after sunset every evening for one night per recording site. We obtained an average of ca. 100 call sequences per night (and thus per recording site). We recorded the calls of foraging bats onto a ToughBook Laptop (Panasonic, New Jersey, USA) using an ultrasonic microphone (CM16/CMPA, Avisoft, Berlin, Germany) connected to an USG 116 Hm soundcard (Avisoft) and the software RECORDER USGH v. 3.4 (Avisoft) at 250 kHz sampling frequency and 16 bit resolution. The microphone was vertically mounted on a tripod 35 cm above ground level. Recordings were triggered manually when a bat was visually detected and consisted of 3 s before and after triggering.

CALL ANALYSIS AND PLAYBACK PREPARATION

Recorded species were identified during call recording by observation with night vision goggles (ATN PVS7-3, ATN, San Francisco, USA; based on body size and foraging style) and afterwards in Selena software (Animal Physiology, University of Tübingen, Germany; FFT 256, frequency resolution 125 Hz and auto padding) based on call shape and frequency of the spectrogram. We excluded recordings if visual observation and call analysis did not match.

In total, across all six recorded bat species, we obtained a total of 1478 recordings of 6 s duration. For the playbacks, we selected 1-s segments with a good signal-to-noise ratio containing either only search phase calls or only feeding buzzes. The number of selected segments differed between playback species and call types (search calls and feeding buzzes) and mostly ranged from 11–32 segments, except for *H. savii* (2 feeding buzz segments) and *N. noctula* (70 search call segments). We created final playback files of 10 s duration by replicating each segment of 1 s duration. Final playback files were high-pass filtered at 15 kHz and normalized to −3 dB full scale of the playback system. As control stimuli we used ten different pure-tones of 10 s duration ranging from 20 kHz to 65 kHz in 5 kHz steps. Altogether, we had seven different playback types (six test playbacks, i.e., two call types from three species, and one control). All playbacks were conducted at 250 kHz sampling frequency and 16 bit resolution. In Bulgaria, we randomly selected each night five files with search calls and five files with feeding buzzes from each of the three recorded species. Together with the ten control files, this yielded 40 playback files per night. In Germany, we presented 30 playbacks per night by randomly selecting ten files with search calls (out of the 150 files of all three species), ten files with feeding buzzes (out of 150 files of all three species) and ten control stimuli. For each playback session, the selected files (40 in Bulgaria, 30 in Germany) were presented in random order.

PLAYBACK EXPERIMENTS

We conducted playback experiments during May and July in Germany and June in Bulgaria at the foraging sites of four Vespertillionid bat species (one open-space and one trawling species in each country). In Bulgaria, the focal species were *Nyctalus noctula* (open-space forager) and *Myotis capaccinii* (trawling bat). In Germany, the focal species were *Pipistrellus nathusii* (open-space forager) and *Myotis daubentonii* (trawling bat). We presented three types of call recordings to each focal species, namely calls of conspecifics (i.e., belonging to the same species) and of two different heterospecifics. The two heterospecific species differed in their foraging ecology (one open-space or trawling forager). Therefore, each focal species had playbacks from conspecifics, from one heterospecific species with the same and one with a different foraging ecology. Playbacks were presented with an ultrasonic loudspeaker (ScanSpeak; Avisoft) and an USG Player 116 soundcard (Avisoft). The loudspeaker had an overall low-pass characteristics of −12 dB between 10 and

110 kHz and a maximum output level of 100 dB SPL (re. 20 μPa) at 1 m distance and was located 1 m from the lakeshore and 50 cm above the ground pointing toward the lake. We positioned a microphone (details see above) next to the speaker to record the focal bats for posterior identification. Additionally, we used a bat detector (100D Petterson, set to heterodyne) and night vision goggles (ATN PVS7-3, San Francisco, USA) to follow the behavior of the focal bat.

We defined the experimental area as a circle with a radius of ten meters around the loudspeaker and used bushes and trees as reference points for distance estimation. Whenever a bat entered the experimental area, a randomly and blindly chosen playback file was presented. Simultaneously, we recorded the echolocation calls of the focal bat and observed its flight behavior visually. The bats behavior was scored during the experiment in the field. When the bat changed its flight direction toward the loudspeaker, we scored this as a "reaction" to the playback. Otherwise, when the bat did not change its flight direction, this was scored as "no reaction." We only observed one trial where a bat turned away from the loudspeaker, which was excluded. Trials in which a bat was initially flying directly toward the loudspeaker were excluded because a potential reaction could be due to the loudspeaker being a physical obstacle. The species of the focal individual was identified during the experiments visually (night vision goggles; ATN PVS7-3) based on body size and foraging style (i.e., in open space or trawling) and afterwards based on spectrograms of the recorded echolocation calls. Trials were excluded if visual observation and call analysis did not match and if the focal bat did not belong to our focal species (*Nyctalus noctula* and *Myotis capaccinii* in Bulgaria, *Pipistrellus nathusii* and *Myotis daubentonii* in Germany).

DATA ANALYSIS

For each focal species, we counted the number of bat passes showing a "reaction" or "no reaction" to each playback type. Statistical analyses were conducted in R 2.11.0 (R Development Core Team, 2008). Per focal species, we computed two generalized linear models (GLM) for binomial data to test for differences in the number of reacting bat passes. First excluding the reactions to the control stimuli, we calculated GLMs with playback species (three levels) and call type (two levels) as fixed factors to test for species- and call type-specific reactions. For the second GLM, we included the reactions to the control stimuli and used playback type (combining bat species and call type) as a single fixed factor with seven levels to test for further differences between call types. Pair-wise comparisons between factor levels were performed with the *multcomp* package with single-step adjusted *p*-values.

RESULTS

OVERALL REACTION TO CALL PLAYBACKS

We presented four focal bat species the search echolocation calls and feeding buzzes of three con- and heterospecific bat species as well as sinusoidal control stimuli. We counted the number of bat passes showing a reaction to the playback, defined as a change of flight direction toward the loudspeaker. All four focal species reacted in less than 9% of the trials to the control stimuli (**Figure 2**). In contrast, there was a large variation in the response of different focal species to the different playbacks; bats reacted in 4–53% of the trials to search calls and in 10–100% of the trials to feeding buzzes. The minimal adequate GLM for *Nyctalus noctula* (**Figure 2A**) as focal species included both fixed factors playback species and call type, and the interaction between both factors. For the other three focal species (*Myotis capaccinii*, *Pipistrellus nathusii*, *Myotis daubentonii*; **Figures 2B–D**), the minimal adequate GLM included the fixed factors playback species and call type, but not their interaction.

REACTION TO DIFFERENT CALL TYPES (SEARCH CALLS AND FEEDING BUZZES)

Call type was included in the minimal adequate GLM of all four focal species; thus, call type influenced the number of reacting bat passes. For each focal species, we conducted *post-hoc* multiple comparisons between the overall reactions to different call types (including all playback species). Although call type was included in the minimal adequate GLM of *N. noctula*, its overall reaction did not differ between search and buzz calls (adj. $p = 0.446$, *post-hoc* tests with manual contrasts to account for factor interaction). The three other focal species (*M. cappaccinii*, *P. nathusii*, *M. daubentonii*) reacted more strongly to buzz calls than to search calls (Tukey *post-hoc* tests, adj. $p = 0.034$–<0.001).

REACTION TO DIFFERENT PLAYBACK SPECIES

Playback species was included in the minimal adequate GLM of all four focal species; thus, the playback species influenced the number of reacting bat passes. For each focal species, we conducted *post-hoc* multiple comparisons between the overall reaction to different playback species (including search calls and feeding buzzes). The overall reaction of *N. noctula* did not differ between playback species (adj. $p = 0.821$–1.000), despite playback species being included in the minimal adequate model. The three other focal species (*M. cappaccinii*, *P. nathusii*, *M. daubentonii*) reacted overall stronger to conspecific playbacks than to heterospecific playbacks (Tukey *post-hoc* tests, adj. $p = 0.03344$–<0.001). In contrast, their reaction did not differ between the heterospecific species (Tukey *post-hoc* tests, adj. $p = 0.2368$–0.8433).

INTRA- AND INTERSPECIFIC REACTION TO SPECIFIC PLAYBACK TYPES

Nyctalus noctula (**Figure 2A**) reacted strongly to conspecific feeding buzzes (100%, $N = 7$; **Figure 2A**). However, it reacted rarely to conspecific search calls or to any heterospecific call type (13–25%) and it did not react at all to the control (0%). Nevertheless, none of these differences were significant (**Figure 2A**, Tukey *post-hoc* test, adj. $p = 0.983$–1). Due to this pattern, the minimal adequate model included both factors and their interaction, while the *post-hoc* tests showed that *N. noctula* does not generally react differently to any playback species or call type. *Myotis capaccinii* (**Figure 2B**) also reacted most strongly to conspecific feeding buzzes (88%, $N = 16$), and reacted equally strongly to buzzes of the heterospecific *H. savii* (45%, $N = 11$; Tukey *post-hoc* test, adj. $p = 0.287$). Both differed from the reaction to the control stimuli (7%, $N = 55$; adj. $p = 0.0453$–<0.001). Furthermore, the reaction of *M. capaccinii* to conspecific feeding buzzes was also stronger compared to conspecific search calls (28%, $N = 25$; adj. $p = 0.016$), which did not differ from

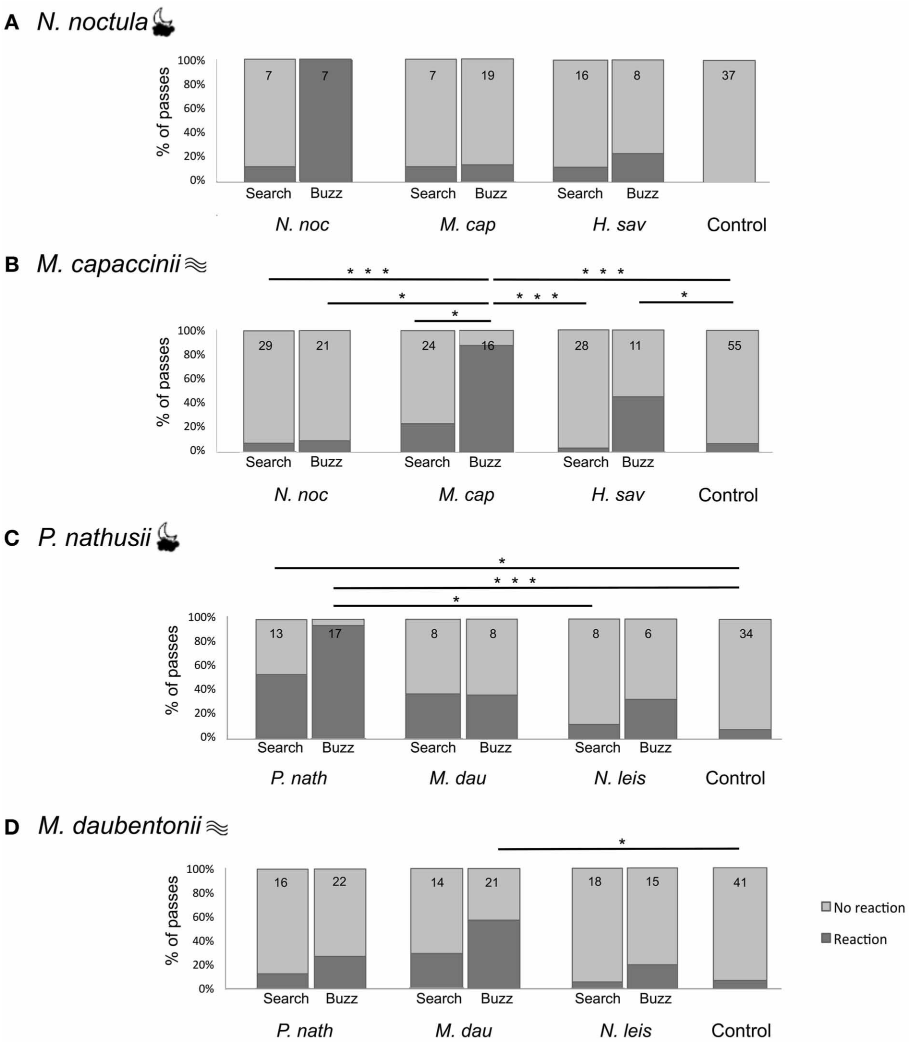

FIGURE 2 | Percentage of bat passes showing a reaction (dark gray) and no reaction (light gray) in response to the playback of echolocation calls of different species. (A) Reactions of *N. noctula* and **(B)** *M. capaccinii* in Bulgaria. **(C)** Reactions of *M. daubentonii* and **(D)** *P. nathusii* in Germany. The focal species is indicated at the top left of each panel, with symbols indicating its foraging style [cloud (☁) = open-space forager; waves (≋) = trawling forager]. The playback species is indicated below the bar plots. The small numbers in each bar are the number of recorded passes. Lines and asterisks indicate significant differences between playback types (*$0.05 > p \geq 0.01$; **$0.01 > p \geq 0.001$; ***$p < 0.001$) based on multiple comparisons with Tukey contrasts between all seven different playback types.

the control stimuli (7%, $N = 55$; adj. $p = 0.216$). Likewise, *M. capaccinii* did not react to any calls of *N. noctula* (7%, $N = 29$; 9%, $N = 21$), which did not differ from the reaction to the control stimuli (adj. $p = 1.000$), but differed from the reaction to conspecific feeding buzzes (adj. $p = 0.001$–<0.001). *Pipistrellus nathusii* (**Figure 2C**) reacted most strongly to conspecific feeding buzzes (94%, $N = 17$) and equally strongly to conspecific search calls (54%, $N = 13$; adj. $p = 0.268$). Both reactions differed from the reactions to the control stimuli (9%, $N = 34$, adj. $p = 0.0375$–<0.001). The reactions to the calls of *M. daubentonii* (38%, $N = 8$) and the feeding buzzes of *N. leisleri* (33%, $N = 6$) were intermediate between the reactions to conspecific calls and the control, but not significantly different to either of them due to the small sample size. *Myotis daubentonii* (**Figure 2D**) reacted, like the other species, most strongly to conspecific feeding buzzes (55%, $N = 22$), which differed significantly from the control stimuli (7%, $N = 41$, adj. $p = 0.00397$). The remaining reactions to conspecific search calls and heterospecific calls were intermediate between the conspecific feeding buzzes and the control stimuli, without any significant differences.

DISCUSSION

Generally, focal species reacted more strongly to playbacks of echolocation calls than to playbacks of control stimuli, more strongly to feeding buzzes than to the search calls, and more strongly to the calls of conspecifics than to those of heterospecifics. The detailed reaction patterns differed between focal species, with some species potentially showing signs of heterospecific eavesdropping.

REACTION TO DIFFERENT CALL TYPES (SEARCH CALLS AND FEEDING BUZZES)

Bats only emit feeding buzzes just before attacking prey (Kalko, 1995; Bradbury and Vehrencamp, 1998; Schnitzler and Kalko, 2001; Siemers, 2006). Consequently, feeding buzzes provide information on prey availability and the profitability of a foraging patch to bats in the vicinity. The use of this cue potentially increases the chance of the eavesdropper to find food (Barclay, 1982; Fenton, 2003; Gillam, 2007; Dechmann et al., 2009). This information is not present in search phase calls. We thus predicted a call type-specific reaction, which was supported in three species (*M. capaccinii*, *P. nathusii* and *M. daubentonii*) by an overall stronger attraction to feeding buzzes compared to search calls.

The detailed analysis per playback type supported this finding on the intraspecific level for one species. *M. capaccinii* reacted significantly stronger to feeding buzzes than to search phase calls. The data of the other three species (*N. noctula, P. nathusii* and *M. daubentonii*) also showed stronger reactions to feeding buzzes than to search calls. However, these differences were not significant, but still showed different patterns between species. *N. noctula* and *P. nathusii* reacted almost always to conspecific feeding buzzes, but *M. daubentonii* only to about half of the playbacks. *N. noctula* and *M. daubentonii* reacted rarely to search calls while *P. nathusii* reacted to about half of the playbacks. These results suggest an attraction of bats to the feeding buzzes of other individuals from the same species, supporting previews findings about bats using conspecific buzzes as an indicator for food availability (Barclay, 1982; Fenton, 2003; Gillam, 2007; Dechmann et al., 2009).

On an interspecific level, we also found evidence of eavesdropping on the feeding buzzes of heterospecifics in one species. *M. capaccinii* reacted equally to the feeding buzzes of conspecifics and those of *H. savii.* Both reactions differed significantly from the control and other playbacks. The other three focal species reacted sometimes more to heterospecific feeding buzzes compared to search calls, though never strongly and significantly.

REACTION TO DIFFERENT PLAYBACK SPECIES

Three species (*M. capaccinii*, *P. nathusii* and *M. daubentonii*) reacted more strongly to conspecific than to heterospecific echolocation calls, supporting our prediction of species-specific reactions. The overall reaction to different heterospecifics, however, did not differ for any of the focal species. Likewise, none of the focal species reacted overall similarly to calls of conspecifics and of heterospecific with similar foraging ecology. Our prediction of foraging ecology-dependent reaction was thus not confirmed for all calls of a species.

INTRA- AND INTERSPECIFIC REACTION TO SPECIFIC PLAYBACK TYPES

We found no general and unequivocal evidence for interspecific eavesdropping. While the lack of reaction to ecologically dissimilar species is in line with our prediction, it is not supported by a matching reaction to ecologically similar heterospecifics. For example, the open-space foragers *N. noctula* and *P. nathusii* reacted only little to the playbacks of the heterospecific trawling bats *M. capaccinii* and *M. daubentonii*. However, both species also did not react to playbacks of heterospecific open-space foragers (*H. savii* and *N. leisleri*), indicating that they might not react at all to any heterospecific. Only *M. capaccinii* showed a clear attraction to heterospecific echolocation calls, namely to the feeding buzzes of *H. savii*, despite these species' overall difference in foraging habitats (trawling and open-space foragers, respectively). However, *M. capaccinii* does not only forage above water surfaces but also in open airspace (Dietz et al., 2009), which is the typical hunting habitat of *H. savii* (Dietz et al., 2009). *M. capacinii* might thus have reacted to the feeding buzzes of a heterospecific with partially overlapping foraging ecology, which indicated a profitable aerial foraging spot. In contrast to Übernickel et al. (2012), this raises the possibility of interspecific eavesdropping across foraging guilds. However, another possibility is that the reaction of *M. capaccinii* is due to the acoustic similarity of the echolocation calls, particularly the feeding buzzes, of *M. capaccinii* and *H. savii* (Balcombe and Fenton, 1988; Übernickel et al., 2012). To test this, it would be interesting to see if *M. capaccinii* reacts even more strongly to the trawling bat *M. daubentonii*, which is also acoustically similar, yet overlaps more in foraging ecology than *H. savii.*

EAVESDROPPING IN BAT COMMUNITIES

The occurrence and potential benefits of eavesdropping will be determined by multiple factors, including a species' foraging style and social system, the species similarity with sympatric species, and the conditions of its habitat, such as prey availability (Dechmann et al., 2009; Jones and Siemers, 2011; Übernickel et al., 2012). Eavesdropping enables bats to extend their perception beyond the limited detection range of their own echolocation system and to gain information about prey availability, profitable foraging patches, roosting sites and the behavior of other individuals (e.g., Barclay, 1982; Gillam, 2007; Ruczynski et al., 2007; Dechmann et al., 2009). On the other hand, eavesdropping might constitute a cost for the bat that is being eavesdropped upon, potentially leading to competition between interacting individuals. The costs and benefits in a foraging context are determined by the availability of resources. For instance, females of the bat *Noctilio albiventris* eavesdrop on conspecific calls to detect large, but patchily distributed insect swarms (Dechmann et al., 2009). Since the swarms are so large that they cannot be monopolized and exploited by a single individual, eavesdropping does not incur any costs and has the benefit of an increased detection range of the swarms. In contrast, for bats that feed on more distributed prey items, eavesdropping will be costly for the bat that is eavesdropped upon, particularly in times of scarcity and high energy demand, and potentially lead to resource defence (Barlow and Jones, 1997).

We predicted that bat species would react more strongly to ecologically similar species, i.e., species with similar foraging habitats, foraging styles and prey spectra. Such ecological similarity is also reflected in morphological and echo-acoustic similarity between species, which influences their maneuverability, flight speed, bite force, hunting style and prey perception ability (Balcombe and Fenton, 1988; Swartz et al., 2003; Siemers and Schnitzler, 2004). As a consequence, ecologically dissimilar species regularly differ in additional aspects such as their body size, flight speed, foraging style and call shape and frequency, which are all potential explanations for low reaction to playbacks of ecologically dissimilar species. For example, *N. noctula* forages high up in the air and might thus not be attracted to the calls of the smaller species hunting closer to water bodies and background structures (*M. capaccinii*, *H. savii*). The low proportion of reactions from medium (*M. capaccinii*, *M. daubentonii*) and small sized bat species (*P. nathusii*) to the playbacks of the bigger bats (*N. noctula* and *N. leisleri*) can be due to marked body size differences, which again correlate with differences in maneuverability, flight speed, bite force and prey spectrum. Balcombe and Fenton (1988) suggested that bats react most to calls that are acoustically similar to their own calls, based on the idea that acoustic similarity reflects ecological similarity. This idea is confirmed by the attraction of *M. cappaccinii* to the feeding buzzes of *H. savii*, which have feeding buzz calls that are similar both in frequency and repetition rate (**Figure 1**). However, we did not find a reaction to the playback of heterospecific echolocation calls in other species pairs with a similar amount of acoustic similarity in the feeding buzzes (e.g., *N. noctula* and *M. cappaccinii* or *M. daubentonii* and *N. leisleri*). The species-specificity of echolocation calls is more pronounced for search calls than for feeding buzzes, which allows the possibility that bats were not able to tell species identity based on feeding buzzes alone. Further studies separating the effects of ecological and acoustic similarity would thus be interesting.

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Groups of bats improve sonar efficiency through mutual suppression of pulse emissions

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How bats adapt their sonar behavior to accommodate the noisiness of a crowded day roost is a mystery. Some bats change their pulse acoustics to enhance the distinction between theirs and another bat's echoes, but additional mechanisms are needed to explain the bat sonar system's exceptional resilience to jamming by conspecifics. Variable pulse repetition rate strategies offer one potential solution to this dynamic problem, but precisely how changes in pulse rate could improve sonar performance in social settings is unclear. Here we show that bats decrease their emission rates as population density increases, following a pattern that reflects a cumulative mutual suppression of each other's pulse emissions. Playback of artificially-generated echolocation pulses similarly slowed emission rates, demonstrating that suppression was mediated by hearing the pulses of other bats. Slower emission rates did not support an antiphonal emission strategy but did reduce the relative proportion of emitted pulses that overlapped with another bat's emissions, reducing the relative rate of mutual interference. The prevalence of acoustic interferences occurring amongst bats was empirically determined to be a linear function of population density and mean emission rates. Consequently as group size increased, small reductions in emission rates spread across the group partially mitigated the increase in interference rate. Drawing on lessons learned from communications networking theory we show how modest decreases in pulse emission rates can significantly increase the net information throughput of the shared acoustic space, thereby improving sonar efficiency for all individuals in a group. We propose that an automated acoustic suppression of pulse emissions triggered by bats hearing each other's emissions dynamically optimizes sonar efficiency for the entire group.

Keywords: echolocation, sonar, communication, vocalization, noise, bat, acoustic masking, ethernet

INTRODUCTION

Environmental noise degrades the transmission of all animal communication sounds (Ryan and Brenowitz, 1985; Ryan, 1986; Brumm and Slabbekoom, 2005; Jones, 2008), but echolocation by bats is particularly sensitive because bats need to clearly hear their own faint echoes to hunt and navigate (Neuweiler, 2000; Schnitzler and Kalko, 2001). For bats the most significant source of degrading acoustic interference is the echolocation pulses of other bats, and researchers have long puzzled over how echolocating bats avoid interfering with one other's sonar while flying in dense swarms or within noisy crowded day roosts (Griffin, 1958). In order to echolocate efficiently bats maintain precise control over the acoustic and temporal properties of their echolocation pulses (Neuweiler, 2000; Schnitzler and Kalko, 2001; Schnitzler et al., 2003; Smotherman, 2007), and in some cases this includes adaptations for echolocating in the presence of other bats. Some bats display a jamming avoidance behavior in which they change their outgoing call pitch in order to minimize overlap in bandwidth (Ratcliffe et al., 2004; Ulanovsky et al., 2004; Gillam et al., 2007; Bates et al., 2008; Tressler and Smotherman, 2009; Necknig and Zahn, 2011), and some increase pulse amplitude in the presence of background noise (Simmons et al., 1978; Tressler and Smotherman, 2009; Tressler et al., 2011). These relatively minor changes in pulse acoustics have so far only been documented in pairs of bats and are considered unlikely to be effective for much larger groups of bats because their vocal parameters are tightly constrained by highly specialized laryngeal and respiratory mechanics (Metzner and Schuller, 2007), a finely tuned auditory system (Popper and Fay, 1995), and would force bats to alter pulse characteristics away from optimal parameters for foraging and navigation (Schnitzler and Kalko, 2001). In light of these limitations other more comprehensive answers are needed to explain how bats echolocate in groups.

An alternative to jamming avoidance behavior is for bats to modulate the timing of their pulse emissions to minimize temporal overlap with another bat's echolocation pulses. Many animals acutely regulate the timing of their vocalizations to minimize acoustic interference, including frogs (Loftus-Hills, 1974; Zelick and Narins, 1985; Moore et al., 1989), birds (Ficken and Ficken, 1974; Knapton, 1987; Brumm, 2006; Planque and Slabbekoorn, 2008), and primates (Egnor et al., 2007). Although echolocation serves a different function than these other forms of vocal communication it is possible that bats echolocating in small groups utilize some sort of antiphonal emission strategy to promote emitting pulses out of phase with one another as a means for minimizing temporal overlap with conspecifics, and there is evidence from the field that bats modify emission timing in the presence of other bats (Obrist, 1995). We recently investigated whether solitary free-tailed bats shifted the timing of their pulse emissions in response to artificial acoustic stimuli mimicking the emissions of nearby conspecifics (Jarvis et al., 2010). Bats were found to postpone pulse emissions by roughly 80 ms every time they heard an artificial pulse. We hypothesized that under natural conditions this behavior could promote antiphonal emissions and might also lead to slower pulse emissions in social settings. The potential benefits of antiphonal calling are straightforward, but how this might be managed for even modest sized groups of 5–10 bats is difficult to imagine. Furthermore, if the acoustic suppression of pulse emissions did result in slower pulse emissions for the entire group it was unclear how this could be managed without significantly degrading sonar performance. Here we directly test whether bats emit pulses more slowly in groups than when alone, and if so whether this behavior supports an antiphonal calling strategy that helps bats avoid interfering with one another.

Free-tailed bats are often found hunting insects alone or in small groups of two or three individuals at a particular foraging site, but they also migrate together in dense swarms of tens to thousands of bats and establish day roosts housing hundreds to millions of individuals. In these large densely populated roosts and particularly during emergence from the caves (Gillam et al., 2010) it seems unlikely that any combination of changes in the acoustics or timing could effectively mitigate the interfering effects of the surrounding din. How exactly do free-tailed bats respond to the background noise generated by many continuously echolocating neighboring bats? We predicted that in high population densities free-tailed bats would abandon any attempts to coordinate their temporal emission patterns in favor of emitting pulses more frequently to compensate for information lost due to mutual interference. This was tested using artificial acoustic stimuli simulating the acoustic impacts of progressively larger group sizes.

The results described here indicate that pairs and small groups of 3–10 bats do indeed suppress each other's emissions, but not in support of an antiphonal emission strategy. Instead we find that free-tailed bats appear to adjust pulse emission rates to maximize pulse efficiency, which requires balancing the need to extract more information from the environment by emitting more pulses while minimizing the relative proportion of those pulses producing ambiguous echoes. Drawing upon lessons learned from the study of how information flows through communications networks (Shannon, 1948; Abramson, 1970; Tanenbaum, 2003) we will show how a population density-dependent suppression of pulse emission rates can theoretically improve sonar efficiency in noisy crowded social conditions by improving information throughput of the shared acoustic space. However, when population density grows to the point where the likelihood of an overlap occurring becomes greater than the likelihood of producing an unambiguous echo, the bats switch to emitting pulses at higher rates than when alone. This second strategy may increase the probability of sporadically producing unambiguous echoes or may exploit auditory integration mechanisms that build the auditory scene from bits and pieces of many incomplete or distorted echoes (Moss and Surlykke, 2001; Moss et al., 2006). Free-tailed bats thus adapt their sonar pulse emission rates to differing social contexts via two discreet behavioral responses, slowing pulse emissions to aid coordination in small groups and speeding pulse emissions in dense noisy conditions.

MATERIALS AND METHODS

ANIMALS

These experiments utilized captive wild-caught male and female Mexican free-tailed bats (*Tadarida brasiliensis Mexicana*). All husbandry and experimental procedures were in accordance with National Institutes of Health guidelines for experiments involving vertebrate animals and were approved by the local Institutional Animal Care and Use Committee (TAMU animal use protocol #2007–254). The bats were kept in an artificial habitat with a reversed light cycle and temperature varying daily and seasonally to simulate natural condition. Animals were provided a diet of mealworms supplemented with vitamins and minerals and water was available *ad-libitum*.

ACOUSTIC RECORDING AND PLAYBACK APPARATUS

For all experiments bats were placed in a 10 × 10 × 20 cm plastic-coated 1/4″ steel mesh cage which was then positioned in the center of a 6 × 3 × 1.5 meter room lined with sound-absorbing four-inch acoustic foam. The room was kept dark and the temperature was maintained around 30° Celsius during recording sessions. Experiments were performed during the first 4 h after the animals' subjection sunset (12:00–16:00 Zeitgeber time). Vocalizations were recorded with a Brüel & Kjær type 4939 free-field 1/4″ microphone (Brüel & Kjær, Nærum, Denmark) positioned 10 cm from edge of the cage and oriented toward the center. The bats' vocalizations were digitized and analyzed using the hardware and software package Datapac 2K2 (RUN Technologies, Mission Viejo, CA). Pulses were automatically discriminated from background by applying a fixed threshold to the waveform envelope. To account for potential under-sampling due to temporal overlap between simultaneously uttered pulses we visually inspected spectrograms and made corrections by hand as necessary.

Acoustic stimuli were produced with a Vifa 1″ Tweeter (model # BC25SC55-04) powered by a Sony amplifier (model # STR-DE598) which provided a maximum output of ≈80 ± 6 dBs from 15 to 50 kHz. The speaker was mounted 10 cm from and oriented toward the bat's cage. The microphone and loudspeaker were separated by a piece of sound-absorbing foam adjusted daily to minimize the recorded amplitude of the stimulus relative to the amplitude of the bats' pulse emissions. The stimuli for these experiments were digitally created with the TDT OpenEX software v5.4 (Tucker-Davis Technologies, Alachua, FL), and the analog signal was generated by TDT System III RX6 hardware (Tucker-Davis Technologies, Alachua, FL).

EXPERIMENT 1: DO ECHOLOCATING BATS SUPPRESS THE PULSE EMISSIONS OF THEIR CONSPECIFICS?

Individuals or groups of 2–10 naïve bats were recorded echolocating while crawling around the steel mesh cage positioned in the center of the anechoic recording chamber. The mean pulse emission rate per bat was calculated as the total number of pulses detected divided by total duration of the recording and the number of individuals placed in the cage. To determine whether an artificial stimulus altered pulse emission rates solitary bats were presented with artificial downward frequency-modulated sounds mimicking the echolocation pulses of free-tailed bats(Jarvis et al., 2010) at a repetition rate of five pulses per second, similar to naturally behaving bats.

EXPERIMENT 2: DOES MUTUAL SUPPRESSION LEAD TO REDUCED INCIDENCES OF OVERLAPPING PULSE EMISSIONS?

To determine whether the prevalence of overlapping pulse emissions occurred less frequently than predicted based on random chance we compared the real rate of overlaps occurring between two bats with Monte Carlo simulations of pairs of bats echolocating together. Real rate of overlaps was measured by manually counting the numbers of overlapping pulses occurring in randomly selected 10-s time epochs collected from 141 separate recordings of pairs of bats. We defined an overlap event as any instance when a second pulse appeared in the spectrogram within 10 ms of the onset of a previous pulse. Pulse durations typically varied from 4 to 8 ms and the returning echoes perpetuated in the chamber for at least 5 ms beyond the end of the first pulse. Under natural conditions the period over which another bat's emissions might overlap with the time course of a returning echo likely extends well beyond the 10 ms limit used here, but we will show that the results presented here are easily adapted to reflect more liberal time windows to accommodate different species or habitats. Monte Carlo simulations of pairs of bats echolocating together were generated using 100 randomly chosen 10-s epochs of acoustic recordings from isolated naïve bats, which gave 4950 discreet simulated cross-pairings. For each real and simulated epoch we measured the mean pulse rate and number of overlaps occurring within the 10 s epoch and from this determined the probability distribution of overlaps as a function of mean pulse rate. It was not possible to discriminate between the echolocation pulses of real bats recorded in pairs reliably enough to measure each individual bat's pulse emission rate. Finally, based on the assumption that simultaneous emissions always have the potential to create ambiguities in the perception and interpretations of succeeding echoes, we define *pulse efficiency* as the mean proportion of emitted pulses that did not overlap with another bat's emissions and therefore likely produced unambiguous echoes. Pulse efficiency was calculated by subtracting the expected interference rate (overlaps per second) from mean pulse emission rate.

EXPERIMENT 3: HOW DO BATS RESPOND TO THE PRESENCE OF CONTINUOUS NOISE?

To measure the behavioral response to continuous noise we measured the effects of a prolonged broadband noise stimulus on pulse emission rates. Preliminary experiments indicated that the bat's pulse emission rates typically declined over the 20–30 min time-course of an experimental session regardless of stimulus type, preventing us from directly comparing extended recordings of bats echolocating in noisy vs. silent conditions. Furthermore, individual call rates varied significantly across days, making it difficult to achieve statistically significant results when comparing stimulus conditions across days. Therefore, to control for daily fluctuations and the systematic short-term decline in emission rates seen over the course of initial recordings, bats were exposed to a time-varying noise stimulus composed of 10-s blocks of white noise alternated with 10-s of silence. An iterative process led us to compromise upon 10-s stimulus epochs because this timeframe was at least two orders of magnitude longer than their typical inter-pulse intervals and yet short enough that there was no detectable time-dependent reduction in mean call rate within each epoch. Preliminary trials with longer epochs of up to 2 min produced qualitatively similar results. This stimulus pattern will hereafter be referred to as the "continuous" noise stimulus to distinguish it from the periodic noise-burst stimuli used in Experiment 1 and our previous study (Jarvis et al., 2010). For each trial the total number of echolocation pulses uttered was pooled from all experimental (stimulus ON) and silent (stimulus OFF) conditions and both mean emission rate and relative proportion of pulse's uttered was calculated for the noise On and noise Off conditions. To test if the bats responded differently to noise when alone vs. in the presence of other bats, experiments were conducted in two separate sessions. In the first session, recordings were carried out with groups of either four or eight bats placed in the same cage and collectively exposed to the continuous noise stimulus. Following this, each bat from the group was isolated and recorded individually while being exposed to the same series of stimuli. Data were normalized as the total percentages of pulses occurring in silence vs. noise.

EXPERIMENT 4: AT WHAT TEMPORAL RATIO OF NOISE TO SILENCE DOES THE NOISE PROMOTE FASTER EMISSIONS?

Six solitary bats were exposed to stimuli of varying duty cycles constructed by alternating a 10 ms burst of broadband noise with silent intervals of variable length. For example 10 ms of noise alternating with a 90 ms silent period gave a 10% duty cycle; other silent intervals were 40 ms (20% duty cycle), 10 ms (50% duty cycle), 3.3 ms (a 75% duty cycle), and 1.1 ms (a 90% duty cycle). Each bat was recorded for six 12-min exposures to each duty cycle. During these recording sessions, the stimulus was switched on and off every 2 min, allowing the stimulus blocks to be interspersed with blocks of silence. The total number of echolocation pulses uttered was pooled from all 6 min of experimental (stimulus ON) and silent (stimulus OFF) conditions during each session. Different duty-cycle stimuli were presented in pseudorandom order to balance for time and order effects.

STATISTICAL ANALYSIS

All result are expressed as mean ± standard deviation. Statistical analyses were performed with Sigma Stat v.9.0 (Systat Software, San Jose, CA). For Experiment 1 non-parametric *t*-tests and a Kruskal-Wallis One-Way analysis of variance on ranks was used to investigate the effect of population density on average pulse rate, and a least-squares method was used to determine the best curve fit. For Experiments 2 and 3, a Two-Way analysis of variance test was performed to investigate the effects of noise and social conditions on pulse emission rates. For Experiment 4, a Two-Way analysis of variance using Holm-Sidak multiple comparison tests was performed to determine the effects of stimulus condition and duty cycle on emission rates.

RESULTS

EXPERIMENT 1: DO ECHOLOCATING BATS SUPPRESS THE PULSE EMISSIONS OF THEIR CONSPECIFICS

There was a significant reduction in mean emission rates when bats were echolocating in pairs vs. when they were alone (**Figure 1A**, Mann-Whitney test. $T = 930$, $n_1 = 28$, $n_2 = 57$, $p = 0.011$). There was also a significant reduction in pulse emission rates when bats echolocated while the loudspeaker played back an artificial stimulus mimicking the presence another free-tailed bat (**Figure 1A**; $t = 2.045$, $df = 35$, $p = 0.048$). **Figure 1B** plots the significant effects of increasing bat density on the mean pulse emission rates ($H = 90.199$, $df = 7$, $P = 0.001$). The negative relationship between bat density and mean pulse emission rate was best fit by an inverse first order non-linear regression [$F_{(1,\ 6)} = 93.97$, $p < 0.0001$, $R^2 = 0.94$] that decayed toward an asymptote equivalent to ~20% of the mean emission rates for naïve solitary bats, or roughly 1 pulse per second.

EXPERIMENT 2: DOES MUTUAL SUPPRESSION LEAD TO REDUCED INCIDENCES OF OVERLAPPING PULSE EMISSIONS?

Comparing real groups of bats to Monte Carlo simulated groups of bats revealed that the bats' echolocation behavior was strongly altered by social context. Real pairs of bats emitted significantly

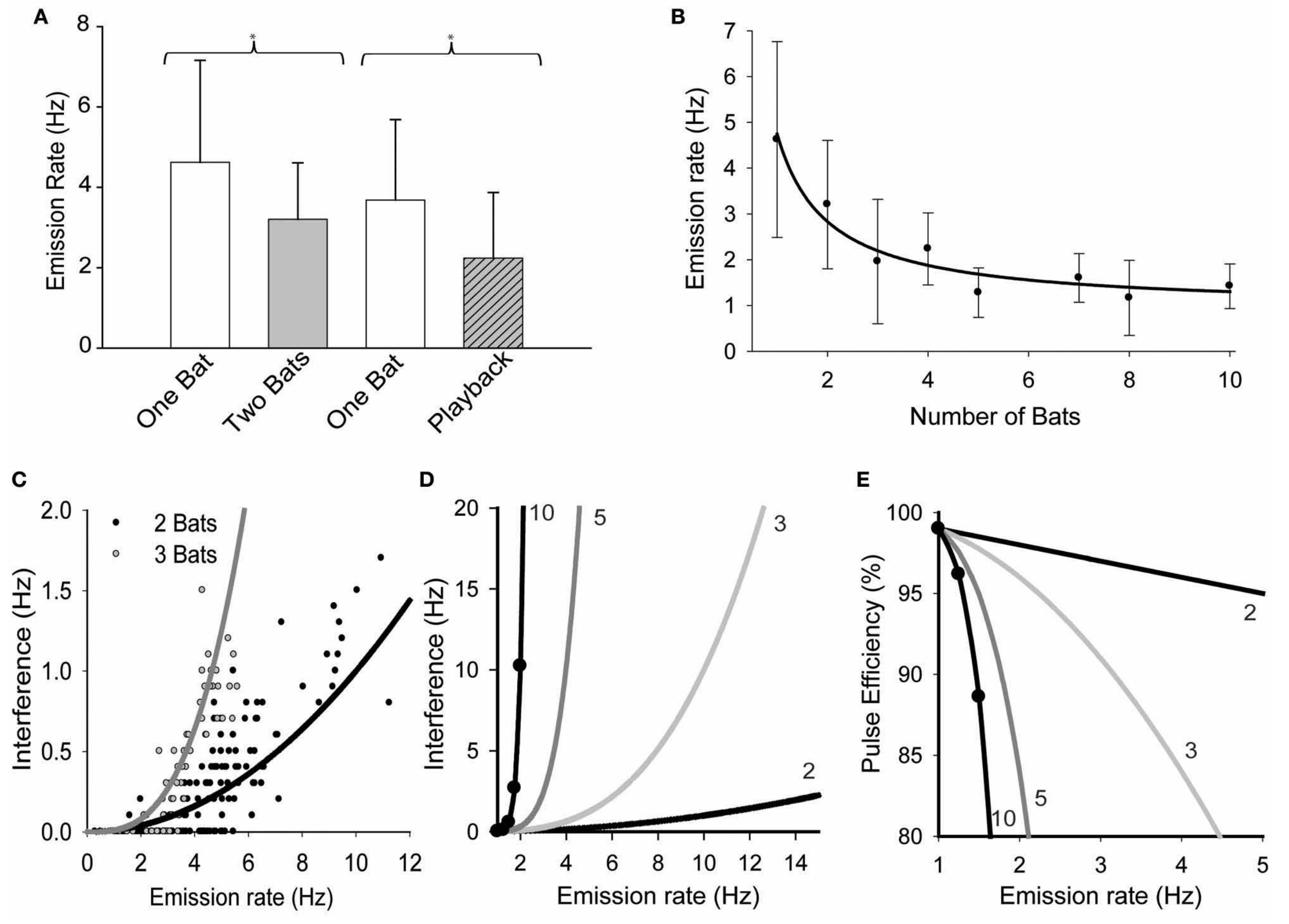

FIGURE 1 | The effect of group size on pulse emission rates. **(A)** Bats' mean pulse emission rates recorded alone vs. when echolocating in pairs, and then again for alone vs. while echolocating with a speaker simulating the presence of another bat echolocating (playback). **(B)** Average emission rates per bat plotted vs. the total number of bats in the group. Pairwise multiple comparisons indicated that mean pulse emission rates for groups of 3 or more bats were significantly lower than solitary bat emission rates ($Q = 5.033$, $p < 0.05$). Data were fit with a first order linear regression (solidline, $y = 0.92 + 3.82/x$). **(C)** Plot of mean pulse rates vs. the rate at which overlaps occurred (interferences) for pairs ($n = 141$) and triads ($n = 56$) of bats. Both sets of data were well fit by the same simple power function of the form $y = r\tau^n$, where $r =$ mean emission rate (Hz), $\tau =$ overlap window duration (ms) and $n =$ number of bats. [$r^2 = 0.71$, $F_{(1,\ 140)} = 344.9$, $P < 0.001$]. Extending the functions derived from **(C,D)** illustrates the expected effect of pulse emission rates on mutual interference rates for groups of 2, 3, 5, and 10 bats. **(E)** These functions were then used to predict the effect of pulse emission rates on the proportion of pulses expected to generate unambiguous echoes, or $y = 1 - r\tau^n$ (pulse efficiency) for different group sizes.

fewer pulses per second than simulated pairs (4.6 ± 2.1 Hz, $n = 141$ vs. 6.0 ± 3.1 Hz, $n = 4950$, respectively, $P < 0.0001$) and also emitted overlapping pulses significantly less frequently than simulated pairs (0.29 ± 0.37 Hz vs. 0.38 ± 0.38 Hz, $P < 0.0001$). Analyses also revealed that real pairs produced a higher percentage of epochs with no instances of overlap (48%) than simulated pairs (15%) suggesting that real pairs of bats were successfully avoiding overlaps better than expected by chance alone. However, this observation could simply be a product of reduced pulse emission rates, since the number of overlaps per second was strongly correlated with mean pulse emission rates per epoch for both real and simulated bats ($R = 0.83$, $p < 0.0001$ and $R = 0.75$, $p < 0.0001$, respectively). To investigate this we examined whether the reduction in interferences was independent of pulse emission rates. It was hypothesized that if bats actively avoided overlapping with one another's emissions, then the data from real bats should reflect a change in the correlation between interference rates and pulse emission rates. This was found not to be true; although real pairs of bats emitted fewer pulses per second neither the mean overlap rate nor the slope of the correlation varied significantly over the overlapping range of emission rates ($P > 0.05$). Alternatively if the probability of two or more bats' emissions overlapping in time was random, then the interference rate was predicted to follow a simple power function of the form $r\tau^n$, where r is the mean emission rate, τ is the empirically defined overlap window duration (10 ms), and n is the number of bats. **Figure 1C** plots how frequently real bats echolocating in pairs or triads emitted overlapping pulses (labeled *Interferences*, quantified as overlaps per second) as a function of the mean pulse emission rate. Both data sets were well fit by the function $r\tau^n$ [$r^2 = 0.71$, $F_{(1,\ 140)} = 344.9$, $P < 0.001$], indicating that interferences had occurred randomly and their propensity was predictably based on mean emission rates and population density and that the bats were not timing their pulse emissions to avoid overlaps with one another. **Figure 1D** extends this function to illustrate how pulse emission rates are predicted to influence interference rates for groups as large as 10 bats. The graph demonstrates that bats in modest group sizes of five or more are faced with a daunting increase in the probability that their pulse emission will overlap with those of neighboring bats. **Figure 1E** uses the same functions to estimate pulse efficiency ($1 - r\tau^n$) as a function of pulse emission rate. This provides an estimate of the relative proportion of emitted pulses that would likely return unambiguous echoes over a natural range of pulse emission rates, illustrating that pulse efficiency is expected to decrease steeply with increasing population density and faster emission rates.

EXPERIMENT 3: HOW DO BATS RESPOND TO THE PRESENCE OF CONTINUOUS NOISE?

When exposed to "continuous" blocks of broadband noise, the bats emitted pulses more frequently while the noise was present than during the intervening silent periods (**Figure 2A**) regardless of whether they were recorded individually or in groups [Two-Way ANOVA, $F_{(1,\ 40)} = 143.8$, $p = 0.001$]. There was also a significant interaction effect between the social and noise conditions [$F_{(1,\ 40)} = 8.937$, $p = 0.005$] arising because bats called more frequently in noise than silence but less frequently in groups than alone, indicating that these effects were combinatorial and not mutually exclusive. Social condition had no significant effect upon the response to sustained noise stimuli. The mean pulse emission rates were lower for groups vs. solitary conditions but increased in noise under both conditions (group rates were 1.5 ± 0.9 Hz in silence vs. 1.8 ± 1.3 Hz in noise; solitary rates 1.8 ± 0.8 Hz vs. 2.3 ± 1.0 Hz in noise). Although the general behavior was consistent with previous results the overall range of pulse emission rates during these experiments was less than in earlier experiments because the bats were no longer naïve to the recording chamber and had habituated to the experimental procedure.

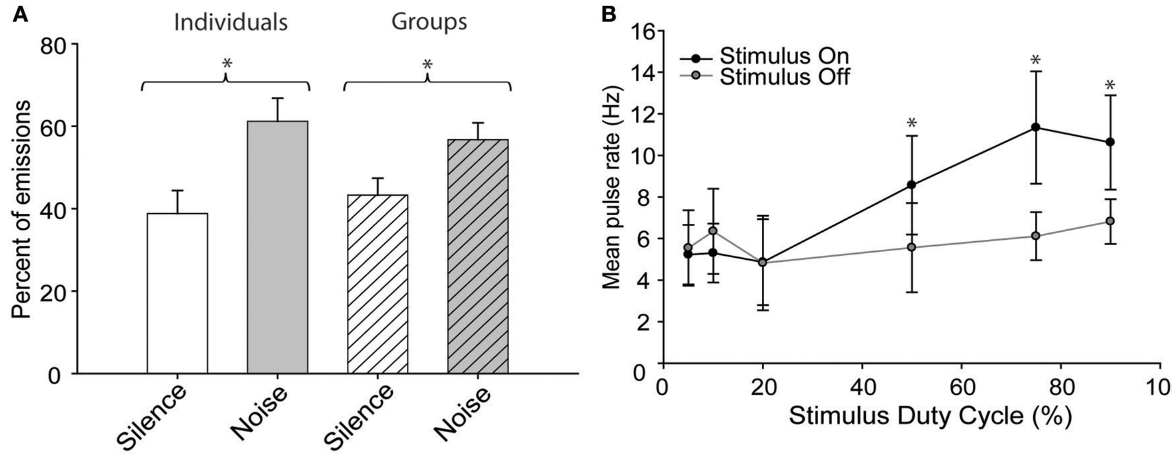

FIGURE 2 | The effect of continuous noise stimuli on pulse emission rates. (A) Bats emitted pulses more frequently in the presence of continuous background noise than during intervening silent periods. The effect was similar whether recording from individuals or groups of bats. **(B)** The effect of stimulus duty cycle on the mean pulse emission rates of solitary bats. Error bars indicate standard deviation; asterisks indicate statistically significant differences from intervening silent periods ($P < 0.01$).

EXPERIMENT 4: AT WHAT TEMPORAL RATIO OF NOISE TO SILENCE DOES THE NOISE PROMOTE FASTER EMISSIONS?

The above experiments demonstrate that free-tailed bats respond differently, depending on whether the interfering noise stimulus is continuous or periodic. Specifically, bats emit pulses less frequently in periodically noisy conditions but more frequently in the presence of sustained noise. To better estimate the point at which bats treat a noise as continuous vs. periodic, a subset of bats were exposed to a series of noise burst stimuli presented at duty cycles ranging from 5 to 95% and we compared pulse rates during stimulus presentations to the rates obtained during intervening silent periods (**Figure 2B**). Stimulus duty cycle had a significant effect upon pulse emission rates [Two-Way ANOVA, $F_{(1,\ 70)} = 14.888$, $p = 0.001$] with was a statistically significant interaction effect between the noise status (on/off) and stimulus duty cycle [$F_{(5,\ 70)} = 5.123$, $p = 0.001$]. *Post-hoc* tests determined that while there was no significant difference in pulse rates among the 5, 10, and 20% duty cycle conditions, duty cycles at or above 50% caused a significant increase in pulse emission rates relative to silent conditions [Holm-Sidak method; 50%, $t = 2.652$, $p = 0.05$; 75%, $t = 4.613$, $p = 0.05$; 90%, $t = 3.355$, $p = 0.05$; $F_{(5,\ 70)} = 8.872$, $p = 0.001$]. There was no significant difference in emission rates across duty cycles at or above 50%, indicating that the bats responded similarly to all of these stimuli as if they were continuous noise.

DISCUSSION

Mexican free-tailed bats live in large dense colonies consisting of hundreds to millions of individuals (Simmons et al., 1978; Ratcliffe et al., 2004). They are highly social animals that spend a large part of their time echolocating in close proximity to other echolocating bats. It is assumed that high population densities present significant challenges for an active sonar system, since signal degradation and perceptual ambiguities are expected to arise from interferences derived from other bats' echolocation pulses. Whether or not bats utilize behavioral strategies for mitigating this interference is unknown. We previously reported that free-tailed bats responded to brief noise bursts by postponing the emission of subsequent echolocation pulses (Jarvis et al., 2010). We speculated that this behavior might improve sonar performance in social conditions by encouraging an antiphonal emission strategy among pairs or small groups of bats. The results presented here dismiss that hypothesis, instead demonstrating that the suppression caused by hearing one another's pulses does not lead to temporal coordination of pulse emissions among pairs or triads of bats. Monte Carlo simulations support the conclusion that overlaps occurred randomly and pairs or triads of bats performed no better than chance at avoiding overlap with each other's emissions.

It was also hypothesized that the acoustic suppression of pulse emission might lead to the generalized suppression of pulse emissions in groups. This was confirmed. Bats slowed their pulse emission rates in response to hearing either the echolocation pulses of real bats or artificial echolocation pulses. Increasing bat density resulted in greater suppression of emissions, indicating that the suppressive effects were additive in nature. If neighboring bats suppress each other's pulse emissions but this suppression does not promote an antiphonal emission strategy, what then is the benefit of this behavior? Here we propose that lessons learned from modern communications networks may explain how slowing pulse emissions can improve a bat's sonar performance when echolocating within a group.

The *ALOHA* system was an inaugural experiment in computer networking designed to link multiple independent users spread across the Hawaiian Islands to a central mainframe computer via a shared UHF radio channel (Abramson, 1970). Signals were randomly transmitted to and from a central computer in time-limited bursts or "packets" of information in a completely unsynchronized manner which led to "collisions" among users transmitting at the same time, causing the loss of both signals. Error detection algorithms were instituted that allowed users to know when their signals had collided, and a simple retransmission protocol was incorporated independently by users that continually resent signals until a successful transmission occurred. This resulted in an uncoordinated competition for channel time that degraded the overall flow of information for all users. To improve network efficiency ALOHAnet's architects investigated how often collisions occurred and how to best to guide user behavior to optimize information flow through the network while also improving transmission efficiency for each user (Abramson, 1970). Network performance was characterized by its total information *throughput* as a function of overall *traffic load*.

Abramson and colleagues showed that as channel traffic increased the rate of collisions among user transmissions increased exponentially and consequently the probability of a successful transmission decreased exponentially (Abramson, 1970). For any single user the immediate probability (p) of a successful transmission was predicted by $p = e^{-2\lambda}$, where λ was a product of the number of users (n), mean transmission rate (r), and signal duration (τ). Channel throughput (S) was used as a measure of how efficiently information is transmitted through a shared communication channel. Maximum possible throughput for any shared channel is achieved only when all user transmissions are perfectly coordinated to utilize 100% of the channel time without any collisions, and is effectively unachievable without comprehensive central coordination. Since a channel's capacity to transmit information can also be underutilized, S is ultimately a function of both channel usage and p, thus $S = \lambda e^{-2\lambda}$, reflecting the compromise between transmission rate and interference rate. **Figure 3A** illustrates how this function could be applied to a group of bats sharing a common acoustic space, except that in this analogy the acoustic space represents a shared communication channel. All the bats sharing the space are transmitting and receiving their echolocation pulses over the same shared channel, and each bat is likely to lose information when its transmissions collide with another bat's transmissions. For analytical purposes we assume that any overlapping pulse emissions result in the total loss of both transmitted signals, but this may not be entirely true for bats. For free-tailed bats we define r = mean pulse emission rate, τ = overlap window (10 ms), and then $\lambda = n_{\text{bats}}\ r\tau$. For any given population density greater than one it can be shown that there is an optimum mean pulse emission rate where all bats would presumably benefit

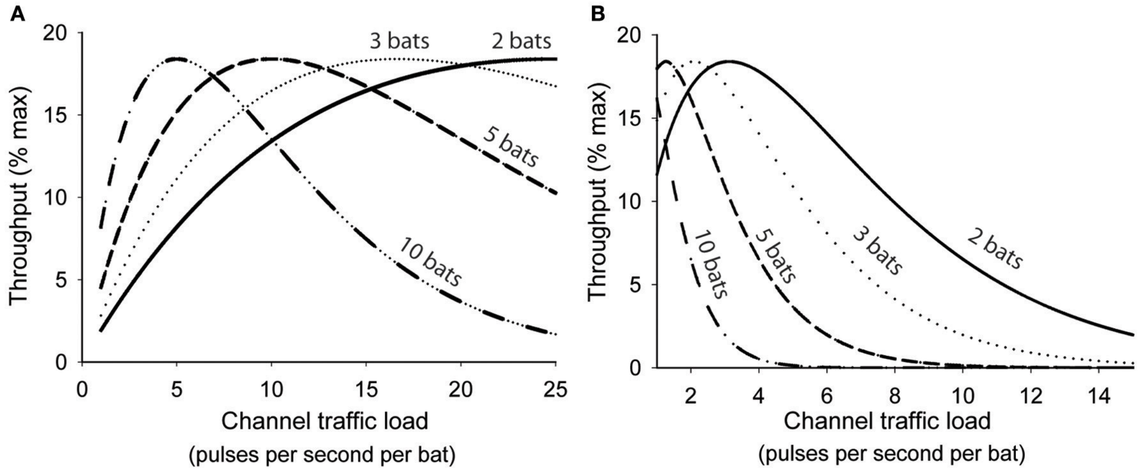

FIGURE 3 | Interactive effects of population density and emission rates on theoretical information throughput (S) of a shared acoustic communication channel following the function $S = \lambda e^{2\lambda}$, where $\lambda = r\tau^n$. (A) calculates information throughput assuming a conservative overlap window (τ) of 10 ms while **(B)** assumes an empirically-determined overlap window of 80 ms. In both graphs throughput is maximized at progressively slower emission rates as group size increases. In B the peak S is achieved at an optimum emission rate 3.25 Hz/bat for pairs of bats, 2.0 Hz/bat for triads, 1.25 Hz/bat for groups of five, and 1 Hz/bat for groups of ten.

from increased pulse efficiency, deriving the most information possible from their echolocation pulse stream with the least amount of wasted emissions. Increasing pulse emission rates beyond this optimum rate rapidly degrades information throughput of the common airspace because the relative proportion of pulses generating unambiguous echoes steeply declines for all individuals.

The random-access nature of a “pure ALOHA” network such as the one described above was found to constrain network throughput to a maximum value of 0.5/e, or roughly 18.4% of the theoretical maximum achievable capacity (Abramson, 1970; Kleinrock and Tobagi, 1975). Since interferences automatically trigger re-transmissions, such random-access networks are inherently unstable due to a positive feedback loop wherein retransmissions lead to a progressively increasing traffic load and consequently more frequent collisions or interferences. For bats, this means that if all the animals in the group increased pulse emission rates to compensate for lost information due to mutual interference, as might be expected based on their known response to cluttered acoustic environments (Petrites et al., 2009), then their net sonar performance would decline rather than improve. Instead, to maintain even modest throughput efficiency bats would be better off reducing emission rates as *n* increased, else the number of pulses generating unambiguous echoes would rapidly diminish. To combat this phenomenon in ALOHAnet, regulatory protocols were applied to constrain when and how often users retransmitted their data. One of these, known as the “carrier sense multiple access” protocol (CSMA) is relevant to bats because CSMA incorporated a “listen-before-send” algorithm, in which transmitters first checked to see if the channel is free before transmitting, and if not briefly postpone transmissions. This greatly reduced traffic load by reducing the number of collisions and retransmissions, and thereby increased network utilization and information flow for all users. We now hypothesize that acoustic suppression of pulse emission exhibited by free-tailed bats serves a function similar to CSMA in wireless communication networks, effectively improving sonar performance in social settings by optimizing pulse emission rates relative to population density.

The optimum range of pulse emission rates predicted by **Figure 3A** is significantly higher than the emission rates we observed for similarly sized groups of bats (**Figure 1B**). This may be accounted for by differences in the predicted and actual overlap window durations. We used a conservative estimate of 10 ms in our analyses, however, our previous studies indicate that hearing another bat’s echolocation pulses can suppress echolocation pulses for up to 80 ms, suggesting that the effective overlap window is somewhere closer to 80 ms. The actual time window over which returning echoes may be subject to interference should vary predictably with habitat and target distances, but it is possible that in free-tailed bats the general behavior is tuned to a specific range, represented by an echo delay of 80 ms. When we recalculated information throughput values using an 80 ms value for τ (**Figure 3B**) we found optimum pulse emission rates more closely aligned with the empirically obtained emission rates for groups of different sizes. This supports the hypothesis that free-tailed bats are reducing their pulse emissions to optimize information throughput of their shared acoustic channel.

Importantly, pulse emissions were never entirely suppressed. At group sizes of five or more the emission rates approached an asymptotic minimum of ∼1 Hz, equivalent to about 20% of the average pulse rate of solitary bats under identical conditions. This indicates that pulse emissions would never be entirely suppressed by the echolocation pulses of their neighbors regardless of population density. In fact, in contrast to the suppression caused by brief periodic noise bursts, we found that sustained broadband noise increased pulse emission rates. This effect was evident regardless of whether bats were alone or echolocating in groups. Pulse emission rates only increased significantly at stimulus duty cycles greater than or equal to 50%, leading us to conclude that once the noise occupies more than half the available time window they behaved as though the noise was essentially continuous. This is consistent with the idea that once the probability that an emitted pulse will overlap with noise exceeds 50%, the bats behave as though every echo may be compromised by noise. Emitting more pulses per second when echolocating in a constantly noisy environment might increase the probability of sporadically producing unambiguous echoes and may improve echo perception via cognitive mechanisms that allow for integration of auditory cues over many sequential echoes, thereby building a more accurate perceptual map of the auditory scene from bits and pieces of many incomplete or distorted echoes (Moss and Surlykke, 2001; Moss et al., 2006).

CONCLUSION

Solitary bats normally resolve ambiguities in their auditory scene analyses by speeding up their pulse emission rates (Moss et al., 2006; Petrites et al., 2009). Here we propose the counterintuitive hypothesis that echolocating bats cooperatively optimize sonar performance at the group level by *slowing* their pulse emission rates proportional to population density, mirroring protocols developed to optimize information throughput in artificial communications networks (Abramson, 1970). Conspecific bats sharing the same acoustic space must transmit and receive their sonar emissions over a single shared communication channel and therefore face many of the same challenges that constrain wireless communications networks. In artificial systems channel capacity is optimized by regulating the transmission behaviors of users via a common set of rules and constraints that ultimately improves efficiency for all users (Tanenbaum, 2003). Likewise, echolocating bats may have evolved a transmission-delay algorithm similar to those used in communications networks to optimize sonar performance in social contexts. Since these experiments were done with stationary bats, it remains to be seen whether flying free-tailed bats performing challenging sonar-guided navigational tasks also display this behavior, though there is evidence from the field and the lab showing that other species of bats increase interpulse intervals in the presence of other bats (Obrist, 1995; Chiu et al., 2008). During flight pulse emissions are significantly constrained by additional mechanical and physiological factors not present when stationary. From a theoretical standpoint, however, flying bats should have as much if not more to gain as stationary bats from exploiting this strategy. The principle that sometimes less is more may prove to be an important clue toward understanding how bats echolocate together in large groups.

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Metabolic costs of bat echolocation in a non-foraging context support a role in communication

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The exploitation of information is a key adaptive behavior of social animals, and many animals produce costly signals to communicate with conspecifics. In contrast, bats produce ultrasound for auto-communication, i.e., they emit ultrasound calls and behave in response to the received echo. However, ultrasound echolocation calls produced by non-flying bats looking for food are energetically costly. Thus, if they are produced in a non-foraging or navigational context this indicates an energetic investment, which must be motivated by something. We quantified the costs of the production of such calls, in stationary, non-foraging lesser bulldog bats (*Noctilio albiventris*) and found metabolic rates to increase by 0.021 ± 0.001 J/pulse (mean ± standard error). From this, we estimated the metabolic rates of *N. albiventris* when responding with ultrasound echolocation calls to playbacks of echolocation calls from familiar and unfamiliar conspecific as well as heterospecific bats. Lesser bulldog bats adjusted their energetic investment to the social information contained in the presented playback. Our results are consistent with the hypothesis that in addition to orientation and foraging, ultrasound calls in bats may also have function for active communication.

Keywords: Chiroptera, energetic costs, *Noctilio albiventris*, cue, signal, fitness

INTRODUCTION

Information use has been proposed as key adaptive behavior (Danchin, 2004), with communication systems arising when it is important for two individuals to intentionally exchange this information to the benefit of both (Seyfarth and Cheney, 2003; Seyfarth et al., 2010). Animals can intentionally transmit information in the form of "signals" and the resulting active communication should be the core mediator of animal interactions. However, information between animals can also be transmitted via inadvertently produced "cues" which can alter the behavior of an active receiver as well. Cues and signals share several key features: a communicator or sender, information (signal or cue), and a recipient (Danchin, 2004). There is therefore a major distinction between inadvertent cues and intentional signaling and how selection can act on both. According to Maynard-Smith and Harper (2003) a signal is "any act which alters the behavior of other organisms that has evolved because of that fact, and which is effective because of the receiver's response that has also evolved." This requirement that a signal evolved due to its effect on other organisms, is a fundamental difference from cues, which are simply by-products of the producer's action and not under selection for information transfer from either the sender's or receivers viewpoint (Scott-Phillips, 2008).

The long-term currency of communication is Darwinian fitness; the short-term currency is energy or time expended by a sender. If communication is taking place at all, maximizing fitness forces animals to optimize communication and thus selection acts on both, signals (the sender and receiver side) and cues (only on the receiver side). Senders of signals invest energetic costs or time, if the cost to maintain such a signal plays a major role in securing the information content, i.e., the honesty of a signal (Zahavi, 1975, 1977), whereas the receiver can invest considerable energy, time, and predation risks to receive and process both cues and signals and may have to adapt to this in the course of evolution (Bradbury and Vehrencamp, 2011). The role of signals and cues and how the latter may turn into the former is very context-dependent and closely tied to the modality in which they are produced (e.g., sound, vision, olfaction).

One group of animals that constantly and involuntarily produce auditory cues while moving are echolocating bats. Echolocation calls are vocalizations, usually in the ultrasound range above 20 kHz, enabling bats to orientate and forage at night. Echolocation has been described as "autocommunication" (Bradbury and Vehrencamp, 2011) or "communication about the environment surrounding oneself" (Simmons, 1977), with the same bat operating as both signaler and receiver. Echolocation is under strong selection, because call structure, frequency, and intensity are largely determined by the type of prey, amount of background clutter and phylogenetic history of species. Thus, it has been assumed that there would be little or no adaptive plasticity allowing additional communicative information to be contained in them (Schnitzler et al., 2003), although the idea of communicative elements being contained in them is not new (Möhres, 1967). Recently, evidence has been accumulating that bats also act and react in the presence of and in response to other echolocating bats (reviewed in Jones and Siemers, 2010). Echolocation may in fact have evolved from social vocalizations (Fenton, 1984), and the potential for communication may be much higher than previously assumed.

Metabolic costs of acoustic signaling are about eight times that of the silent animal in several taxa (Ophir et al., 2010), and the cost of echolocation can be even higher. For example, in an experimental situation, a stationary 6 g pipistrelle bat looking for food, but adapted to foraging for insects on the wing, spends approximately 0.067 J/pulse (Speakman, 1989) and a 17 g *Eptesicus fuscus* metabolizes 0.197 J/pulse (Speakman et al., 2004). It is unknown how much stationary echolocation is used in a natural scenario, but this would add up to 1.3 and 3.9 J/s, respectively, at up to 20 calls per second, very high compared to *E. fuscus*' daily energy expenditure of about 30 kJ (Kurta et al., 1989, 1990). In contrast, there seems to be no additional cost of echolocation in flying bats (Speakman, 1991), and one should assume that stationary bats should rarely echolocate especially when not trying to locate food. We do not know how much spontaneous echolocation is used in stationary bats under natural conditions, but if bats do intentionally produce these energetically costly calls in non-foraging contexts, instead of active information transfer, this might indicate that these calls not only serve as cues, but might be energetically costly signals in bat communication.

COST OF ECHOLOCATION IN BAT COMMUNICATION—A CASE STUDY OF *Noctilio albiventris*

Noctilio albiventris is a Neotropical bat species that roosts in groups of 5–20 individuals in our study area, Gamboa, Panama (09.078N°; 079.418°W). Radio-telemetry data revealed that group members coordinate flight to forage together (Dechmann et al., 2009), and playback experiments demonstrated that this allows eavesdropping on inadvertent information contained in "feeding buzzes," calls produced during attempts to capture prey (Dechmann et al., 2009). A sudden increase in feeding buzzes is a cue that indicates a profitable feeding patch to eavesdropping group members. These results showed non-opportunistic use of cues in a social context, a behavior otherwise only described in dolphins (Lammers and Au, 2003; Lammers et al., 2003). An additional set of playback experiments with captive *N. albiventris* indicated that echolocation calls may be used not only for eavesdropping on the wing, but also in an exclusively social context (Voigt-Heucke et al., 2010). Stationary, non-foraging *N. albiventris* responded with more social behaviors including more echolocation calls to playbacks of orientation echolocation calls of their own species than those of other species, and even more intensively to calls of unfamiliar conspecifics than individuals of their own social group. This was surprising because due to the high energetic costs of producing ultrasound echolocation calls (in contrast to social calls, which may also be produced in the ultrasound range), non-flying bats were not expected to echolocate more than necessary for orientation or localizing food. In addition, the cue used for eavesdropping by foraging bats are feeding buzzes and not the orientation calls used in the experiment described above.

To investigate if echolocation may also serve as an active signal in bat communication, we first measured the energetic costs of echolocation in non-foraging, non-flying bats. We then used the data published in Voigt-Heucke et al. (2010) to quantify the investment in different social contexts. If echolocation serves a communicative function, we expected metabolic rates to increase significantly in *N. albiventris* producing ultrasound calls in response to the calls of another bat.

METHODS

ENERGETIC COST OF ECHOLOCATION IN *Noctilio albiventris*

We caught adult *Noctilio albiventris* between 7 and 9 pm during March 2009 and 2010 with mistnets (Ecotone, Poland) when they were returning with full bellies to known daytime roosts after foraging in the vicinity of the village Gamboa, Panama. After determining sex, age, reproductive state, and forearm length in mm with calipers (Mahr, Germany) and weighing them to the nearest 0.25 g (Pesola, Switzerland), bats were transferred to a nearby laboratory in soft cloth bags. There, they were placed in the metabolic chamber (1l volume) of a respirometry setup (see below). The metabolic chamber, which was lined with wire mesh, allowed the animals to roost on the side of the container in a natural position, but did not allow them to fly. The chamber was padded with rubber foam to avoid reflections of echolocation calls. An infrared-sensitive video camera (Sony, Japan) confirmed that calling bats were not moving except for turns of the head. The measurements were made in a dark silent room at ambient humidity and temperature (25°C) and the bat's calling activity was monitored and recorded from outside the room on a computer screen. To record echolocation calls, we placed an Avisoft condenser ultrasound microphone CM16/CMPA (Avisoft Bioacoustics, Germany) near the bat's head. The microphone was connected to an Avisoft UltraSoundGate 116Hme, which directly recorded onto a laptop computer with the Avisoft software Recorder USGH version 3.4. Recordings were performed with a 16 bit resolution and a 250 kHz sampling rate.

Some bats remained silent for up to 1 h after being placed in the chamber and all remained silent at least 10 min. And many bats never spontaneously called at all. We measured resting (i.e., immobile and not calling) and calling (i.e., echolocating, but not moving more than the head) metabolic rate and released the bats after they had vocalized ($n = 7$; mass: 25.8 ± 3.2 g; six females, one male). Bats that had not vocalized after 1 h ($n = 2$) were released without recording. All bats were released at the daytime roost before midnight of the capture night.

We measured the oxygen consumption and carbon dioxide production of calling and silent *N. albiventris* using an open-flow, push-through respirometry system. Ambient air with a humidity of about 85–95% was pumped at a flow rate of 1 l min^{-1} via a mass flow controller (TR-FCI, Sable Systems, Las Vegas, NV) and a multiplexer (V2-0, Sable Systems) into the chamber. Reference values were taken before and after the animals were placed in the chamber. After dehumidifying inlet air with a Peltier-Effect

Condenser (PC-1, Sable Systems), we measured CO_2 concentration from a sub-sample (CA 1B, Sable Systems). We used drierite to scrub off potential remaining water from the air, and then measured oxygen concentration.

We used the equation by Bartholomew and co-authors (1981) to measure instantaneous oxygen consumption rate

$$FE_{o_2}(eq) = FE_{o_2}(t-1) + \left[\frac{FE_{o_2}(t) - FE_{o_2}(t-1)}{1 - e^{(-v>>/v)\Delta t}}\right]$$

where FE_{o_2} is the oxygen consumption in the outlet air, $FE_{o_2}(eq)$ is the equilibrium value, V is the volume of the respirometry system, $v>>$ is the flow rate through the system, and Δt is the interval between measurements at times *t* and *t*-1. The denominator of the equation was determined empirically with Datacan (Sable Systems). The rate of oxygen consumption was calculated using Equation (3b) of Withers (1977).

We converted oxygen consumption rate into energy turnover by utilizing the caloric equivalent of protein oxidation (Voigt et al., 2010). After having fed on their insect diet Noctilio used proteins as a metabolic fuel (Voigt et al., 2010). The caloric equivalent for endogenous carbohydrate or fat oxidation is almost the same: 19.6 kJ/lO2 for fat oxidation, 21.1 kJ/lO2 for carbohydrate oxidation (and 18.8 Kj/lO2 for protein oxidation; Penzlin, 1989). The acoustical recordings were started simultaneously with the measurements of oxygen consumption. We counted the number of calls per second throughout metabolic measurements. As the microphone was at a distance of only 5 cm to the bat's head, call intensity very much depended on the orientation of the animal's head, Consequently, it was not possible to quantify sound pressure levels.

Oxygen consumption was not measured with the same animals that were used by Voigt-Heucke and coauthors to assess the behavioral responses to echolocation playbacks (see below). Thus, a mean cost per echolocation call was calculated based on our data.

CALL RATE IN RESPONSE TO DIFFERENT SOCIAL CONTEXTS (ALL DATA FROM Voigt-Heucke et al., 2010)

All data cited in this paragraph are from the cited study, for more details on behavioral response data collection to different playbacks, including animal housing, preparation of the playback files etc. see the original paper (Voigt-Heucke et al., 2010). In summary: four types of playback stimuli were used to quantify the reaction of *N. albiventris* to echolocation calls in a social context. Stimulus categories were orientation calls from (1) familiar conspecifics (group members, $n = 15$ individuals from three social groups), (2) unfamiliar conspecifics (non-group members, $n = 5$), (3) heterospecifics that share roosts with *N. albiventris* (*Molossus molossus*, $n = 5$), and (4) heterospecifics that do not share roosts (*Uroderma bilobatum*, $n = 5$). Here, the experimental animals were 20 experimentally naïve individuals from the three "familiar conspecifics" groups. Bats were allowed to habituate to the experimental situation for at least 30 min before the start of experiments. The bats' behavioral response to the playback was then filmed and their acoustic response recorded. Each bat was tested in five trials. Stimulus categories were presented in random order during these five trial sessions, and only one trial was conducted per night with each bat to avoid habituation.

Each playback trial consisted of three phases: a pre-playback phase (2 min), a playback phase (8 s) and a post-playback phase (5 min). The pre-playback phase started when bats had been hanging motionless and silent for at least 2 min. For analysis the echolocation response rates (n/5 min) of each bat during the 5 min post-playback phase was assessed.

STATISTICAL ANALYSIS—COST OF ECHOLOCATION IN A SOCIAL CONTEXT

To describe the relationship between call rate (*n*/10 s) and oxygen consumption we built a generalized linear mixed model in *lme4* (Bates et al., 2011). We included call rate as a fixed factor and individual as random intercept factor to correct for differences between individuals. As they bats were caught after foraging (which can add about one third to their mass), and the estimations were done with oxygen consumption of one, but response rates of another set of individuals, we corrected for individual and did not additionally include mass after running a simulation with mass that did not affect the results. We ran a model with random slope and intercept and compared it to the random intercept only model (with full fixed factors) using REML estimation as suggested by Schielzeth and Forstmeier (2009). We then bootstrapped the model 10,000 times using the *arm* package to obtain the distribution of the likelihood ratio (Gelman et al., 2011). As the random slope model was no better than the random intercept only model we estimated the fixed effects with a random intercept only model and ML for the estimates. *P*-values for the fixed effects were estimated with a Markov Chain Monte Carlo (MCMC) approach in *languageR* (Baayen, 2011). All analyses were performed in R 2.13 (R-Development-Core-Team, 2009).

We used the equivalent of oxygen consumption per echolocation pulse (assessed by us), to extrapolate the response costs of 20 *N. albiventris* from Voigt-Heucke et al. (2010). We log(x + 10)-transformed the response costs and calculated a repeated measures analysis of variance to test for difference in response costs (number of calls/10 s) to the stimuli "familiar (FC)" and "unfamiliar conspecifics (UC)," and "cohabitant (CH)" and "non-cohabitant heterospecifics (NCH)." *Post-hoc* Tukey–Kramer tests were used for pair-wise differences between the energy costs of stimuli responses. All tests were two-tailed with an assumed alpha value of 5%. Data are presented as mean ± one standard deviation if not otherwise stated.

RESULTS

ENERGETIC COSTS OF ECHOLOCATION IN *Noctilio albiventris*

We recorded the energy consumption of seven bats calling during bouts lasting 18–586 s that produced a maximum of 29 calls/s. The mean (±stdev) metabolic cost of non-calling non-foraging, stationary bats was 51.9 ± 5.7 ml O_2 h^{-1}. When calling costs ranged between 50 and more than 120 ml O_2 h^{-1} (**Figure 1**, **Table 1**). We found a significant positive relationship between call rate and oxygen consumption (**Figure 1**): rate of oxygen consumption (ml O_2 h^{-1}) $= 51.13 + 0.38 \times$ call rate (n/10 s).

COST OF ECHOLOCATION IN A SOCIAL CONTEXT

Calculating oxygen consumption at the pulse rates the bats responded to the social playbacks with, using the equation from the mixed model showed that the bats adjusted their response depending on the presented stimulus [$F_{(3,\,57)} = 3.48$; $P = 0.0257$; **Table 2**]. The energetic costs of responses were significantly higher when exposed to unfamiliar conspecifics than to both types of heterospecifics (**Table 2**).

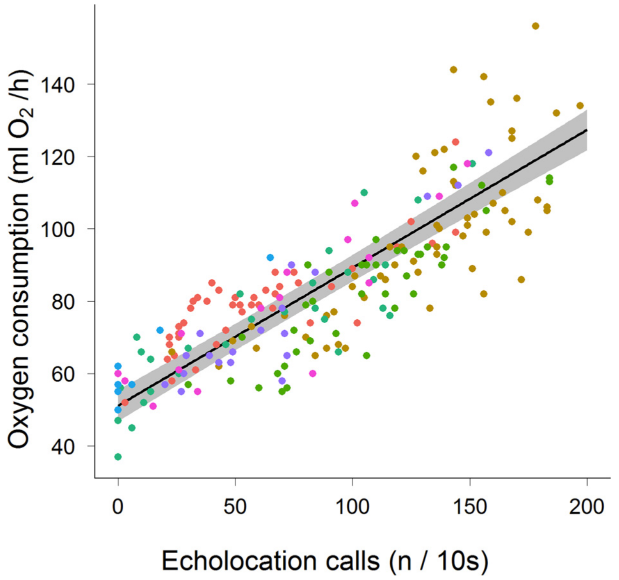

FIGURE 1 | Metabolic rate (ml O_2/h) for seven *Noctilio albiventris* (indicated by different colors) in relation to echolocation pulse rate (n/10 s). The mean metabolic rate as estimated by the model is the black line with the 95% credible interval indicated by the gray area.

Table 1 | Model estimates of the relationship between rate of oxygen consumption (ml O_2/h) and call rate (n/10 s).

	Estimate ± std error	***t* value**	**Lower 95%**	**Upper 95%**	**pMCMC**
Intercept	51.14 ± 2.25	22.75	46.15	56.26	0.0001
Call rate	0.38 ± 0.018	21.79	0.35	0.42	0.0001

pMCMC, probability calculated with Markov Chain Monte Carlo approach.

Table 2 | Pair-wise comparisons using Tukey–Kramer *post-hoc* tests.

comparison	**q**	***p*-Value**
FC-UC	2.41	n.s.
FC-CH	1.46	n.s.
FC-NCH	1.45	n.s.
UC-CH	3.88	<0.05
UC-NCH	3.86	<0.05
CH-NCH	0.18	n.s.

FC, familiar conspecific; UC, unfamiliar conspecific; CH, cohabitant heterospecific; NCH, non-cohabitant heterospecifc; q, Tukey-Kramer statistic; n.s., non-significant. Data from Voigt-Heucke et al. (2010).

DISCUSSION

We estimated the metabolic costs of the spontaneous ultrasound response in non-foraging, stationary bats, in response to different social stimuli, and showed that echolocation in *N. albiventris* incurs substantial energy costs. Calling bats spent 0.0213 J/pulse, about 2–5 times more energy than silent bats. This is lower than previously recorded for other species, most likely due to larger size of Noctilio, but still substantially higher than non-calling energy expenditure. Bats increase call rates significantly in response to playbacks of unfamiliar conspecifics (Voigt-Heucke et al., 2010) and thus adjust their energetic investment according to the social information perceived in the presented playback, an indicator of active signaling. Jamming avoidance or increased call rates to improve foraging efficiency in a competitive situation, cannot explain the bats' costly response in an experimental situation. Female mice are more interested in the odors of unknown than of known males (Kavaliers, 2003) and, similarly, bats respond more strongly to the calls of unfamiliar conspecifics than heterospecifics (Voigt-Heucke et al., 2010) which translates into significantly higher costs. The exact purpose of this remains unknown, but several non-exclusive interpretations are possible. The calls may convey information about the sender's identity, sex, or quality, but they may also be a dominance, aggression, or appeasement gesture.

Most animals produce sounds specifically for communication, a typical example being bird song. In contrast, bat echolocation calls are primarily produced for orientation and foraging and are under strong selection for adaptation to this niche specific purpose. Nonetheless evidence has been accumulating that the communicative potential of bat echolocation is high [reviewed in Jones and Siemers (2010), Knörnschild et al. (2012)]. The ultrasound calls of bats, which are adapted to foraging on the wing are very costly when the bats are presented with food in a stationary situation (Speakman, 1989; Speakman et al., 2004). In contrast, calls emitted on the wing during foraging and orientation are not costly (Speakman, 1991) probably due to the timing of call emission with the wing beat upstroke and exploiting the power generated by the resulting muscle contractions. All studies that try to determine the communicative function of echolocation, including our own, have used the number of echolocation calls as response variable (Kazial, 2004, 2008; Yovel et al., 2009; Schuchmann and Siemers, 2010; Voigt-Heucke et al., 2010; Schuchmann et al., 2012). An increase in call rates on the wing could be interpreted as an attempt to be more competitive in a foraging situation, and flying *Noctilio albiventris* in the field do indeed react to playbacks of feeding buzzes of unfamiliar individuals by approaching them (Dechmann et al., 2009; Übernickel et al., 2012). In the proper experimental context, changes in echolocation rates could even be interpreted as an intentionally produced vocalization with the goal to alter the behavior of the caller or to indicate, for example, individual identity, sex, group membership, or species, fulfilling at least one of the conditions for the definition of a signal as proposed by Maynard-Smith and Harper (2003), however, this had not been tested before.

The amount of time bats during our measurements of oxygen consumption spent echolocating varied from just two short bouts of less than 10 s (a female) to 50 or more 10 s bouts (also a female). Our sample of seven bats was composed of a male and pregnant as well as non-reproductive females, showing a high correlation between call rate and oxygen consumption, which gives us confidence that these data are a good enough representation of the species' behavior and energy investment to indicate that an investment is in fact being made.

Based on our results, we advocate that depending on the context, echolocation calls may either be used as cues produced by foraging conspecifics, i.e., eavesdropping on feeding buzzes; or intentionally produced costly signals. Whereas it has often been shown that signals, such as mating calls can also serve as cues for other con- and heterospecifics, our data are consistent with the hypothesis that even though echolocation calls are mainly strongly selected autocommunicative signals in an ecological context, they may in addition be actively produced signals for social communication.

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Social calls of flying big brown bats (*Eptesicus fuscus*)

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Vocalizations serving a variety of social functions have been reported in many bat species (Order Chiroptera). While echolocation by big brown bats *(Eptesicus fuscus)* has been the subject of extensive study, calls used by this species for communication have received comparatively little research attention. Here, we report on a rich repertoire of vocalizations produced by big brown bats in a large flight room equipped with synchronized high speed stereo video and audio recording equipment. Bats were studied individually and in pairs, while sex, age, and experience with a novel foraging task were varied. We used discriminant function analysis (DFA) to classify six different vocalizations that were recorded when two bats were present. Contingency table analyses revealed a higher prevalence of social calls when males were present, and some call types varied in frequency of emission based on trial type or bat age. Bats flew closer together around the time some social calls were emitted, indicating that communicative calls may be selectively produced when conspecifics fly near one another. These findings are the first reports of social calls from flying big brown bats and provide insight into the function of communicative vocalizations emitted by this species.

Keywords: big brown bat, communication, competition, *Eptesicus fuscus*, foraging, inter-bat distance, social calls

INTRODUCTION

Since the pioneering studies of Griffin and Webster, it has been recognized that many bats produce high frequency calls and use information carried by returning echoes to localize objects in their environment (Griffin, 1958; Griffin et al., 1960). Research has also shown that bats emit vocalizations in social contexts (see Fenton, 1985; Pfalzer and Kusch, 2003). For example, Suthers (1965) described a distinctive call produced by fishing bats (*Noctilio leporinus*) to avoid in-flight collisions. In addition, vocalizations produced by bats have been reported to serve mating-related functions (e.g., Bradbury, 1977: *Hypsignathus monstrosus*; Lundberg and Gerell, 1986: *Pipistrellus pipistrellus*; Davidson and Wilkinson, 2004: *Saccopteryx bilineata*), to recruit conspecifics (e.g., Wilkinson and Boughman, 1998: *Phyllostomus hastatus*; Arnold and Wilkinson, 2011: *Antrozous pallidus*), to respond to bats calling from a roost (e.g., Chaverri et al., 2010: *Thyroptera tricolor)*, to avoid physical aggression (Leippert, 1994: *Megaderma lyra*), and to defend foraging patches (e.g., Rydell, 1986: *Eptesicus nilssoni*; Barlow and Jones, 1997: *Pipistrellus pipistrellus*). Despite these studies, few examples of communicative vocalizations emitted by flying, foraging bats have been reported. Examining such vocalizations, in concert with information about bat sex, age, foraging context, and inter-bat interactions, can provide insight into the functions of social calls in bats.

Social calls emitted by bats during flight might serve to repel or attract other foragers. For example, calls produced by *Pipistrellus pipstrellus* when food density is low have been shown to repel conspecifics (Barlow and Jones, 1997), whereas calls emitted by female *Phyllostomus hastatus* coordinate group foraging (Wilkinson and Boughman, 1998). Alternatively, calls might influence mating and therefore should occur most frequently at the time of year when animals are engaged in reproductive behaviors. For example, male *Tadarida brasiliensis* produce songs during a limited period each spring (Bohn et al., 2009). Finally, calls with an appeasement function (Gadziola et al., 2012) would be expected to be produced by vulnerable individuals, such as juveniles, to avoid aggressive encounters with other bats, as has been proposed for calls emitted by *Megaderma lyra* (Bastian and Schmidt, 2008).

Eptesicus fuscus is a temperate, aerial-hawking insectivore that is widespread in North America (Kurta and Baker, 1990). Female *E. fuscus* form maternity colonies in the spring and early summer, and the bats "swarm" (Fenton, 1969) and mate at hibernation sites before hibernating for the winter. This species forms non-random associations with roost-mates (Willis and Brigham, 2004; Metheny et al., 2008), and multiple individuals can be found foraging at the same site, indicating that bats have opportunities to communicate while foraging. Two studies have reported that *E. fuscus* can learn a novel foraging task or food location by interacting with knowledgeable conspecifics (Gaudet and Fenton, 1984; Wright et al., 2011).

Echolocation by *E. fuscus* has been studied extensively (e.g., Simmons and Vernon, 1971; Masters et al., 1991; Surlykke and Moss, 2000). Some research indicates that echolocation signals themselves can serve a communicative function, such as revealing information about identity, age, and sex (Masters et al., 1995; Kazial and Masters, 2004; Grilliot et al., 2009; Jones and Siemers, 2010; Knörnschild et al., 2012). However, most studies of social calls in this species have focused on mother-infant communication or vocal development (e.g., Gould, 1971, 1975; Gould et al., 1973; Moss, 1988; Monroy et al., 2011). A recent study of roosting or crawling bats indicated that social call production varies with behavioral context (Gadziola et al., 2012), but, to date, we know of no description of social calls from flying big brown bats, although Barbour and Davis (1969) noted that *E. fuscus* are known to emit an "audible chatter" (p. 130) when flying near each other.

In this study, we document the occurrence and the context of social calls emitted by big brown bats flying together in a large behavioral test room. We manipulated context by varying prey-capture skill level, age, and sex of bat pairs and then used recordings of high-speed video and audio to determine the position of each individual before and after emitting social calls. If calls served a mating related function, we expected them to be emitted primarily in late August or September when spermatogenesis peaks and mating in this species typically begins (Kurta and Baker, 1990) and to be produced by males flying in the presence of females. If calls served to recruit or repel individuals to or from a food source, we expected a higher rate of calls when at least one skilled forager was present. Finally, we predicted that calls related to appeasement would be most common when juveniles were present. Here we test these predictions and describe the repertoire of social calls emitted by flying big brown bats.

MATERIALS AND METHODS

SUBJECTS AND EXPERIMENTAL SET-UP

Thirty-six *Eptesicus fuscus* obtained from the wild under a Maryland Department of Natural Resources collecting permit and two born in captivity served as subjects in this study. This research was conducted with approval from the Institutional Animal Care and Use Committee at the University of Maryland. At the time of testing, 24 animals were adults ($\geq$1 year old; 17 F, 7 M), and 14 were juveniles (estimated ages at start of testing: 21–51 days ($X \pm SD = 34 \pm 10$); 6 F, 8 M). Based on their ages, the juvenile bats should not have been reproductively capable during most or all of the experimental period. Bats always had access to water and were maintained on a reverse 12:12 h light:dark cycle (lights off from 08:30 to 20:30). When not flying, they were housed in cages containing three to four bats each.

We flew pairs of big brown bats in the presence of a single, non-shareable prey item (tethered mealworm—larval *Tenebrio molitor*) in a 7 × 6 × 2.5 m anechoic flight room. As bats flew, we recorded 8 s segments of synchronized audio and video data using two high-speed (240 frames/s in 2005–2006; 250 frames/s in 2007) infrared-sensitive video cameras (in 2005–2006: Kodak MotionCorder Analyzers, Model 1000, Eastman Kodak Company, San Diego, CA, USA; in 2007: Photron PCI-R2, Photron USA, Inc., San Diego) and two ultrasound-sensitive microphones (UltraSound Advice, London, UK) amplified (UltraSound Advice) and recorded at 250 kHz/channel (Wavebook, IOTech, Cleveland, OH, USA). The room was lit with low-intensity and long wavelength overhead lighting (>650 nm, red filters, Reed Plastics, Rockville, MD, USA) and two red light-emitting diode (LED) headlamps to minimize availability of visual cues [see Chiu et al. (2008) and Wright et al. (2011) for additional details]. Recordings from 415 one-bat and 528 two-bat trials involving 83 pairs of bats were then examined.

Bat pairs fell into three categories: (1) one individual had learned to take the tethered mealworm, while one was naïve (mixed trial type; July–September 2006 and July–August 2007; 36 pairs), (2) both individuals were naïve (naïve trial type; July–September 2006 and July–August 2007; 40 pairs), or (3) both individuals had learned to take tethered mealworms (skilled trial type; July–August 2005 and July–August 2006; 7 bat pairs). While some naïve individuals in mixed trials began to learn the task, previously naïve individuals were no longer paired with other bats once they learned to capture the mealworm (Wright et al., 2011). We recorded all individuals in paired bat trials, and each bat flew with an average of 4.5 other bats (range: 1–11 partners; median: 4 partners). A test day began with both bats being released simultaneously (skilled pairs) or in some cases with a naïve bat resting on the wall when another bat was released (naïve and mixed pairs). For skilled and mixed pairs, we recorded prey capture and the previous 8 s. On a given test day, once the mealworm was taken, another was immediately presented to the same pair of bats until 10–20 mealworms had been consumed. For naïve pairs, bats were flown for a fixed period of time (7 min.) based on the time it took trained bats to consume 10–20 mealworms, and 8 s recording segments were saved throughout this time period, as described in Wright et al. (2011). Skilled pairs were captured in between each mealworm presentation, while mixed and naïve pairs flew freely during this time. Bats occasionally landed on the flight room wall during trials but were usually flying. In addition to two-bat trials, we recorded single-bat trials from 22 naïve and eight skilled bats. Please see Chiu et al. (2008) and Wright et al. (2011) for additional details.

IDENTIFICATION AND CLASSIFICATION OF CALL TYPES

By inspecting spectrograms and listening to audio files slowed by a factor of 10–20, we identified calls that differed in time-frequency structure from frequency-modulated (FM) echolocation calls produced by big brown bats. We did not employ a frequency cut-off regarding which calls to include, but we excluded vocalizations resembling buzzes [feeding buzz pulses drop below 20 kHz, have short duration (<1 ms), and have short pulse interval (PI; <8 ms)] because these calls were typically produced when bats were feeding, landing or investigating objects in the room, and their potential social function could not be separated from echolocation function. Other, low frequency calls were, however, included in the data set presented here. We considered emission of calls only in the presence of conspecifics as evidence that calls serve a social function.

We first categorized calls by consistent patterns in time-frequency structure. This method resulted in seven call types: (1) upward frequency-modulated (UFM)—end frequency exceeds start frequency by $\geq$5 kHz without additional change in frequency; (2) U-shaped (U)—dominant frequency decreases by ≥5 kHz, then increases again to between 50 and 150% of the start frequency; (3) chevron-shaped (CS)—dominant frequency increases by ≥5 kHz, then decreases again to between 50 and 150% of the start frequency; (4) short frequency-modulated (SFM)—short duration, narrow bandwidth calls with ending frequency ≥18 kHz, duration ≤6 ms, and bandwidth ≤20 kHz; (5) long frequency-modulated (LFM)—an initial downward sweep, and duration (3.75–82.7 ms) longer than typical echolocation calls produced by big brown bats in a confined space (>3.7 ms, mean duration of echolocation calls in our single bat recordings)—these calls appeared in two varieties: short (chirp-like FM sweeps virtually always paired with a long LFM) and long (elongated quasi-constant frequency portion after initial frequency drop) and often occurred in pairs or trios; (6) quasi-constant frequency (QCF)—dominant frequency is within 5 kHz of the start frequency; and (7) frequency-modulated bout (FMB)—a sequence of 3–4 frequency-modulated (FM) sweeps that were longer in duration than typical echolocation calls (mean duration of FMB pulses: 9.2 ms, compared with echolocation call durations ≤4 ms) sometimes followed by several short, buzz-like calls (short duration calls with relatively short PI; **Figure 1**). FMB refers to a specific pulse type and the fact that it occurs in a sequence of 3–4 such pulses. Not all FMBs were followed by buzz-like pulses; therefore, the presence of such pulses was not considered a defining characteristic of this call type.

To quantify the accuracy of this call classification system, we conducted a discriminant function analysis (DFA) assuming unequal covariances and using start frequency (kHz), end frequency (kHz), mid-frequency (frequency in the middle of the call's start and end time; kHz), and call duration (ms). For call types with more than one pulse (FMB, some LFM), we took the mean values of all pulses within the sequence and used these data in the DFA. We did not include the short, buzz-like calls that often occurred at the end of FMB, for the reason noted above. Due to the small number of U calls recorded ($n = 26$), we excluded this call type from the DFA and all subsequent quantitative analyses.

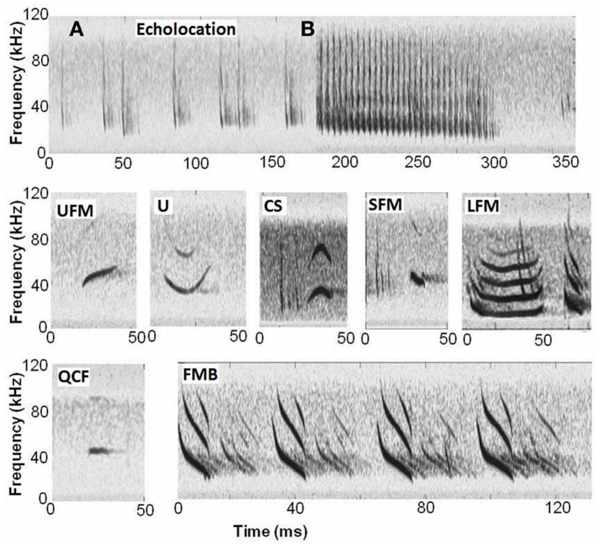

FIGURE 1 | Calls recorded in a flight room. (A) Standard echolocation calls (two bats flying); **(B)** feeding buzz with the second bat echolocating; UFM, upward frequency-modulated; U, U-shaped; CS, chevron-shaped; SFM, short frequency-modulated; LFM, long frequency-modulated (double-LFM showing long and short varieties of the call type); QCF, quasi-constant frequency; and FMB, frequency-modulated bout with only the initial FM sweeps shown (four pulses and their echoes are shown). Note that for several of the examples above (e.g., CS, SFM, and LFM), echolocation calls from the other bat present in the trial are also visible.

CALLER IDENTIFICATION AND CALL CONTEXT

To rule out the possibility that calls of a given type were produced exclusively by one individual, we calculated the minimum number of individuals emitting each call type by examining the number and composition of pairs from which calls were recorded. In addition, we used a combination of video and audio data to identify, when possible, which bat had emitted each vocalization using the following criteria: (1) the social call was visible in the spectrogram of both audio channels, (2) at least one bat was in view of both cameras during the time the call was emitted, and (3) both individuals were identifiable during the trial (see Chiu et al., 2008). Particularly in naive bat trials wherein no bat was catching the prey, we often did know which bat was which during a given recording: we might determine that one social call was emitted by "Bat A" while another call was emitted by "Bat B," but we could not always determine whether Bat A was the adult female or the juvenile male (for example) in that recording. Therefore, caller identification was not possible for all calls. For call types emitted by more than five known callers, we compared the number of callers of each sex with the proportion of bats we tested that were female (61%) or male.

To determine the context in which calls were given, we investigated whether call occurrence was independent of trial type, bat age, and bat sex. Because we could not always determine which bat emitted a call, and we recorded few trials per pair in some cases (range: 1–25 trials per pair; median: 5 trials), we accounted for variation in the number of calls emitted by each individual by examining the data on a per-trial basis. Specifically, we compared the number of trials containing at least one instance of a given social call type. We excluded juvenile-juvenile trials from these analyses because all 25 trials included one bat in common and only one such trial contained any social call. Data included trials from every combination of sex (female–female: $N = 126$ trials; female–male: $N = 256$ trials; male–male: $N = 121$ trials) and trial type (naïve: $N = 181$ trials; mixed: $N = 170$ trials; skilled: $N = 152$ trials).

We examined the relationship between each factor (age, sex, and foraging experience) and call prevalence, using contingency tests, for each call type. For SFM, we found a significant interaction between trial type and sex, so we tested for effects of trial type within trials with the same sex combination. Because all bats tested in skilled trials were adults, we could not test for age effects in those trials. Instead, we tested for age (adult–adult: $N = 69$ trials; adult–juvenile: $N = 282$ trials) effects within naïve and mixed trials combined for UFM, CS, SFM, LFM, and QCF calls. We recorded too few FMB from naïve and mixed trials to conduct this analysis. Because tests regarding these factors were all drawn from the same data set, we used a sequential Bonferroni correction to assign significance for each of the 19 comparisons made. For call types with significant differences based on trial type or sex, we conducted pairwise comparisons (e.g., female-male vs. male-male trials, or naïve vs. skilled trials). For these comparisons, we used a sequential Bonferroni correction within each factor for each call type (three comparisons for each combination).

FLIGHT BEHAVIOR

Using a custom Matlab program that allowed us to mark and plot the three-dimensional flight trajectories of each bat (see Chiu et al., 2008), we determined in-flight inter-bat distances between animals. We calculated inter-bat distances for the 1 s surrounding the time of social calls (mean of the 500 ms before the start and after the end of each social call), as well as the mean inter-bat distance for the entire 8 s trial in which each social call was recorded. Only video frames with both bats flying in the calibrated volume of the two cameras were included in the analyses. Therefore, animal position data was not available for every social call or for every frame within each 8 s recording, and we sometimes had less than 1 s of video position data surrounding a social call.

We had unequal and sometimes sparse numbers of recordings from each pair of bats and could not always determine caller identity. Therefore, we examined data on a per-trial (recording) basis and only included call types with position data available for 10 or more calls. We averaged the mean inter-bat distances for all calls of a given type within a single recording, and then used paired *t*-tests to compare mean inter-bat distance at the time of calls vs. entire 8 s recordings for each call type.

RESULTS

CALL CLASSIFICATION

In 187 two-bat trials, recorded from 32 bats comprising 53 pairs, we identified seven distinct social call types shown in **Table 1**. We recorded a total of 764 vocalizations or call groups, henceforth referred to as social calls, which were distinct from echolocation calls. Only call types with at least 60 examples were included in the DFA; hence U calls were excluded.

Considering that the results from cross-validation DFAs using half of the data for training were very similar (92–94% correct classification) to those using all of the data at once, we report the results from the entire data set. Based on the results of this DFA, 94.9% of calls were correctly classified [MANOVA: Wilk's lambda $= 0.007$, $F_{(20,\ 2419)} = 413.03$, $P < 0.0001$]. Individual call types were correctly classified as follows: UFM, 92.1%; CS, 93.5%; SFM, 96.7% LFM, 97.5%; QCF, 80.3%; and FMB, 99.5% (**Figure 2**). The first canonical dimension explained 80.6% of the variation, while the next three dimensions explained 10.2, 5.9, and 3.3%, respectively. Inspection of the standardized coefficients (**Table 2**), which indicate how the variables are weighted to form each canonical axis, indicates that most (91%) of the variation among call types is due to differences in frequency, given that duration contributes very little to the first two axes. Based upon the DFA results, we treated these call types as distinct for subsequent analyses.

The mean duration of FMB (not including buzz-like calls) was 79.8 ms, with an average of 3.47 calls per bout (virtually always

Table 1 | Call parameter values for each call type.

Call type	Start frequency *X* ± *SD* (kHz)	Mid-frequency *X* ± *SD* (kHz)	End frequency *X* ± *SD* (kHz)	Duration *X* ± *SD* (ms)	Percentage of 528 recordings in which call(s) occurred	Total calls recorded
Upward frequency-modulated (UFM)	48.0 ± 7.8	53.4 ± 6.7	62.8 ± 9.6	15.0 ± 4.8	8.5	140
U-shaped (U)	50.8 ± 7.4	42.8 ± 8.2	51.1 ± 10.8	16.9 ± 6.6	3.03	26
Chevron-shaped (CS)	47.7 ± 9.0	55.4 ± 8.3	44.3 ± 10.3	16.6 ± 5.4	6.06	92
Short frequency-modulated (SFM)	39.0 ± 5.5	30.8 ± 4.4	25.6 ± 4.4	3.5 ± 1.2	9.7	91
Long frequency-modulated (LFM)[#]	42.6 ± 9.1	21.7 ± 5.8	18.1 ± 4.8	23.8 ± 13.6	7.6	163 (223 pulses)
Quasi-constant frequency (QCF)	44.1 ± 12.0	43.7 ± 13.3	41.9 ± 14.0	12.7 ± 5.2	5.5	66
Frequency-modulated bout (FMB)[#^]	69.2 ± 10.9	33.4 ± 8.1	17.3 ± 4.7	9.2 ± 0.8	35.2	186 (645 pulses)

[#] *The mean of all pulses within a call/bout was used when calculating means and SDs.* [^] *Values are for the first 3–4 calls per bout and do not include the shorter duration, buzz-like calls that often follow.*

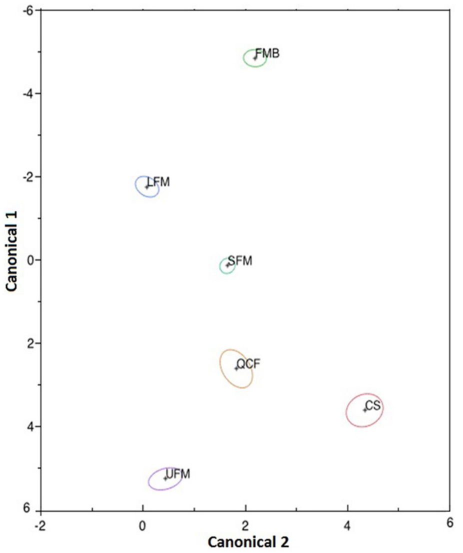

FIGURE 2 | Plot of the first two canonicals, which together explain 90.8% of the variation in the data, for each call. Each point represents the centroid for a given call type. Ellipses show the 95% confidence interval around each centroid. See **Table 2** for standardized coefficients that indicate how the call parameters contribute to canonical 1 and 2. Overall, 95% of calls were correctly classified to type. Call type abbreviations: UFM, upward frequency-modulated; CS, chevron-shaped; SFM, short frequency-modulated; LFM, long frequency-modulated; QCF, quasi-constant frequency; and FMB, frequency-modulated bout.

Table 2 | Standardized coefficients for the discriminant function analysis.

Canonical	Start frequency	End frequency	Mid-frequency	Duration
1	−1.386	0.819	0.803	0.129
2	−0.491	−1.411	2.095	−0.099
3	0.829	0.758	−0.732	−0.559
4	0.449	−0.007	0.177	0.837

3 or 4 calls). The mean duration of LFM was 37.4 ms, with an average of 1.36 calls per sequence (110 single calls, 46 doublets, and seven triplets).

CALL CONTEXT

Calls were produced at various times during 8 s recordings. Because recordings from skilled trials (and most mixed trials) ended with one bat taking the mealworm, the social calls recorded occurred during these 8 s segments. In naïve trials, no bat was taking the mealworm, so emitted calls were recorded at various 8 s intervals throughout the trial period.

Contingency tests (**Table 3**) show that type of trial, sex, and age each influence when five of the six social call types (separated by the DFA) are produced. In general, more social calls were produced when males were present, with the highest prevalence of calls occurring in male-male trials. FMB were produced exclusively by males and were never recorded from a naïve pair of bats. With regard to trial type, CS calls were more common in naïve than mixed or skilled trials and more common in mixed than skilled trials, and QCF calls were more common in mixed and naïve trials than skilled trials. In addition, SFM and FMB were significantly more prevalent in skilled trials compared with naïve or mixed trials, and FMB were more common in mixed than naïve trials. With regard to sex, UFM, QCF, and FMB were significantly more common in male-male than female-male or female-female trials, and FMB were also significantly more common in female-male pairs vs. female-female pairs (no FMB was recorded from any female-female pair). Finally, UFM calls were more likely to occur in adult–juvenile vs. adult–adult trials (**Table 3**, **Figure 3**). LFM calls were emitted independent of trial type, sex, or age.

Based on position data, we assigned 335 calls of the six types separated using the DFA to a specific vocalizing bat. Social calls were emitted by males and females, and juveniles and adults. These 335 calls were attributed to 14 individuals (six juveniles initially naïve to foraging task and eight skilled adults; nine males and five females). Of these calls, UFM were emitted by six males (three juveniles, three adults) and no female; CS were emitted by four males and one female (four juveniles, one adult); SFM were produced by four males and three females (all adults); LFM were

Table 3 | Differences in call prevalence based on trial type, sex combination, and age combination as determined by Pearson's Chi-Square statistics.

	UFM	CS	SFM~	LFM	QCF	FMB
Trial type	—	**N > Mi > S**	**S > Mi S > N**	—	**Mi > S N > S**	**S > Mi > N**
Sex	**MM > FM MM > FF**	—	—	—	**MM > FM MM > FF**	**MM > FM > FF**
Age^	**AJ > AA**	—	—	—	—	N/A

*Bold lettering indicates comparisons that are significant after a sequential Bonferroni correction. > indicates that the given call type was more common in the context to the left of the symbol.—indicates no significant difference for that comparison. N, naïve; Mi, mixed; and S, skilled trial type; MM, male–male; and FM, female–male; and FF, female–female trials; AA, adult–adult; and AJ, adult–juvenile. See **Figure 3** for distribution of calls across trial types and sex and age combinations.*

^Data pertaining to age refers only to naïve and mixed trial types.

~Because we found a significant interaction between sex and trial type for SFM, we tested for type effects within female–male (FM) and male–male (MM) trials separately. The data shown above for SFM refer to FM trials; there was no significant difference in trial type within MM trials only, and we had insufficient data to test within FF trials.

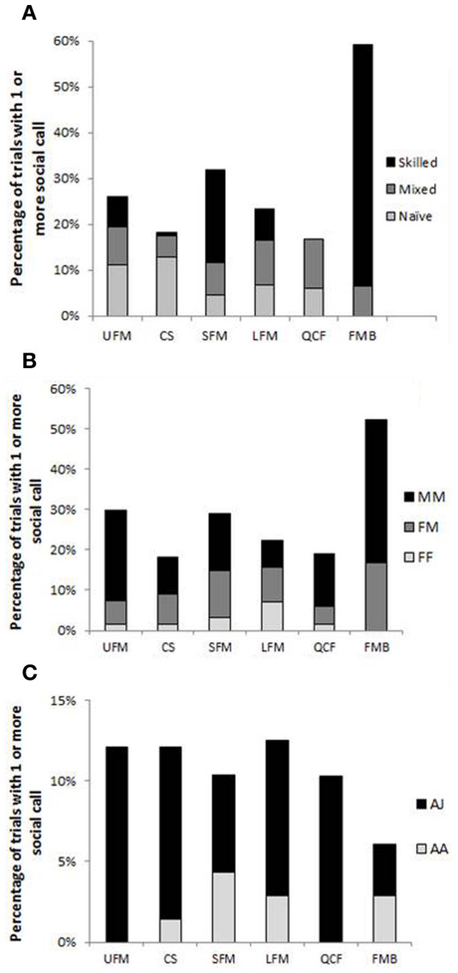

FIGURE 3 | Percentage of trials from each trial type (A), sex combination (B), and age combination (C) containing at least one instance of social calls of each type. See **Table 3** for related statistics. Because all skilled trials ($N = 152$) contained only adults, skilled trials are excluded from panel **(C)**. Mixed ($N = 170$) and naïve ($N = 181$) trials are mostly from adult–juvenile pairs, which is why calls from this age combination appear so much more common than social calls from adult–adult pairs in the figure. MM, male–male ($N = 121$); FM, female–male ($N = 256$); FF, female–female ($N = 126$); AJ, adult–juvenile ($N = 282$); and AA, adult–adult ($N = 69$ naïve and mixed trials) trial types. Call type abbreviations: UFM, upward frequency-modulated; CS, chevron-shaped; SFM, short frequency-modulated; LFM, long frequency-modulated; QCF, quasi-constant frequency; and FMB, frequency-modulated bout.

given by two males and three females (two juveniles, three adults); QCF were emitted by two males (one juvenile, one adult) and no female; and FMB were emitted by six males (one juvenile, five adults) and no female. Males were significantly more likely to emit UFM ($N = 32$ calls) and FMB ($N = 168$ calls) calls ($X_1^2 = 9.4$, $P = 0.002$ for each). Each call type was emitted by at least six individuals (based upon calls attributed to a certain bat and on bat pair composition), and with the exception of SFM, which were never assigned to a juvenile, every call type was emitted at least once by a juvenile, an adult, and a male.

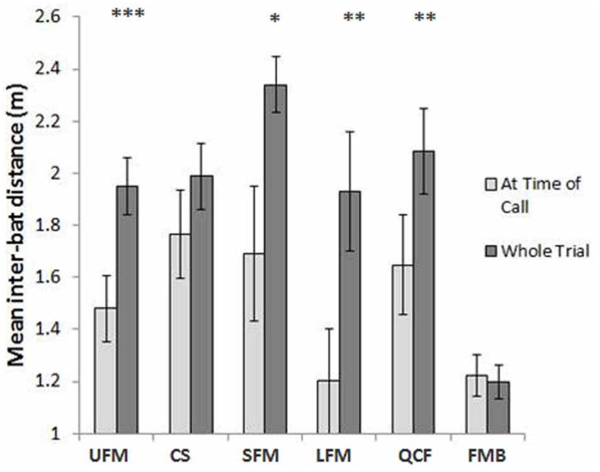

FIGURE 4 | Mean inter-bat distances before and after ("at time of call") social calls were emitted and for trials containing these types of social calls overall. * Indicates $P < 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.0001$. Error bars represent one standard error. Call type abbreviations as in **Figure 3**. The mean inter-bat distance for entire trials is smaller for trials containing FMB compared with other call types because almost all FMB were recorded from skilled trials, and skilled bats competing for prey tend to fly closer together and exhibit increased following/chasing behavior compared with naïve bats (Wright et al., 2011). The closer distances are not necessarily related to FMB production.

FLIGHT BEHAVIOR RESPONSE TO CALLS

Bats flew closer together around the time some call types were produced. Analyses show that UFM, SFM, LFM, and QCF were produced when individuals flew near each other. Bats flew significantly closer during the 1 s surrounding emission of these calls compared with complete recordings for UFM [$N = 61$ calls, paired $t_{(28)} = 4.85$, $P < 0.0001$], SFM [$N = 55$ calls, paired $t_{(26)} = 2.34$, $P = 0.028$], LFM [$N = 25$ calls, paired $t_{(7)} = 4.40$, $P = 0.0031$], and QCF [$N = 25$ calls, paired $t_{(15)} = 2.97$, $P = 0.0096$; **Figure 4**]. When most LFMs were produced, at least one bat was resting on the wall or out of camera view. Both bats were flying and in view of the cameras when only 15% of LFMs were emitted, so the data pertaining to inter-bat distance for this call type represents only a small portion of LFMs recorded in this study. We found no significant difference regarding inter-bat distance for CS [$N = 41$ calls, paired $t_{(19)} = 1.68$, $P = 0.11$] or FMB [$N = 72$ calls, paired $t_{(45)} = 0.347$, $P = 0.73$; **Figure 4**].

DISCUSSION

Vocal interactions mediate a variety of behaviors in bats (see Fenton, 1985), yet there have been relatively few descriptions of social calls emitted by flying bats, and even fewer where the identities and flight paths of individuals were known. In this paper we quantitatively differentiate six types of social calls from pairs of flying big brown bats, *Eptesicus fuscus*, and find that they occur nonrandomly depending on several factors. Each call type was emitted by several individuals, and prevalence of some call types differed depending on trial type, sex, and/or age. Some call types were also emitted more often when bats were in close proximity or when bats skilled at prey capture were flying, indicating that some calls likely influence foraging behavior as described below.

CALL CONTEXT AND FLIGHT BEHAVIOR

For call types that covaried with sex (UFM, QCF, and FMB), trials with more than one male were always more likely than female-only trials to contain social calls, with male-male trials yielding the highest prevalence of social calls. Frequency-modulated bouts (FMB) were produced exclusively by male bats. Despite this male bias in call production, we did not find evidence to support an exclusive mating-related function for any call type. First, we found no call type in September that was not also recorded in July and August. In Maryland, the peak of spermatogenic activity for *E. fuscus* is in August, and mating occurs between September and March (Kurta and Baker, 1990). While it is possible that captive bats might not maintain mating seasonality, our captive bats show a marked decrease in activity during the time they would naturally hibernate, indicating that they are still influenced by seasonal changes. Second, calls were emitted with either sex present rather than only in the presence of the opposite sex.

We did find support for the hypothesis that some calls are related to foraging. Specifically, SFM and FMB were emitted more frequently in trials in which bats had experience taking tethered insects. Considering that only one prey item was available, bats were actively competing for food, making it unlikely that these calls served to recruit conspecifics, as has been reported for *Phyllostomus hastatus* (Wilkinson and Boughman, 1998). Notably, we recorded FMB exclusively when at least one bat was knowledgeable in the foraging task. While additional work is needed to reveal the role of FMB, this call may serve a food defense function, as was demonstrated for a foraging-related social call produced by pipistrelle bats (Barlow and Jones, 1997).

Bats flew closer together 500 ms before and after the production of UFM, SFM, LFM, and QCF than during the 8 s recordings containing these calls (**Figure 4**). The tendency of bats in this study to fly closer together when emitting social vocalizations may indicate that they selectively produce calls when they are near a conspecific, or that there is a greater need for communication when flying in close proximity. For instance, if a call's function is food-related, call emission might not be necessary unless the competitor is close to the caller or the prey item. If the function of a call is to warn another bat to keep its distance or to reduce potential aggression, the same idea would hold true.

While some call types appear to be foraging-related, CS calls were recorded significantly more often in trials with two naïve bats, and bats did not fly closer together before and after emission of CS calls compared with other times. Higher prevalence of this call type in naïve trials (when no prey capture occurred) indicates that its occurrence is not positively related to foraging. Instead, foraging situations may reduce the frequency of its emission, possibly because bats are instead producing other foraging-related social calls. Additional possible functions of CS calls include appeasement or conveying aggression (e.g., Leippert, 1994; Gadziola et al., 2012), but further research is needed to determine their purpose.

AGE AND CALL PREVALENCE

While the data relating inter-bat distance to call type can include only events when both bats were flying and in view of both cameras, many calls were emitted when at least one bat was out of view (either flying or resting on the wall). Anecdotally, we observed juvenile bats resting on the wall emitting social calls, often audible to the human ear, each time the other bat approached it as it circled the room. Given that LFM was the only call type with a mean end frequency below 20 kHz (**Table 1**) that was commonly recorded when juveniles were present, it is likely that many of these calls were LFMs, which closely resemble calls recorded by Gadziola et al. (2012) in an appeasement context. Gadziola et al. (2012) state that appeasement calls "appear to promote social contact" between individuals (p. 11). When we recorded LFMs, both bats were flying and visible during call emission for only a small percentage of calls. Considering our observations and the results in Gadziola et al. (2012), it is possible that juveniles resting on the wall were emitting appeasement calls when approached by flying adults. It should be noted, however, that regardless of the function of LFM calls, they are not emitted exclusively by juveniles, and there was no significant difference in LFM prevalence in adult–juvenile compared to adult–adult trials. While the structure of LFM calls resembles that of isolation calls produced by *E. fuscus* pups, our findings do not indicate that this call is age-limited. Emission of isolation calls in *E. fuscus* is reported to decline by week 4 (Moss, 1988; Monroy et al., 2011), yet 49% of the 45 LFM calls positively attributed to an individual bat were produced by adults, and 85% of trials ($n = 40$) containing LFM calls were recorded from bats >28 days of age, including 30% of trials with only adult bats present.

QCF calls were never recorded in adult-only trials, while all call types were recorded in adult–juvenile trials. In addition, we found a higher prevalence of UFM calls in adult–juvenile trials compared with adult–adult trials. Because we did not always know the identity of the caller, we cannot say whether these results represent juveniles emitting more social calls, adults producing more social calls in the presence of juveniles, or both. One possible explanation is that juvenile-adult dyads create a different social dynamic than adult pairs, perhaps resulting in increased likelihood of appeasement-related calling by juveniles.

There is a paucity of literature reporting social calls from *E. fuscus*, but papers on vocal development in pups, and including some calls from adults, describe vocalizations resembling CS, LFM, and QCF calls (Moss, 1988) or U and LFM calls (Monroy et al., 2011). Some of the calls we recorded also show similarities to those Gadziola et al. (2012) recorded from crawling/roosting adult and juvenile bats. For example, their DFMs syllable, which was recorded in an aggression context, is structurally similar to our SFM, except that the former were usually emitted as a multi-syllabic call. Low frequency, multi-harmonic, calls resembling those described as aggressive calls by Gadziola et al. (2012; e.g., rBNBs and rBNBl) were not emitted by flying *E. fuscus* in our study but were often emitted when bats were being handled by humans. The time-frequency characteristics of these calls are distinct from short duration (0.5–1 ms) buzz-like calls, which we excluded on the basis that social buzzes may not be easily distinguished from feeding, inspection, or landing buzzes.

Gadziola et al. (2012) recorded calls very similar to our LFM calls, including couplets of calls (DFMl, shalDFMl, DFMl-QCFl, and DFMl-QCF-UFM), in an appeasement context. Additional call types were similar in some attributes (e.g., call shape) but not in others (e.g., call frequency) to the calls described in this paper. In general, the calls Gadziola et al. (2012) recorded from crawling/roosting bats were lower in frequency than the vocalizations we recorded from flying bats of the same species. Bats in flight may be more likely to employ social calls with frequencies overlapping with those of their echolocation pulses so as to use the echo return information from social vocalizations. Another possible explanation for use of higher frequency social calls in flight is that flying bats might reflexively increase the tension on their vocal membranes as they would to produce sonar calls. That some calls were recorded exclusively in a flying or a crawling/roosting context highlights the breadth of potential information bats could convey via communicative vocalizations and provides further evidence of context-specific use of such calls.

While relatively few papers present social calls from flying, foraging bats, each of the call types described here shares some spectral attributes with communicative calls recorded from other bat species in various contexts. For example, *Desmodus rotundus* isolation calls and calls emitted by mothers searching for their young (Fenton, 1985), as well as the alarm calls of *Tadarida brasiliensis* (Bohn et al., 2008), each contain portions that rise in frequency, as does our UFM. Chevron-shaped (CS) calls are produced by juvenile *Pteropus poliocephalus* (Nelson, 1964) in an isolation and location context, as well as by *Saccopteryx bilineata* in their territorial song (Behr and von Helversen, 2004) and by *T. brasiliensis* in directive and face rub calls (Bohn et al., 2008). Double-note calls emitted by *Myotis lucifugus* in maternity colonies and during swarming contain a portion resembling our U call (Barclay et al., 1979). As noted, our LFM resembles isolation calls, including those of *M. lucifigus* (Barclay et al., 1979), as well as showing similarity to a marking call of *T. brasiliensis* (Bohn et al., 2008), and social calls emitted by *M. bechsteinii* in maternity roosts and in flight (Pfalzer and Kusch, 2003) and *Pteronotus parnelli* (Kanwal et al., 1994). Our SFM and QCF calls bear some resemblance to the irritation and mounting calls, respectively, of *T. brasiliensis* (Bohn et al., 2008), and *P. parnelli* also produce lower frequency QCF calls in a social context (Kanwal et al., 1994). Finally, our FMB is similar in structure to individually-specific contact calls emitted by *Antrozous pallidus* (Arnold and Wilkinson, 2011). It should be noted that while the calls we describe here share some structural similarities with calls emitted by other species, the frequency ranges may not overlap. The variety of call types emitted, with calls of similar shape being used in very different contexts by different species, indicates that caution must be used when attempting to generalize call function based on spectral features alone.

This study uncovered a rich repertoire of social calls produced by flying big brown bats, *Eptesicus fuscus*, one of the most studied bats in North America. We found that males produced more social calls and that bats flew in closer proximity when emitting UFM, SFM, LFM, and QCF calls. By varying the context in which pairs of bats flew, we were able to determine that some call types are produced in a foraging-related context. These findings highlight the importance of inter-individual acoustic communication in bats as they forage, and lay the foundation for future research on the functional role of bat social calls in a variety of settings, both in the lab and the field.

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From the ultrasonic to the infrared: molecular evolution and the sensory biology of bats

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Great advances have been made recently in understanding the genetic basis of the sensory biology of bats. Research has focused on the molecular evolution of candidate sensory genes, genes with known functions [e.g., olfactory receptor (OR) genes] and genes identified from mutations associated with sensory deficits (e.g., blindness and deafness). For example, the *FoxP2* gene, underpinning vocal behavior and sensorimotor coordination, has undergone diversification in bats, while several genes associated with audition show parallel amino acid substitutions in unrelated lineages of echolocating bats and, in some cases, in echolocating dolphins, representing a classic case of convergent molecular evolution. Vision genes encoding the photopigments rhodopsin and the long-wave sensitive opsin are functional in bats, while that encoding the short-wave sensitive opsin has lost functionality in rhinolophoid bats using high-duty cycle laryngeal echolocation, suggesting a sensory trade-off between investment in vision and echolocation. In terms of olfaction, bats appear to have a distinctive OR repertoire compared with other mammals, and a gene involved in signal transduction in the vomeronasal system has become non-functional in most bat species. Bitter taste receptors appear to have undergone a "birth-and death" evolution involving extensive gene duplication and loss, unlike genes coding for sweet and umami tastes that show conservation across most lineages but loss in vampire bats. Common vampire bats have also undergone adaptations for thermoperception, via alternative splicing resulting in the evolution of a novel heat-sensitive channel. The future for understanding the molecular basis of sensory biology is promising, with great potential for comparative genomic analyses, studies on gene regulation and expression, exploration of the role of alternative splicing in the generation of proteomic diversity, and linking genetic mechanisms to behavioral consequences.

Keywords: echolocation, hearing, vision, olfaction, taste, perception

INTRODUCTION

Bats perceive the world by using a wide range of sensory mechanisms, some of which have become highly specialized (Altringham and Fenton, 2003). Vision is ineffective in complete darkness (although many pteropodids rely largely on vision in dimly lit conditions); hence most bats use echolocation for orientation, and often for prey detection and localization. The literature on the sensory biology of bats is therefore dominated by research on echolocation (Griffin, 1958; Thomas et al., 2004; Jones, 2005). Echolocation is now understood in depth from neurobiological mechanisms (Pollak and Casseday, 1989; Popper and Fay, 1995) through to behavioral and ecological correlates of signal design (e.g., Kalko and Schnitzler, 1998; Schnitzler and Kalko, 1998; Jones and Holderied, 2007). Bats use ultrasound and lower frequency sound for communication, and have evolved rich repertoires of social calls (e.g., Clement et al., 2006; Ma et al., 2006; Bohn et al., 2009; Carter et al., 2012). Considerable advances are being made to understand the role of sound in communication (Jones and Siemers, 2011; Puechmaille et al., 2011). In contrast, the roles of others senses in the lives of bats are less well-understood, even though these senses can be of fundamental importance. Ecological aspects of vision, olfaction, touch, and thermoperception are reviewed by Altringham and Fenton (2003) who concluded that "*with some notable exceptions, our knowledge about vision and olfaction has not advanced greatly since Suthers's (1970) review, compared to the enormous strides made in studies on echolocation.*" This stems partially from the great difficulty in observing and measuring these senses in wild, nocturnal flying mammals such as bats.

Recent years have seen considerable progress in our understanding of the genetic basis of sensory perception, attributable in part to advances in molecular genetics technologies and the associated abundance of new comparative sequence data. Most recent work has focussed on "candidate genes" associated with specific sensory traits. Candidate genes are genes known to be involved in pathways that affect phenotypes; sequencing these in individuals with unusual or different phenotypes can help identity mutations that can be related to adaptation (Stapley et al., 2010). For example, sequencing genes that possess mutations associated with non-syndromic deafness in humans has been valuable in identifying genes likely to be important in audition in other mammals, including bats, and understanding the molecular adaptations and mutations associated with auditory specialization and disease predisposition (Kirwan et al., 2013). One of the aims of this paper is to review studies on candidate genes associated with sensory perception in bats, and to show how these studies have elucidated our understanding of evolutionary processes, especially positive selection, convergent evolution and sensory trade-offs in which specialization in one sensory modality may result in reduced neural (and consequently genetic) investment in other senses (Harvey and Krebs, 1990). The identification of candidate genes is a first step in elucidating molecular mechanisms underpinning the sensory biology of bats.

In this paper we review advances in our knowledge of the genetic basis of sensory behavior in bats. We consider echolocation at the levels of both signal production and reception. We then describe how sequencing studies of genes associated with vision, olfaction, taste and thermoperception have revealed remarkable cases of convergent evolution, sensory trade-offs and novel adaptations. Gene symbol nomenclature is dynamic, and in this review we have followed the symbols used by the authors of the research papers on bats, though always presenting the symbols in lower case as is recommended for non-human homologues. Some of these gene symbols differ from those in the official nomenclature (see www.genenames.org), and the symbols used in the original papers on bats are listed alongside the official gene symbols and the approved gene names can be determined from **Table 1**. With molecular methods advancing rapidly, we conclude by outlining approaches that can potentially build on findings from candidate gene studies. We conclude by considering future opportunities for further developing this field, which has been one of the most fast-moving and exciting in research on bats in recent years.

Table 1 | Genes referred to in the text.

Sense	Gene symbol	Approved name
Echolocation	*FoxP2*	Forkhead box P2
	Slc26a5 (*Prestin*)	Solute carrier family 26, member 5 (Prestin)
	Kcnq4	Potassium voltage-gated channel, KQT-like subfamily, member 4
	Tmc1	Transmembrane channel-like 1
	Dfnb59 *(Pjvk)*	Deafness, autosomal recessive *59*
	Cdh23	Cadherin-related 23
	Pcdh15	Protocadherin-related 15
	Otof	Otoferlin
	Wnt8a	Wingless-type MMTV integration site family, member 8A
	Fos	FBJ murine osteosarcoma viral oncogene homolog
	Chrna10	Cholinergic receptor, nicotinic, alpha 10 (neuronal)
	Myo15A *(Myo15)*	Myosin XVA
	Ush1g	Usher syndrome 1G (autosomal recessive)
	Strc	Stereocilin
	Tectb	Tectorin beta
	Otog	Otogelin
	Col11a2	Collagen, type XI, alpha 2
	Gjb2	Gap junction protein, beta 2, 26kDa
	Cldn14	Claudin 14
	Pou3f4	POU class 3 homeobox 4
	Myo6	Myosin VI
Vision	*Rh1*	Rhodopsin
	Crx	Cone-rod homeobox
	Sag	S-antigen; retina and pineal gland (arrestin)
	Opn1sw *(SWS1)*	Opsin 1 (cone pigments), short-wave-sensitive
	Opn1mw *(M/lws)*	Opsin 1 (cone pigments), medium-wave sensitive
Olfaction	*OR*	Used to refer to the family of olfactory receptor genes
	Trpc2	Transient receptor potential cation channel, subfamily C, member 2
Taste	*Tas1r1*	Taste receptor, type 1, member 1
	Tas1r2	Taste receptor, type 1, member 2
	Tas1r3	Taste receptor, type 1, member 3
Thermoperception	*Trpa1*	Transient receptor potential cation channel, subfamily A, member 1
	Trpv1	Transient receptor potential cation channel, subfamily V, member 1

Nomenclature follows HUGO Gene Nomenclature Committee (www.genenames.org). Names used in papers cited in the text are given in brackets after the approved gene name. Approved names are for human genes, except for Trpc2 where the gene has become pseudogenized in humans where the mouse homologue (Mouse Genome Informatics—www.informatics.jax.org) is listed.

ECHOLOCATION

To better understand the implications of molecular studies for the evolution of echolocation, it is necessary to appreciate the current view on phylogenetic relationships among bat families. Evidence from a wide range of gene sequencing studies supports the hypothesis that bats using laryngeal echolocation (i.e., which produce signals in the larynx) are paraphyletic. Bats in the family Pteropodidae do not use laryngeal echolocation (though bats in one genus—*Rousettus*—echolocate by tongue clicking), but belong to the suborder Yinpterochiroptera that also includes laryngeal echolocators from the families Megadermatidae, Craseonycteridae, Rhinopomatidae, Hipposideridae, and Rhinolophidae (Teeling et al., 2005; Meredith et al., 2011). Some of these bats, notably the horseshoe bats (Rhinolophidae) and Old World leaf-nosed bats (Hipposideridae) arguably possess the most sophisticated echolocation systems known of all organisms. Indeed the close evolutionary relationship between the Pteropodidae and the families Rhinolophidae and Hipposideridae is surprising given that the latter have a particularly specialized sonar involving the emission of long constant frequency (CF) calls permitting the classification of insect prey, combined with broadband sweeps for localizing targets and the ability to adjust the frequency of emitted calls to compensate for Doppler shifts induced by their flight speed (Schnitzler, 1968; Trappe and Schnitzler, 1982; Hiryu et al., 2005). All the other 15 families of bats that use laryngeal echolocation, including the recently proposed Miniopteridae (see Miller-Butterworth et al., 2007) and Cistugidae (see Lack et al., 2010), are classified in the suborder Yangochiroptera (**Figure 1**; see also Teeling et al., 2000, 2005; Jones and Teeling, 2006; Meredith et al., 2011).

This phylogenetic arrangement of bats raises two alternative scenarios about the evolution of laryngeal echolocation. Either echolocation had evolved in the common ancestor of all extant bats, and was subsequently lost in the Pteropodidae [with echolocation evolving secondarily by tongue-clicking in cave roosting bats in the genus *Rousettus* (Möhres and Kulzer, 1956; Yovel et al., 2011)], or echolocation evolved independently (possibly even on several occasions) in the Yinpterochiroptera and the Yangochiroptera (**Figure 2**). It seems reasonable to assume that molecular genetic analyses should be helpful in discriminating between these hypotheses: the independent evolution of echolocation may have resulted in different genetic mechanisms being recruited for echolocation in different lineages of bats, while a single origin predicts that extremely similar genetic mechanisms will underpin echolocation in all bats and molecular loss-of-function should be evident in the pteropodids (predictions reviewed in Teeling et al., 2012). Anatomical evidence suggests that several bat species known from fossils in the Eocene were likely to have used echolocation, hence the ability to echolocate has been present in most bats during all of their known fossil history (Simmons et al., 2008; Teeling, 2009a; Teeling et al., 2012).

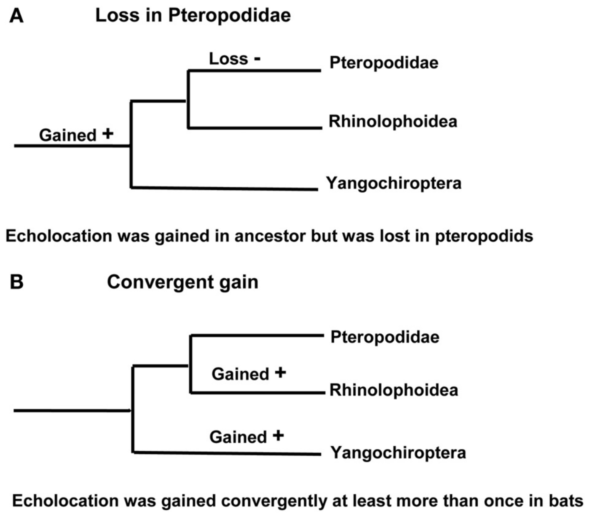
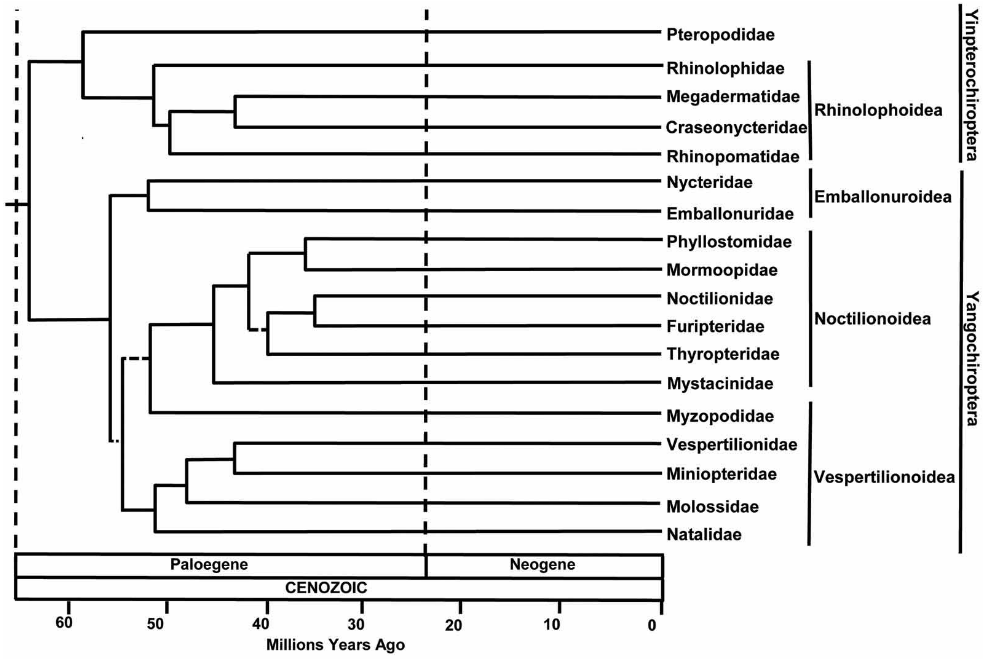


FIGURE 2 | Alternative hypotheses for the evolution of laryngeal echolocation. (A) Phylogenetic tree showing a single loss in the Old World fruit bats. **(B)** Phylogenetic tree in which echolocation was acquired independently by more than one lineage.



Dotted branches indicate clades not recovered with high support in Meredith et al. 2012.

FIGURE 1 | Most recent phylogenetic arrangement of bat families based on dating and consensus analysis of amino acid and DNA analyses. Dotted lines represent branches not recovered with high support. See Meredith et al. (2011) for details. A newly suggested family, Cistugidae, would be basal to the Vespertilionidae in this tree, having diverged from this family approximately 35 MYA (Lack et al., 2010). Reproduced with permission from the American Association for the Advancement of Science.

Reviews of some of the candidate genes likely to be involved in echolocation have been conducted by Maltby et al. (2009), Teeling (2009b), and Teeling et al. (2012), and the reader is referred to these for more detail.

VOCALIZATION

FoxP2 is a gene coding for a transcription factor associated with vocalizations and sensory-motor integration. Briefly, mutations in *FoxP2* affect production and comprehension of language in humans (see review by Fisher and Marcus, 2006) and two adaptive substitutions in *FoxP2* that occurred since humans split from a common ancestor with chimpanzees suggest that *FoxP2* was important in the evolution of human language (Enard et al., 2002). Although *FoxP2* is highly conserved in most mammals studied, it shows high levels of diversity, as well as evidence of divergent selection, in echolocating bats (Li et al., 2007; Zhang et al., 2013). Li et al. (2007) found exons 7 (likely to be important in the evolution of language in humans) and 17 to be especially divergent in bats compared with other mammals, and a recent whole-genome analysis detected even higher divergence in Exon 3 of *FoxP2* in *Myotis davidii* compared with the mammalian consensus sequence (Zhang et al., 2013). Because echolocation involves vocal behavior and extreme sensory-motor coordination it seems likely that the accelerated evolution of *FoxP2* in echolocating bats is related to the evolution of diverse types of echolocation strategies and their integration with subsequent motor behavior such as manoeuvring in flight (Li et al., 2007). However, molecular evolutionary analyses of two highly variable exons in *FoxP2* did not provide unequivocal insights into whether laryngeal echolocation evolved on more than one occasion in bats (also see Teeling et al., 2012). Moreover, to date there is no clear reason for the variation seen in *FoxP2* in bats. Examination of existing genome data suggests this gene is present as a single copy and, therefore, we can rule out duplication and neofunctionalization as a potential source of diversification. One explanation might be that *FoxP2* was recruited into the pathways underpinning echolocation early in the evolution of bats, and that observed sequence variation simply reflects the fact that echolocation is itself a highly variable trait that has undergone considerable divergence and convergence over the course of tens millions of years.

Gene silencing of *FoxP2* by lentivirus-mediated RNA interference is feasible (Chen et al., 2013), and opens opportunities for direct tests of whether *FoxP2* expression affects echolocation behavior in bats. Knockdown experiments show how *FoxP2* in the basal ganglia nucleus area X is important for accurate vocal imitation in birds (Haesler et al., 2007). Working with the CF echolocating bat, *Hipposideros armiger*, Chen et al. (2013) substantially reduced the typically high levels of *FoxP2* expression in the anterior cingulate cortex (ACC) of the brain, an area involved in motor control and important in vocalization (Paus, 2001). *FoxP2* silencing disrupts Doppler shift compensation in *H. armiger* confirming that it plays an important role in echolocation (Metzner and Schuller, 2009; Metzner and Zhang, 2009). These studies also found that *FoxP2* expression was higher in the suprageniculate nucleus and the ACC in the brains of bat species that use laryngeal echolocation (*Rhinolophus ferrumequinum*, *H. armiger* and *Myotis ricketti*), whereas in species without laryngeal echolocation (*Rousettus leschenaultii* and *Cynopterus sphinx*) expression was stronger in the olfactory tubercles (Metzner and Schuller, 2009; Metzner and Zhang, 2009). The identification of downstream neural targets affected by FoxP2 in bats remains as an exciting challenge; attempts to identify these binding targets in human neuron-like cells have revealed that FOXP2 either represses or activates gene expression at promoter sites involved in the modulation of synaptic plasticity, neurodevelopment, neurotransmission, and axon guidance (Vernes et al., 2007, 2011).

HEARING

A number of recent studies have focussed on candidate genes associated with audition. The membrane motor protein Prestin drives mechanical amplification of sound in the outer hair cells (OHCs) of the cochlea. Prestin functions by directly converting voltage to displacement and consequently acts several orders of magnitude faster than enzymatically-driven proteins (Zheng et al., 2000). Knockout studies of mice suggest that Prestin may enhance auditory sensitivity 100-fold (i.e., by 40 dB) by electromotility resulting from its mechanical elongation and contraction (Liberman et al., 2002). Molecular evolutionary studies identified positive selection acting on anion-transporter genes in the *Slc26* family, resulting in the evolution of the *Prestin* gene (formally known as *Slc26a5*) on the evolutionary branch leading to mammals: subsequently *Prestin* has been under strong purifying selection in many mammalian lineages (Franchini and Elgoyhen, 2006).

Phylogenetic tree reconstructions based on *Prestin* amino acid sequences recover an erroneous monophyletic group containing echolocating Yinpterochiroptera and Yangochiroptera lineages, rather than the accepted species tree in **Figure 1** (Li et al., 2008). This startling result, coupled with the absence of any detectable relaxed selection acting on *Prestin* in non-echolocating fruit bats, suggests that the Prestin protein may have evolved convergently in echolocating lineages. More recently, *Prestin* sequences from echolocating bats and dolphins have also been found to contain convergent amino acid residues (Li et al., 2010; Liu et al., 2010a,b), (**Figure 3**) and appear to be concentrated in areas of the protein involved in voltage sensing (Li et al., 2010). In total, Liu et al. (2010a) found 10 amino acid sites in *Prestin* that appear to have evolved convergently in echolocating rhinolophoid bats and toothed whales providing one of the most compelling examples of convergent sequence evolution yet described (see Christin et al., 2010 for a review of other cases).

Positive selection acting on *Prestin* was also detected in rhinolophoid bats that use Doppler shift compensation and which emit calls with long CF components (Li et al., 2008). Prestin confers auditory selectivity as well as enhancing sensitivity (Zheng et al., 2002), and this is probably important for bats that use Doppler shift compensation as they possess especially sharp hearing (auditory foveae) to separate pulses from echoes by frequency and enhance the detection of fluttering targets (Schnitzler and Denzinger, 2011). Although the moustached bat, *Pteronotus parnelli* (Mormoopidae), from the New World has independently evolved an echolocation system that uses long CF signals and Doppler shift compensation (DSC), it shares most amino acid changes in Prestin with its congeners and with phyllostomid bats

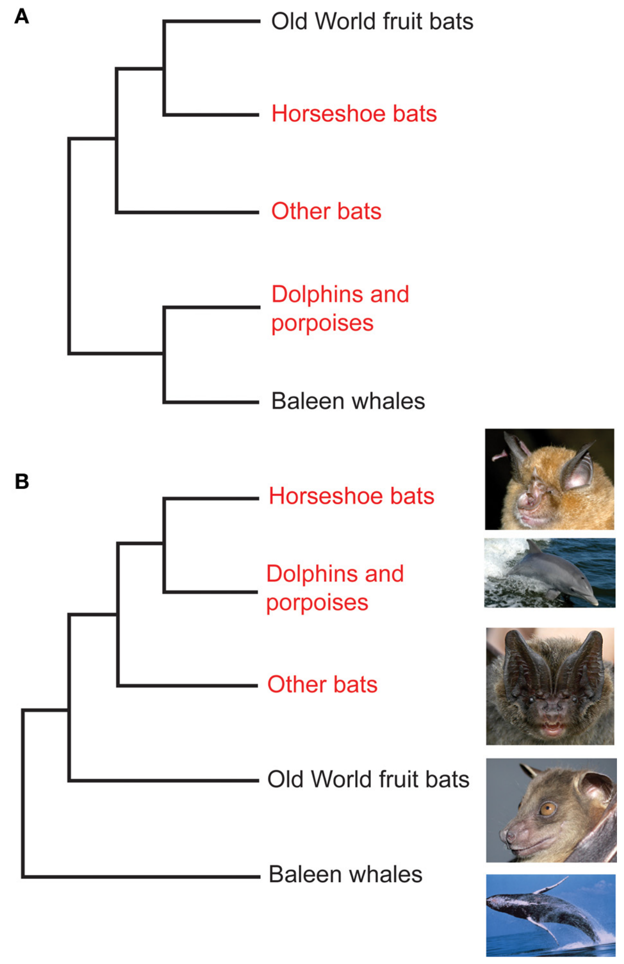


FIGURE 3 | Convergent evolution of prestin sequences in echolocating bats and cetaceans. (A) Phylogeny of Old World fruit bats (Pteropodidae), horseshoe bats and their close relatives, other bat lineages studied by Liu et al. (2010a,b) and Li et al. (2010), dolphins and porpoises, and baleen whales as determined from large-scale molecular sequence analyses. **(B)** The arrangement that arises from analysis of the *Prestin* gene. Bat groups highlighted in red use laryngeal echolocation and cetacean groups highlighted in red exhibit biosonar behavior. In **(A)** these echolocating taxa are paraphyletic—non-echolocating Old World fruit bats are sister to echolocating horseshoe bats, and echolocating dolphins and porpoises are sister to non-echolocating baleen whales. In **(B)** all echolocating taxa form a monophyletic group, and dolphins and porpoises are the sister group of horseshoe bats. Photographs are species studied by Liu et al. (2010a,b) and Li et al. (2010). From top to bottom they are the greater horseshoe bat *Rhinolophus ferrumequinum* (G. Jones), the bottlenose dolphin *Tursiops truncatus* (NASA), the Beijing barbastelle *Barbastella beijingensis* (J. R. Flanders), the greater short-nosed fruit bat *Cynopterus sphinx* (G. Jones), and the humpback whale *Megaptera novaeangliae* (NOAA). From Jones (2010) reproduced with permission from Elsevier.

that do not use DSC rather than with rhinolophoid bats (Shen et al., 2011). Hence the adaptive changes found in *Prestin* of rhinolophid bats are not necessary for CF echolocation and DSC in *P. parnelli*, and different evolutionary trajectories in *Prestin* evolution occur for this specialized form of echolocation.

Positive selection acting on *Prestin* in rhinolophid bats that use DSC could result from the extreme selectivity used in auditory processing by these bats, or could arise because these bats emit calls with relatively high frequencies relative to their body size (Jones, 1999). The extent of protein evolution appears to be linked to the evolution of high-frequency hearing (Rossiter et al., 2011). In particular, there are more non-synonymous mutations in *Prestin* in whale and bat species that emit higher frequency vocalizations (and are therefore assumed to be more sensitive to higher frequencies), and in toothed whales, and the relationship remains even after accounting for phylogenetic relatedness (Liu et al., 2010b).

The gene *Kcnq4* encodes a protein that acts as a voltage-gated potassium channel involved in the regulation of electrical signaling. It is expressed in the OHCs, especially at the basilar part of the cochlea (Kharkovets et al., 2000). Mutations in *KCNQ4* in humans can cause the progressive loss of high frequency hearing (Kharkovets et al., 2006) hence its evolution in bats is of especial interest. The molecular evolution of *Kcnq4* in bats shows several parallels with patterns seen in *Prestin*. Echolocating bats form a monophyletic group in the *Kcnq4* nucleotide and amino acid sequence trees, and five amino acid sites are shared between echolocating bats in both suborders [Yinpterochiroptera and Yangochiroptera (Liu et al., 2011)]. Reconstruction of ancestral sequences suggests that bats in the two suborders evolved mutations at two amino acid sites in parallel. Moreover the number of amino acid replacements is positively correlated with assumed frequency of best hearing in both the Yangochiroptera and the Rhinolophoidea (Liu et al., 2011).

Liu et al. (2012) independently confirmed the monophyly of bats that use laryngeal echolocation in gene trees based on Kcnq4 amino acid (but not nucleotide) sequences, and identified eight shared substitutions among lineages that may have evolved under parallel evolution. Surprisingly, none of the eight parallel substitutions identified by Liu et al. (2012) match those identified by Liu et al. (2011). Again, the arguments for parallel evolution were developed in part because there was no evidence for relaxed selection acting on *Kcnq4* during the evolution of Old World fruit bats that do not use laryngeal echolocation.

Mutations in the genes *Tmc1* and *Pjvk* (formally known now as *Dfnb59*) result in non-syndromic hearing loss in mammals. *Tmc1* encodes a transmembrane protein found in inner and OHCs in the cochlea, and may function in moving molecules to the plasma membrane, or may provide intracellular regulatory signals during hair cell development (Marcotti et al., 2006). *Pjvk* encodes the protein pejvakin, and mutations in the gene cause auditory neuropathy in humans and vestibular defects in mice (see Davies et al., 2011). As is the case with *Prestin*, phylogenetic trees based on coding sequences of both genes group echolocating bats as a monophyletic clade (Davies et al., 2011). Some genetic convergence between whales and bats that use echolocation is also apparent (Davies et al., 2011). Convergent amino acid changes in bat clades that use high-frequency signals in echolocation support the hypothesis that both genes may be associated with high-frequency hearing, and parallel mutations in *Tmc1* shared between *R. ferrumequinum* and *P. parnellii* imply convergent evolution associated with CF echolocation and DSC in this case (Davies et al., 2011).

Although much research has focussed on genes involved in voltage motility, Shen et al. (2012a) investigated genes (*Cdh23* and *Pcdh15*) associated with hair bundle motility in OHCs, and *Otof*, which encodes a protein that may trigger membrane fusion in ribbon synapses in inner hair cells and potentially functions in transmitting auditory signals to the brain. Mutations in all these genes are again associated with deafness in humans. *Otof* shows strong expression in the auditory cortex of adult bats that use laryngeal echolocation (*Miniopterus schreibersii*) compared with *Rousettus leschenaultii* that echolocates by tongue clicking [which is a sophisticated but non-laryngeal form of echolocation (Yovel et al., 2011)]. Parallel evolution in all three genes was suggested for three groups of echolocating mammals (Yinpterochiroptera, Yangochiroptera, and toothed whales) (Shen et al., 2012a). The authors suggest that parallel evolution has hence occurred in a number of auditory processes—voltage motility, cochlear amplification and neural transduction—and that the processing of echolocation signals involved coevolution of genes that are involved in a number of pathways during auditory processing. It is remarkable that multiple genes involved in different auditory processes have shown independent evolution in three groups of echolocating mammals (Shen et al., 2012a). Recent sequencing of the genomes of an echolocating and a non-echolocating bat (Zhang et al., 2013) suggested that further echolocation-related genes include *Wnt8a* and *Fos*.

Despite these above findings, it is important to emphasize that cases of sequence convergence in which substitutions lead to erroneous phylogenetic groupings are still rare and most genes, including hearing genes, are expected to recover the recognized species tree. Liu et al. (2012) analysed the molecular evolution of *Chrna10*, a gene that encodes the α10 nicotinic acetylcholine receptor subunit important role for mediating synaptic transmission between medial olivocochlear fibers and OHCs, and for the inhibition of somatic electromotility (Elgoyhen et al., 2001). Trees based on *Chrna10* amino acid sequences resembled the species trees rendering bats that use laryngeal echolocation paraphyletic (Liu et al., 2012). Kirwan et al. (2013) undertook phylogenetic and selection analyses of 11 genes implicated in hearing (*Myo15 (Myo15a)*, *Ush1g*, *Strc*, *Tecta*, *Tectb*, *Otog*, *Col11a2*, *Gjb2*, *Cldn14*, *Kcnq4* [which was reported as showing parallel evolution by Liu et al. (2012)], *Pou3f4*) and found good support for the paraphyly of echolocating bats across these loci as well as a high level of evolutionary conservation. Consequently it is apparent that as expected, only some hearing genes have been modified in bats during the evolution of echolocation, with others being subjected to purifying selection and perhaps being involved in more general aspects of audition rather than in specialized adaptations associated with echolocation. There is no evidence for positive selection acting on *Myo6* in echolocating bats (Shen et al., 2013), despite this gene being associated with hearing loss in humans (e.g., Oonk et al., 2013). Rather the gene is expressed at high levels in the kidneys of pteropodid bats, shows accelerated evolution in this lineage, and may have evolved in relation to the low protein intake from a frugivorous diet (Shen et al., 2013).

In summary, parallel evolution has been suggested for seven genes associated with a number of distinct auditory processing mechanisms in bats that use laryngeal echolocation. Although convergence seems a plausible explanation for similarities in genes seen between echolocating cetaceans and bats, is it really the case that convergent evolution has shaped the evolution of echolocation in yinpterochiroptean and yangochiropteran bats that use laryngeal echolocation? One evolutionary scenario is that the ancestor of all bats did not have the ability to echolocate, pteropodids never acquired it and that laryngeal echolocation convergently arose in the stem echolocating lineages. Another scenario is that laryngeal echolocation arose in the ancestor of all bats, convergently diversified in the extant echolocating lineages and was lost in the pteropodids (see **Figure 2**). A hypothesis of convergent gene evolution might predict that bats using tongue-clicking for echolocation (*Rousettus* species) would also have evolved convergent genetic mechanisms for auditory processing similar to those of laryngeal echolocators given the apparent sophistication of their biosonar (Yovel et al., 2011), although no such signatures have been seen.

Studies on gene convergence often emphasize that there is no evidence for relaxed selection acting on auditory genes in pteropodids that do not use laryngeal echolocation, which would suggest loss of echolocation capabilities, yet is an absence of relaxed selection in hearing genes truly indicative of loss of echolocation in pteropodids? Mammals rely heavily on hearing for survival; there is no non-pathogenic "deaf" phenotype observed in mammals (Kirwan et al., 2013). Therefore, the candidate "hearing" genes studied are under high purifying selection given that key mutations in these genes result in a deaf phenotype. True relaxed selection, which typically results in a loss-of-function mutation over time, should not be evolutionarily permissible. Therefore, given the conserved nature of these genes extensive relaxed selection should not be evidenced in pteropodids, even if echolocation capabilities were lost (Teeling et al., 2012; Kirwan et al., 2013).

In a recent comparative study of bat inner ear structures, Davies et al. (2013a) tackled this question of relaxed selection at the morphological level. The authors found that the cochleae of non-echolocating pteropodids showed little deviation from those of other non-echolocating mammals, whereas the cochleae of echolocating yinpterochiropterans and yangochiropterans were highly modified, and the latter showed evidence of a burst of morphological change following divergence of the two suborders. At the same time, this study revealed no clear support for a loss of echolocation in pteropodids. A related investigation of semicircular canal morphology in echolocating bats found that the two major clades of echolocating species differed in canal size and shape in relation to body mass and cochlear size (Davies et al., 2013b). While these two studies cannot offer firm conclusions about whether laryngeal echolocation evolved more than once in bats, they do hint at independent evolutionary pathways consistent with multiple acquisitions.

How can the fossil record help inform our understanding of the evolution of echolocation? Whether or not the Eocene fossil bat *Onchonycteris finneyi*, dated at 52.5 Mya, was able to echolocate on the basis of anatomical traits has been the subject of considerable debate; in particular the small relative gross cochlea size suggests it could not (Simmons et al., 2008, 2010; Veselka et al., 2010). In contrast, Eocene fossil bats from other genera such as *Icaronycteris* and *Palaeochiropteryx* have been found to possess relatively larger cochleae that are indicative of echolocation capabilities (also see Simmons et al., 2008 and references within). If correct, the proposed placement of these echolocating genera on consecutive branches outside of the crown group of extant echolocating bats would necessitate further gains of echolocation (see Simmons and Geisler, 1998), a scenario that is arguably less parsimonious than a single loss in pteropodids. Such conflicting signals between molecular and morphological datasets regarding the issue of the evolution of echolocation highlight a need for more integrated approaches combining fossil evidence alongside molecular evolutionary analyses. In this regard, the recent and surprising finding that combined large-scale phenomic and gene datasets recover a monophyletic group of echolocating bats (O'Leary et al., 2013) warrants further study. Ultimately, a single origin of echolocation followed by secondary loss in pteropodids would be better supported if fossilized ancestral pteropodids with anatomical characteristics of echolocation were found, or if pseudogenization of genes known to be specific for echolocation could be identified in non-echolocating taxa (Teeling et al., 2012). This is challenging given that pteropodids have a poor fossil record that anatomical features may become damaged during fossilization, and also for the reason that genes associated with echolocation are likely to be variants of genes fundamental to vocalization and hearing in more general contexts. However, it is only through the integration of these different fields that the evolution of echolocation in bats will be elucidated.

VISION

Vision is important for bats, especially for those bat species that do not echolocate. Vision can be effective over greater distances than echolocation and, although the latter provides more acuity (Suthers, 1970), bats use vision for orientation and for finding food (see review by Altringham and Fenton, 2003). Even in echolocating bats, prey detection may be multimodal, involving several senses (including vision), which are used according to perceptual constraints imposed by environmental conditions (Eklof and Jones, 2003). When vision and echolocation provide conflicting cues, visual cues are used preferentially (Chase, 1983; Orbach and Fenton, 2010). Recent research on the genetic mechanisms underpinning vision in bats has mainly focussed on the molecular evolution of light-sensitive pigments. These pigments consist of a membrane-bound G-protein-coupled receptor (an opsin) and a chromophore that undergoes photoisomerization when it absorbs light. Consequent conformational changes in the opsin result in transduction of signals, and thereby photons are transformed into electrochemical signals (Yokoyama and Yokoyama, 1996). Of course night vision has been understudied in bats, and is likely to involve a suite of adaptations in addition to opsin tuning. Hopefully some of the recent molecular evolutionary findings will inspire resurgence in research on behavioral aspects of vision in bats.

RODS

Rods are the dominant photoreceptors in bat retinae (Suthers, 1970). Rods are adapted for vision in conditions where light levels are low, and are the main photoreceptors found in nocturnal mammals. The opsin in rods is known as rhodopsin, and its high sensitivity confers monochromatic vision under dim-light (scotopic) conditions. Zhao et al. (2009a) sequenced approximately 94% of the coding sequence of the rhodopsin (*Rh1*) gene from 15 bat species, and found that the gene was intact in all species studied. The authors determined the spectral tuning of rhodopsin from its amino acid structure. Wavelengths of maximum absorbance (λ_{max}) were inferred as 497–501 nm, with most species having values at the upper extreme of this range (501 nm), fitting with the bats possessing the mammalian consensus compliment of critical amino acids. Rhodopsin has been under purifying selection during mammalian diversification, although rhinolophoid bats using high-duty cycle echolocation (species that emit CF signals with Doppler shift compensation) showed higher ratios of non-synonymous relative to synonymous mutations compared with other bats, perhaps as a consequence of relaxed selection (Zhao et al., 2009a).

Shen et al. (2010) amplified cDNA of *Rh1* from 15 bat species and recovered a different phylogenetic arrangement, with Pteropodidae forming a monophyletic group together with yangochiropterans to the exclusion of the yinpterochiropterans that use high-duty cycle echolocation. The authors argued that multiple incidences of convergent evolution in *Rh1* between yangochiropterans and pteropodids had occurred, though ecological factors that could have brought about such convergence are not clear. The same research team analysed evolutionary patterns in other genes involved in rod vision and adaptation to dimly lit conditions (Shen et al., 2012b). *Crx* is a photoreceptor-specific transcription factor involved in the differentiation of photoreceptor cells. *Sag* functions in desensitization of the photoactivated transduction cascade, and mutations in this gene can cause blindness at night in humans. Molecular signatures consistent with convergent evolution were detected in both genes, and was especially apparent in *Rh1* (two parallel changes in *Crx*, one in *Sag*) between pteropodid (Yinpterochiroptera) and emballonurid (Yangochiroptera) bats. The authors argued that the relatively large eyes found in both these groups of bats might utilize specialized rod-based visual mechanisms that resulted in convergent amino acid substitutions.

CONES

Color vision in mammals is achieved in part by the possession of opsin proteins sensitive to short and medium- to long-wavelengths of light (Yokoyama and Yokoyama, 1996). Most living mammals are dichromatic and have a short-wavelength sensitive (Sws1—official name Opn1sw) opsin that is most sensitive to blue-violet wavelengths, and a medium- to long-wavelength sensitive (M/lws—official name Opn1mw) opsin with peak sensitivity in the red-green part of the spectrum (Peichl, 2005). Several lineages of nocturnal mammal species have lost function in *Sws1*, which has become pseudogenized, rendering color vision impossible (Jacobs, 2013).

Zhao et al. (2009b) sequenced the *Sws1* gene in 32 bat species and the *M/lws* opsin gene in 14 species. Many bat species, like most diurnal mammals, appear at least potentially to be dichromats, with intact Sws1 and M/lws opsins. Why many nocturnal echolocating bats are potential dichromats deserves further research. Although the latter gene was conserved in all species studied, a loss-of-function of *Sws1* through pseudogenization was apparent in rhinolophoid bats that use high-duty cycle echolocation (i.e. species that use long CF signals and use DSC), and in some Old World fruit bats, especially in taxa that roost in caves (**Figure 4**). This loss-of-function appears to have arisen by independent genetic mechanisms in the ancestral nodes of the Hipposideridae and the Rhinolophidae, where stop codons or indels disrupted the open reading frame (ORF) of *Sws1* at different positions. Genetic evidence suggesting a loss of UV vision in bats with high-duty cycle echolocation and in cave-roosting pteropopids has also been supported by immunohistochemical evidence: after bats were stimulated with UV light, Fos-like expression in the primary visual cortex was more apparent in *Cynopterus sphinx* (a tree-roosting pteropodid) and *Scotophilus kuhlii* (uses low duty cycle echolocation) than in *Rousettus leschenaultii* (a cave roosting pteropodid) and *Hipposideros armiger* (uses high duty cycle echolocation) (Xuan et al., 2012).

Why all bats studied retained a functional M/lws opsin is unclear: perhaps the opsin may play a role in processes other than vision, for example the control of circadian rhythms (Zhao et al., 2009b). Ancestral reconstructions of amino acid sequences suggested that the ancestral vertebrate (and bat) short-wave opsin was ultraviolet (UV) sensitive, with a λ_{max} close to 360 nm. Because the Sws1 opsin has been under purifying selection in many bats, it could be that UV vision is important in many (mainly yangochiropteran) species. One phyllostomid (*Glossophaga soricina*) is indeed able to see UV stimuli, and UV signals may reflect strongly from flowers at low light levels (Winter et al., 2003). These recent findings on potentially functional opsins in bats should hopefully spur renewed interest in color vision in bats, and Zhao et al.'s (2009b) findings suggest that yangochiropterans should have better color discrimination abilities than rhinolophoid bats.

It is of interest that loss-of-function in *Sws1* occurs in bats with what is considered the most sophisticated type of biosonar known—high-duty cycle echolocation involving the emission of CF calls and Doppler shift compensation (Zhao et al., 2009b). This finding suggests that bats may be experiencing trade-offs associated with investment in the neural processing devoted to different senses. Such trade-offs have long been identified in investment in brain tissue (Harvey and Krebs, 1990) because of the extreme energetic demands imposed by neural processing (and even by signal production) (Niven and Laughlin, 2008). For example subterranean star-nosed moles show a reduction in the size of the visual cortex and an increase in the size of cortical regions associated with mechanosensory processing compared with the same parts of the brain in terrestrial hedgehogs (Catania, 2005). Obviously the development and maintenance of brain structures must have a genetic basis, and it is fascinating that potential trade-offs between vision and echolocation are now being identified through the process of pseudogenization leading to loss-of-function in sensory genes. Interestingly, the pseudogenization of *Sws1* for vision in the lineage of high duty cycle echolocators is also associated with accelerated evolution of *Prestin* for hearing in that lineage (Li et al., 2008; Zhao et al., 2009b).

OLFACTION

Olfaction is of great importance in the lives of bats. Frugivorous bats often use olfaction for finding food, and nectarivorous species can find flowers from scent cues. Furthermore, many bat species—perhaps all—use olfaction for communication including for mother-pup recognition, recognition of individuals and conspecifics. In some species for which olfaction appears to be of particular importance, specialized scent glands or tufts of hairs are used for the production and application of scent signals (see review by Altringham and Fenton, 2003).

Tetrapods possess two olfactory systems that have distinctive anatomical and neurophysiological bases (though potentially overlapping functions). All vertebrates studied to date, with the exception of some cetaceans (Kishida et al., 2007), possess a "main olfactory system" (MOS) for the detection of volatile stimuli. Smells are detected by olfactory sensory neurons in the olfactory epithelium in the nasal cavity. Olfactory sensory neurons send information to the main olfactory bulb in the brain, which in turn transmits information to the olfactory cortex and other brain regions. The Accessory Olfactory System (AOS) serves to detect fluid-based stimuli via a vomeronasal organ in the vomer (between the nose and the mouth). Nerve connections link the vomeronasal organ to the accessory olfactory bulb, and then signals are transmitted to the amygdala and the bed nucleus of the stria terminalis, and subsequently to the hypothalamus. Many tetrapods (including birds and many primates) lack an AOS, and the vomeronasal organ shows extensive variability in yangochiropteran bats (Bhatnagar, 1980). In a cladistic analysis of 18 bat families, Bhatnagar and Meisami (1998) concluded that the presence of a functional vomeronasal organ in phyllostomid bats, *Miniopterus* (Vespertilionidae) and *Pteronotus* (Mormoopidae) was the result of multiple gains, however, we suggest that multiple losses of an AOS is equally or more plausible.

THE MAIN OLFACTORY SYSTEM

Olfactory receptors (ORs) are expressed in the cell membranes of olfactory sensory neurons located mainly in a small region of the upper nasal epithelium and initiate signal transduction cascades that send nerve impulses to the brain. They belong to the class A rhodopsin-like family of G protein-coupled receptors (Niimura and Nei, 2007). Each OR cell expresses only one odorant receptor, though each receptor can combine with several different odorants. Information from ORs is translated by the brain into a receptor code that represents a specific scent (Rinaldi, 2007).

In general, *OR* genes constitute the largest family of genes in the mammalian genome, for example comprising about 6% of the protein-coding genes in the dog (Lindblad-Toh et al., 2005; Hayden et al., 2010). There is enormous variability in the number of *OR* genes among mammal species—mice have approximately 1500 *OR* genes, humans about 800 (Niimura and Nei, 2003).

FIGURE 4 | Mutations in the short-wavelength opsin gene *SWS1* mapped onto the species tree based on published dated phylogenies of bats. The tree shows substitution rates, indels, and stop codons. Numbers of insertions and deletions are illustrated by downward and upward triangles respectively. Inferred ancestral stop codons are shown by squares. Sequence logos show key changes in spectral tuning amino acid sites in which the height of the amino acid abbreviation is proportional to its posterior probability. Sequences with stop codons are shown in red font, with loss-of-function related to the presence of indels or stop codons illustrated by red branches. Branch lengths represent millions of years (MY), and numbers at nodes represent divergence times in MY. Numbers along terminal branches are ratios of non-synonymous to synonymous mutations after removing indels and stops. Note the loss of function associated with high-duty cycle echolocation and with cave roosting in pteropodids. From Zhao et al. (2009b), reproduced with permission from the National Academy of Sciences USA.

Species that rely heavily on olfaction have large numbers of *OR* genes, whereas animals that specialize in using other senses have fewer functional *OR* genes, and typically high levels of pseudogenization. About half of the *OR* genes in humans are pseudogenes for example (Niimura and Nei, 2007). It is argued that a sensory trade-off exists between vision and olfaction in primates—with many *OR* genes becoming pseudogenized after primates evolved trichromatic color vision (Gilad et al., 2004). A high level of loss-of-function in *OR* genes is apparent in the platypus, which relies largely on mechanoreception and electrolocation for detecting prey, and in echolocating cetaceans (Niimura and Nei, 2007; Hayden et al., 2010).

Given that sensory trade-offs may have resulted in high rates of pseudogenization in other mammals that use specialized senses including electrolocation, echolocation, and trichromatic color vision, it is pertinent to ask whether high rates of pseudogenization are also apparent in echolocating bats. To address this question and explore the evolution of olfaction in bats Hayden et al. (2010) generated new *OR* gene sequence data (∼2000 *OR* gene sequences) from aquatic mammals, semi-aquatic mammals, twelve bat species, and coupled these data with whole genome data from terrestrial mammals, resulting in ∼50,000 OR gene sequences from 50 phylogenetically and ecologically diverse species. They analysed these data using a combination of phylogenetic, principal component, and Bayesian assignment tests, and identified unique signatures of OR gene family usage in bats. They uncovered spectacular examples of *OR* gene losses in three independent lineages of aquatic and semi-aquatic mammals, yet convergent, selective retention of similar functional *OR* families.

Despite the importance of echolocation in the lives of many bats, there was no evidence of a sensory-trade off resulting in extensive "death" of *OR* genes—bats appear to show similar percentages of pseudogenes (10–36%—relatively low levels for mammals in general) regardless of whether they use laryngeal echolocation or not (Hayden et al., 2010—see **Figure 5**). The percentage of *OR* genes that have become pseudogenes in bats is indeed unremarkable for mammals in general [cf. 28% in rat (Nei et al., 2008)], and lower than the ratio in humans (52%—Nei et al., 2008). Echolocating bats did not have more *OR* pseudogenes than non-echolocating bats. Indeed, the lesser horseshoe bat *Rhinolophus hipposideros* uses CF echolocation with Doppler-shift compensation, and only 10% of its *OR* genes are non-functional (Hayden et al., 2010). This species shows loss-of-function in the *SWS1* opsin gene (see above, Zhao et al., 2009b), and so perhaps a trade-off between color vision and echolocation has occurred, although olfaction has remained of importance in the life of this species. In comparison with other bat lineages the number of *OR* genes and the percentage of *OR* pseudogenes is quite low in rhinolophid bats, similar to the putative ancestral mammalian *OR* condition. This suggests that there was no massive "birth and death" of *OR* gene families in this species, most likely resulting from their long history of advanced echolocation capabilities, little reliance on olfaction for prey acquisition but a requirement of olfaction, most likely for communication. The fact that *R. hipposideros* possess olfactory genes that are mostly functional (90%), yet at the same time has a relatively small olfactory bulb (Neuweiler, 2000) could be seen as paradoxical. It follows that both genetic and anatomical data, together with information on the directionality of trait evolution, are all needed to reliably track the evolutionary history of sensory trade-offs.

THE ACCESSORY OLFACTORY SYSTEM

There is also some evidence in support of sensory trade-offs affecting the vomeronasal system in tetrapods, as it has been lost in primates with trichromatic color vision and in birds with tetrachromatic color vision (Zhang and Webb, 2003). *Trpc2* is a gene that can be used to determine vomeronasal sensitivity as it is essential for vomeronasal signal transduction and has

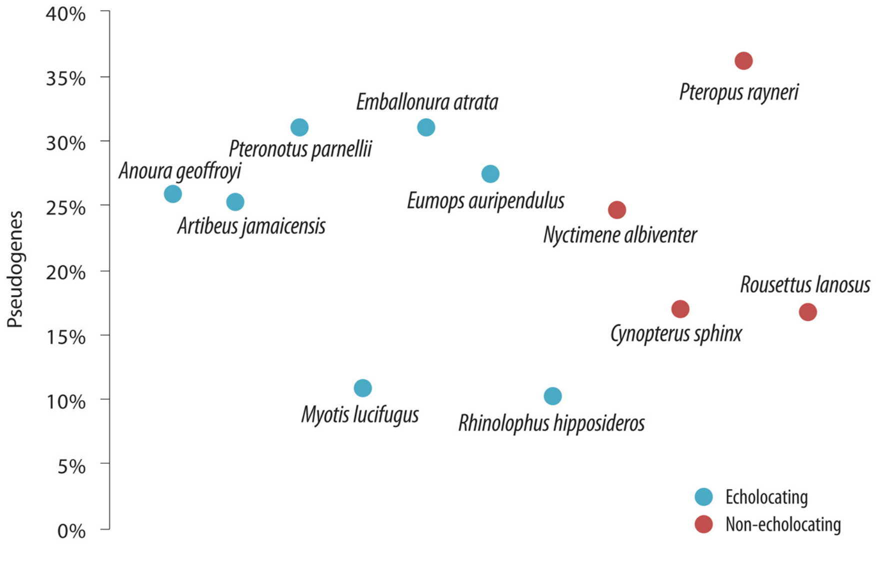


FIGURE 5 | The proportion of olfactory receptor (*OR*) genes that have become pseudogenes in a range of bat species that use laryngeal echolocation (blue symbols) compared with species that do not (red symbols). From Hayden et al. (2010), reproduced with permission from Cold Spring Harbor Laboratory Press.

no known alternative function (Grus and Zhang, 2006). Zhao et al. (2011) sequenced the longest exon (exon 2) of *Trpc2* from 13 bat species and found widespread loss-of-function (**Figure 6**). Multiple indels and premature stop codons were identified in all 10 yinpterochiropterans studied, with some suggestion of independent loss-of-function in Pteropodidae and Rhinolophoidea. Three yangochiropterans studied—*Miniopterus fuliginosus* (Miniopteridae), *Carollia perspicillata* and *Desmodus rotundus* (Phyllostomidae) showed intact exon 2 ORFs and the sequence was under purifying selection (Zhao et al., 2011). Examination of draft genome sequences for *Pteropus vampyrus* and *Myotis lucifugus* suggested that *Trpc2* had been pseudogenized in both species independently (Zhao et al., 2011). These findings are consistent with the anatomical findings of Bhatnagar and Meisami (1998) who reported functional vomeronasal organs in phyllostomid bats and *Miniopterus*, and only otherwise in *Pteronotus* among other bats from 18 families examined.

The extensive loss-of-function of the vomeronasal system in bats does not appear to be related to sensory-trade offs in any obvious way. Loss-of-function is apparent in echolocating and non-echolocating taxa, in dichromatic and monochromatic species, and is not related to the amount of pseudogenization in *OR* genes (Zhao et al., 2011). The only limited evidence for a trade-off occurs in vampire bats, which show loss-of-function in a sweet taste receptor gene but possess a functional vomeronasal system (Zhao et al., 2010a).

TASTE

Taste, or gustation, results from sensations produced when substances react with taste bud receptors in the mouth. There are five primary tastes—sweet, bitter, umami, salty and sour. Genes involved in the last two of these have not been studied in bats. Sweet, umami and bitter are sensed via molecules binding to G protein-coupled receptors (GPCRs) found on the cell membranes of taste buds.

SWEETNESS

Sweetness is useful for the detection of energy-rich foods such as sugars. A family of GPCRs known as Tas1rs functions in the detection of sweet and umami tastes. Only three *Tas1r* genes have been described in mammals, with the Tas1r2 and Tas1r3 heterodimer functioning in the detection of sweetness, and the Tas1r1 and Tas1r3 heterodimer functioning as the umami taste receptor. Hence Tas1r2 is thought to be the only taste receptor specific to sweetness, and *Tas1r2* knockout mice show disrupted responses to sweet taste (Zhao et al., 2003).

Zhao et al. (2010a) sequenced approximately 720 bp of exon 6 from *Tas1r2* in 42 bat species representing a wide range of families and dietary habits. *Tas1r2* evolved in the common ancestor of bony vertebrates, and the sequence analysed has remained conserved and under purifying selection in all bat species studies except for three species of sanguivorous vampire bats (Zhao et al., 2010a). The highly specialized diet of these bats has presumably made the need to discriminate among tastes redundant. Pseudogenization of *Tas1r2* in the three vampire bat species involved different ORF-disrupting mutations, though the relaxation of functional constraints may have already occurred in their common ancestor and the mutations documented in the relatively short portion of *Tas1r2* examined may have been the consequence of neutral evolution following an earlier pseudogenization event that preceded the evolution of sanguivory (Zhao et al., 2010a).

UMAMI

Umami is an appetitive taste, and humans perceive savory or meat-like tastes via umami receptors. Umami may function in the detection of amino acids that may signal nutritious food (Herness and Gilbertson, 1999). Using the same logic as described above for *Tas1r2*, Zhao et al. (2012) sequenced a portion of *Tas1r1* as a probe for the ability to taste umami in bats. Previous studies had shown the gene to be intact in all mammals studied except the giant panda (Zhao et al., 2010b). However, *Tas1r1* was absent, not amplifiable, or pseudogenized in all of 31 bat species studied, implying that the umami taste may have been lost in bats. Why bats—that exploit a wide variety of diets—do not need umami is unclear.

Vampire bats are especially interesting because all three of their Tas1rs appear to be non-functional (Zhao et al., 2012). Vampire bats are therefore unable to taste sweet or umami, and this fits with the lack of ability of common vampire bats *Desmodus rotundus* to learn aversions to harmful foods (Ratcliffe et al., 2003), and their indifference to high sugar concentrations (Thompson et al., 1982). Vampire bats are the only mammals so far known to lack two tastes. It is tempting to speculate that this represents a sensory trade-off with their functional vomeronasal systems and use of infrared heat sensing, though Zhao et al. (2010a) argue that the loss-of-function in Tas1r1 predated the origin of vampire bats. Whether it predated the evolution of sanguivory is of course debatable.

BITTER TASTE

The ability to detect bitter tastes is likely to be adaptive because bitterness is often associated with harmful food items. Whereas the likely consequences arising from molecular evolutionary patterns in sweet and umami tastes are relatively easy to predict because each the GPCRs involved is encoded by a single gene (Shi and Zhang, 2006), the situation regarding bitter taste is more complex. Taste receptors known as T2Rs are responsible for sensing bitterness. Although bitter taste receptors are also GPCRs, *T2R* gene repertoires are extremely variable among species, and as is the case for *OR* genes, evolved by extensive gene duplication and birth-and-death evolution that result in extensive gains and losses of *T2R* genes in all lineages of mammals studied (Dong et al., 2009). Zhuo et al. (2009) examined the *T2R* repertoire in the draft, relatively low coverage (1.7×) genome of the insectivorous little brown bat *Myotis lucifugus*. Twenty-eight *T2R* genes were detected in the bat genome, of which nine appeared intact, eight partial but perhaps still functional, and nine were pseudogenes. This compared with 37 functional genes and 11 pseudogenes in humans, and 37 functional genes and five pseudogenes in the rat. One clade of bat-specific genes was identified, implying that bitter tastants specific to bats may have evolved. Strong positive selection had shaped the evolution of the *T2R* gene repertoire in bats (Zhuo et al., 2009).

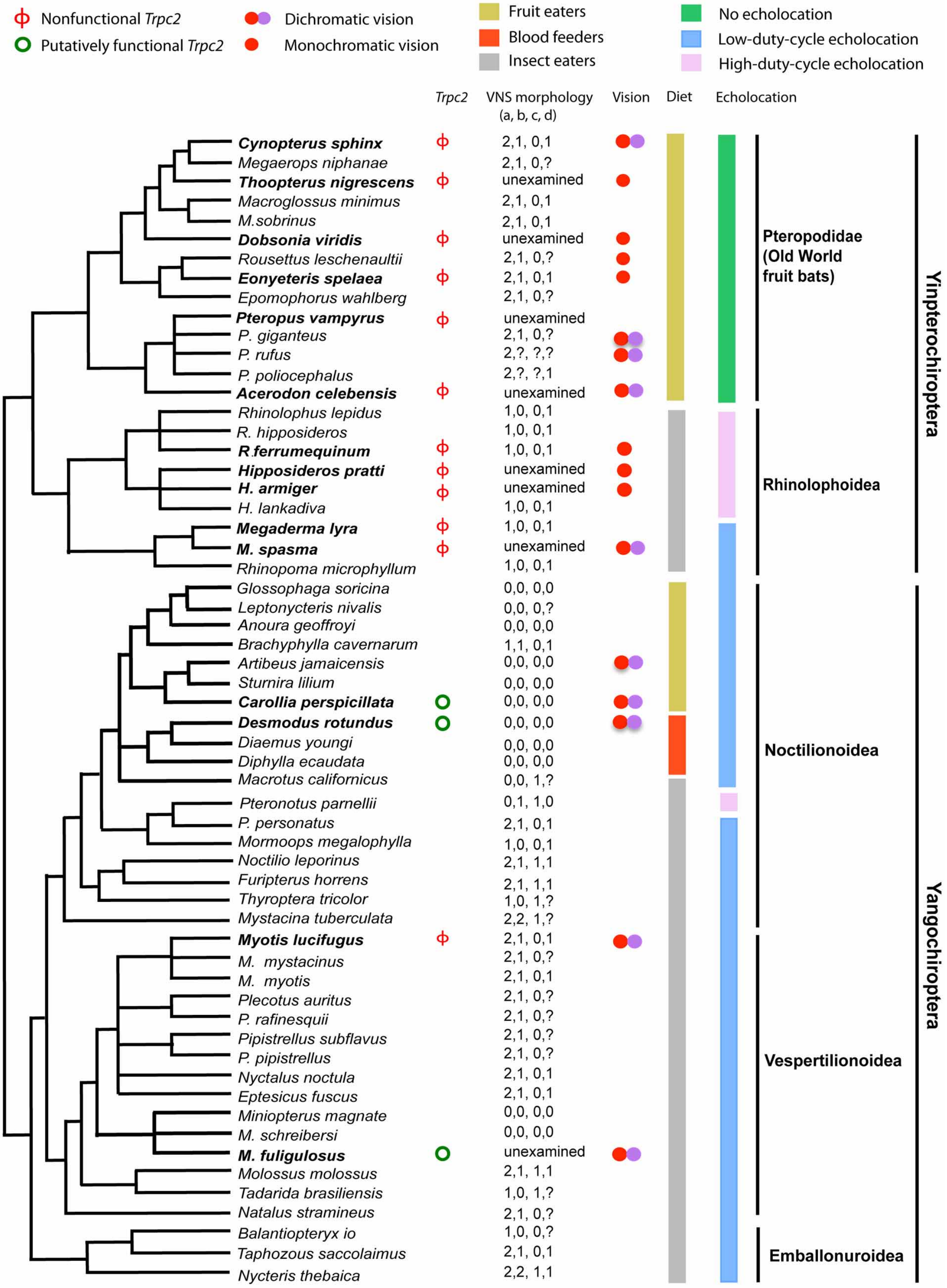


FIGURE 6 | Phylogenetic tree of bats for which information about the vomeronasal system (VNS) is available with information about *Trpc2* functionality, physiology and ecology. Species in bold text had exon 2 of *Trpc2* sequenced by Zhao et al. (2011). VNS morphology—"a" represents vomeronasal epithelial tube (0 = well developed, 1 = rudimentary, 2 = absent), "b" is vomeronasal cartilage (0 = J, C, U, or O-shaped, 1 = bar-shaped, 2 = absent), "c" is information about the nasopalatine duct (0 = present, 1 = absent), and "d" refers to the accessory olfactory bulb (0 = present, 1 = absent). Missing data are coded by "?". Note the limited functionality in *Trpc2*, that genetic functionality corresponds with anatomical functionality, and that functionality occurs in two divergent lineages of bats, suggesting multiple many losses of the VNS across the order of bats. From Zhao et al. (2011) and reproduced with permission from Oxford University Press.

THERMOPERCEPTION

The common vampire bat *Desmodus rotundus* is the only mammal known to possess heat-sensing organs. These bats have three 1-mm diameter pits situated between nasal pads and the noseleaf that are maintained at a cooler temperature than other areas on the face, and are used for the detection of warm temperatures on endothermic prey items that the bats extract blood meals from (Kürten and Schmidt, 1982). Similar structures may exist on the two other species of vampire bats (Altringham and Fenton, 2003).

Vampire bats detect infrared signals by trigeminal nerves that innervate the pit organs in ways that are in some respects convergent with but in other ways radically different from mechanisms of infrared detection by boas, pythons and pit vipers (Kürten et al., 1984; Gracheva et al., 2011). Although both groups use pit organs in the face (albeit in different regions) that are innervated by trigeminal nerves for heat detection, the heat-sensitive channels used by snakes and vampire bats for infrared detection differ significantly. Snakes modify a non-heat sensitive channel (the transient receptor potential A1 or TRPA1 channel) as an infrared detector (Gracheva et al., 2010). Vampire bats produce a shorter version of another member of the TRP family, TRPV1, which includes a small exon that contains a stop codon, by alternative splicing. Alternative splicing can generate a range of distinct RNA variants and consequently proteins with different functions from a single mRNA precursor by the differential joining of 5′ and 3′ splice sites. Gracheva et al. (2011) used an experimental approach—expressing the novel short version of TRPV1 from vampire bats in *Xenopus* oocytes and performing electrophysiological assays—to show the shorter version of the protein is activated at 30°C. Hence the vampire bats maintain the original function of the TRPV1 channel—noxious heat detection at temperatures >43°C, while also obtaining a novel ability to detect body heat for the detection of vital blood meals via the short variant of the protein. This study highlights how thermoperception can arise through mechanisms that involve similar nerve pathways but involve different molecular mechanisms, and illustrates the importance of alternative splicing in the evolution of novel adaptations.

THE FUTURE

Studies to date on the molecular basis of sensory biology in bats have focussed on determining patterns of molecular evolution in candidate genes that have known functions in humans and other model organisms. Often these genes have been targeted because of studies that detected phenotypic defects in humans resulting from mutations, as is the case with genes associated with vocalizations (e.g., dysphasia and dyspraxia resulting from mutations in *FOXP2*) and hearing (e.g., non-syndromic deafness resulting from mutations in hearing genes). Advances in transcriptomics and whole genome sequencing will allow genomic comparisons between mammals with different sensory abilities to be performed at a much larger scale and potentially identify novel genomic regions under sensory selection in bats. Next generation sequencing is making it increasingly possible to identify genetic loci responsible for adaptive evolution in non-model organisms, and the field of adaptation genomics holds great promise (Stapley et al., 2010; Hughes et al., 2013; Zhang et al., 2013).

Differences in gene regulation in bats have been little explored to date. These are likely to be important—for example replacement of the endogenous mouse *Prx1* gene regulatory element with the bat homologue causes limb elongation in mouse embryos by increasing Prx1 expression in the perichondrium, leading ultimately to longer forelimbs in the mice (Cretekos et al., 2008). Differences in patterns and the timing of gene expression, rather than solely changes in the genes themselves may play a major role in the evolution of sensory performance in bats, and yet studies on gene expression and on regulatory genes associated with sensation in bats are still in their infancy.

The importance of alternative splicing in generating proteomic diversity in bats remains largely unknown. Between 40 and 60% of human genes have alternative splice forms, and these comprise one of the major components of functional complexity in the proteomes of humans and other mammals (Modrek and Lee, 2002; Keren et al., 2010). For example, isoforms of the Slo protein expressed in the rat cochlea vary in deactivation kinetics and Ca^{2+} sensitivity, and their occurrence is partly determined by hormonal stress (Xie and McCobb, 1998). The importance of splice variants in bats remains largely unknown; however, Li et al. (2008) identified alternative splice forms of the *Prestin* gene in bat brain and cochlea tissue. Such isoforms might be expected to produce a range of functional outcomes from genes associated with audition in bats. Similarly the importance of other processes contributing toward functional diversity, such as RNA editing (e.g., Garrett and Rosenthal, 2012), is not known for bats and other mammals.

Studies on molecular evolution suggest major differences in the sensory performance of different bat lineages, and set a platform for exciting behavioral experiments. For example, the loss of function of *Sws1* in rhinolophoid bats suggests that these bats should be unable to perceive short wavelengths of light, yet yangochiropterans are dichromats and should have retained this ability. Although we do not know for sure whether intact genes result in the ability to detect short wavelengths (physiological features in the lens may for example influence this), the hypothesis that rhinolophoid and yangochiropteran bats show different abilities in their detection and discrimination between different wavelengths of light seems ripe for testing. Given that bats with intact vomeronasal signal transduction genes are indeed those species known to have functional vomeronasal systems, and that bats with pseudogenized sweet and umami taste receptors are unable to learn taste aversions suggests that linking the genetic basis of sensory behavior to sensory performance has great promise. Research on the sense of touch might also be illuminating. The recent discovery that tactile receptors on bat wings are sensitive to airflow (Sterbing-D'Angelo et al., 2011) makes unraveling genetic mechanisms underpinning the tactile sense in bats an interesting challenge.

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It's not black or white—on the range of vision and echolocation in echolocating bats

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Around 1000 species of bats in the world use echolocation to navigate, orient, and detect insect prey. Many of these bats emerge from their roost at dusk and start foraging when there is still light available. It is however unclear in what way and to which extent navigation, or even prey detection in these bats is aided by vision. Here we compare the echolocation and visual detection ranges of two such species of bats which rely on different foraging strategies (*Rhinopoma microphyllum* and *Pipistrellus kuhlii*). We find that echolocation is better than vision for detecting small insects even in intermediate light levels (1–10 lux), while vision is advantageous for monitoring far-away landscape elements in both species. We thus hypothesize that, bats constantly integrate information acquired by the two sensory modalities. We suggest that during evolution, echolocation was refined to detect increasingly small targets in conjunction with using vision. To do so, the ability to hear ultrasonic sound is a prerequisite which was readily available in small mammals, but absent in many other animal groups. The ability to exploit ultrasound to detect very small targets, such as insects, has opened up a large nocturnal niche to bats and may have spurred diversification in both echolocation and foraging tactics.

Keywords: yinpterochiroptera, yangochiroptera, FoxP2, swiftlet, oilbird, pteropodidae, hearing gene, eocene

INTRODUCTION

Echolocating bats use sonar (echolocation) to navigate in dark environments (Griffin, 1958). Other nocturnal mammals however (including most old world fruit bats) and nocturnal birds rely on other senses (such as vision, olfaction, or whisking) in similarly dark outdoor environments to orient (nearby), navigate (long-range), and forage. At first sight, vision and not echolocation seems the more apt sensory modality to invest in during evolution. Due to the hundreds of thousands parallel sensors (2D in each eye), vision conveys far more spatial information per time unit than echolocation (1D in each ear). Furthermore, bio-sonar information from natural scenes has a much lower angular resolution in comparison with visual information due to the relatively long wavelengths of sound compared to light. This also means that two "acoustic images" taken with a slight angular/positional difference will be much less correlated with each other than two consecutive visual images (Müller and Kuc, 2000). Indeed, all birds including those foraging in dim light, rely on vision when doing so (Thomas et al., 2002) and even those bird species (ca. 25 species) that have evolved bio-sonar seem to use it for orientation only and mainly in caves (Thomassen, 2005; Brinkløv et al., 2013).

Given these facts, why have most bats taken an entirely different path by opting for echolocation during their evolutionary development? Echolocation is surely advantageous over vision in extremely dark or lightless environments such as caves, but many bats customarily emerge from their roosts immediately after sunset at intermediate light levels (1–10 lux) when insect abundance peaks (Swift et al., 1985; Kon, 1989; Jones and Rydell, 1994; Rydell et al., 1996). In these bats, most feeding activity takes place during the first hours, thus many bats spend an important part of their foraging time at crepuscular light levels (>1 lux, Anthony and Kunz, 1977).

The extent to which bats rely on vision or a combination of vision and echolocation while foraging at such intermediate light levels is unknown. The eyes of echolocating bats have been shown to be adapted for nocturnal vision and are believed to impart best performance under ambient light that characterizes dusk (Bradbury and Nottebohm, 1969; Suthers and Wallis, 1970; Hope and Bhatnagar, 1979). Bats thus might rely on vision to a greater extent than commonly believed, but this must still be studied.

Here, we use a theoretical approach together with empirical data in an attempt to compare visual- and echolocation-based sensory performance focusing mainly on the detection range of objects provided by the two modalities. We compare two bat species that start foraging immediately after sunset, each having a different foraging strategy. We examine *Pipistrellus kuhlii* an edge space areal hawker that hunts for very small prey (e.g., mosquitos, Goiti et al., 2003) near clutter (e.g., vegetation) and *Rhinopoma microphyllum* which is an open space aerial hawker preying on large insects (mainly queen ants, Levin et al., 2009) far from clutter. *P. kuhlii* uses frequency-modulated search signals that can start as high as 95 kHz, level out at around 40 kHz, and last around 5–8 ms (**Figure 1A**, Kalko and Schnitzler, 1993), whereas *R. microphyllum* uses multiple harmonic search signals with a fairly constant frequency (quasi constant frequency, QCF) having the strongest harmonic at

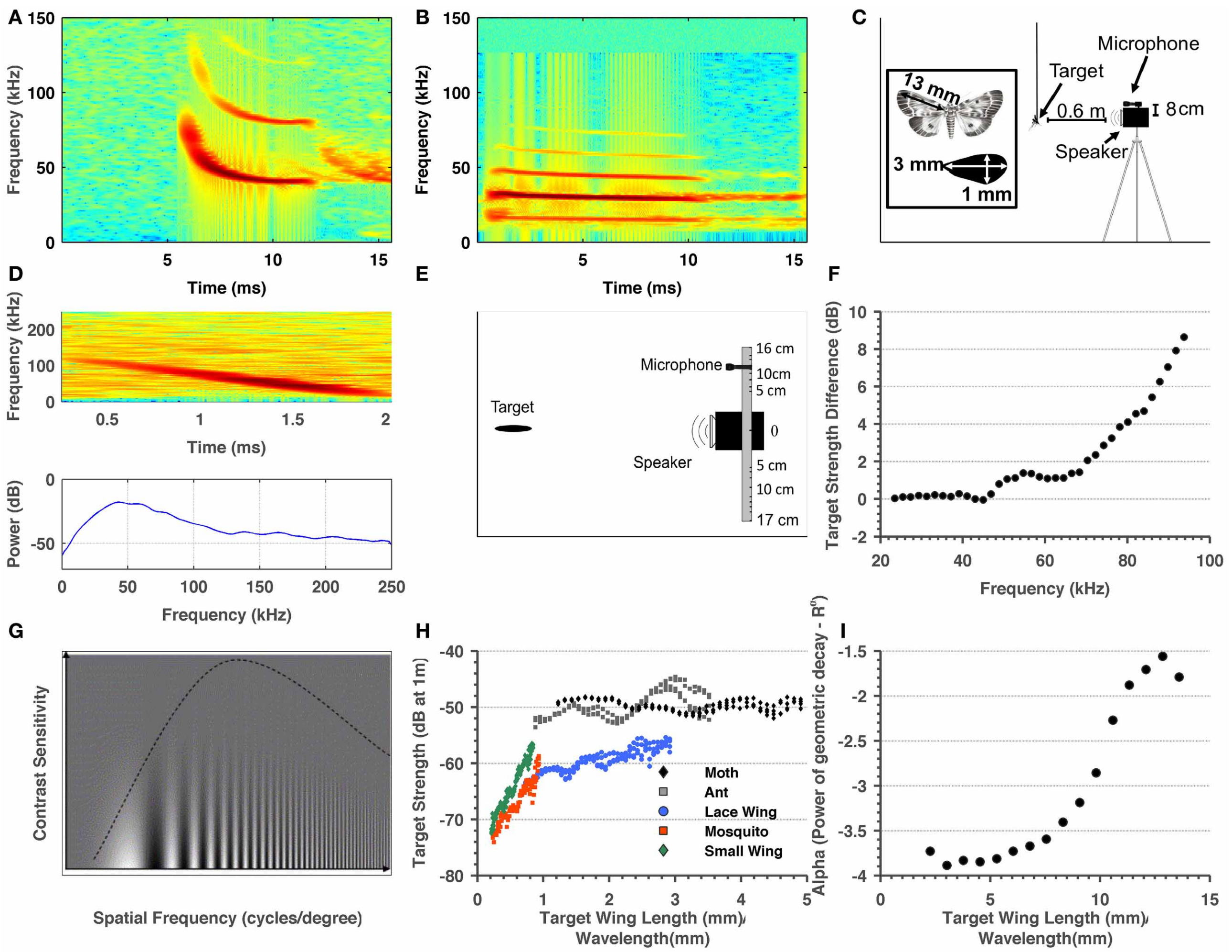

FIGURE 1 | Summary of methods and target-related parameters measured in order to estimate visual and echolocation detection ranges. (A) Spectrogram of a typical *P. kuhlii* search call. **(B)** Spectrogram of a typical *R. microphyllum* search call. **(C)** Sketch of the ensonification setup. **(D)** Spectrogram and spectrum of a single ant echo. **(E)** Sketch of the setup used to measure the directionality of the insects. **(F)** Target strength difference between the sweep echo returning directly (microphone at 0° relative to emitter) and returning from an angle corresponding to 8 cm difference in position between center of microphone and the center of the speaker. These measurements were used to correct target strength measurements. **(G)** Adopted from Beck et al. (2007). The dependency of visual acuity on spatial frequency (X-axis) and the inverse contrast (Y-axis). It can be seen that when contrast is lower (higher on the Y-axis) acuity decreases. **(H)** Target strength estimated for four insects and one artificial object as a function of the ratio between their size and wavelength. **(I)** Alpha—the power of the (2-way) geometric attenuation as a function of the ratio between size and wavelength of the 5 cm object.

28 kHz with a duration of 9–15 ms (**Figure 1B**). Both of these species (only the females in *Rhinopoma*) leave their roosts immediately after sunset when light levels are still high (>10 lux) and profit from at least an hour of hunting before darkness (<1 lux).

Our results suggest that between the two sensory modalities, vision is advantageous for the detection of large objects (e.g., cliffs, trees, etc.) and echolocation is advantageous for detecting small objects such as insects even when there still is some light. We therefore suggest that echolocation is advantageous over vision even in intermediate light levels when hunting for small prey. This finding implies a force that might have pushed the evolution of echolocation and may explain the extreme radiation and specialization found in the echolocation systems of modern bats.

METHODS

Throughout the methods whenever a parameter had to be estimated, we systematically chose parameters that overestimate the visual detection range and underestimate the echolocation detection range, motivated by the notion that if our results show any advantage of echolocation, the real advantage is probably more salient. Moreover, since we did this for several parameters, it is improbable that an error in the estimation of one single parameter would shift the general tendency we found (although it might shift the exact detection ranges).

ENSONIFICATION AND SOUND RECORDING

Equipment

Unless stated otherwise in all experiments described below, ensonifications of various targets were performed using a ScanSpeak ultrasonic dynamic speaker (Avisoft) connected to an UltraSoundGate player116 DA converter (Avisoft). Playbacks were performed with a sampling rate of at least 500 kHz. Recordings were performed using a condenser CM16 ultrasound microphone (Avisoft). Recordings were digitized using an UltraSoundGate 116 Hm device (Avisoft) and stored onto a laptop. Sampling rate was always 500 kHz. All analysis was performed with Matlab (R2012a). Ensonifications were always performed in a sound-isolated room with acoustic foam on all walls ("the experimental room"). Frequency responses and beams of the speaker and microphones can be found in the Avisoft website: http://www.avisoft.com/

Target strength measurements

All ensonifications were performed in a $3 \times 4 \times 2.5\,\mathrm{m}^3$ acoustically isolated room with all walls (and floor) covered with acoustic foam to minimize echoes (**Figure 1C**). Four real insects (moth—*Noctuidae*, ant—*Camponotus*, lacewing—*Chrysopidae*, and mosquito—*Chironomidae*) along with a small wing-shaped cardboard cutout were glued to the tip of a 250 μm diameter optical fiber and hung from the ceiling at the center of the experimental room. The lengths of the insects' wings (the long axis, **Figure 1C**) were 18, 13, 10, 3.5 mm correspondingly. The cutout was used to estimate how much of the target's strength can be attributed to the wings. It had an elliptic shape with a long diameter of 3 mm and a short diameter of 1 mm. The speaker was mounted on a tripod at the same height as the object, 60 cm away from it with its center of beam pointing toward the object (adjusted using a laser pointer). The signal emitted was a 2 ms linear FM chirp starting from 100 kHz down to 20 kHz. The recording microphone was placed on top of the speaker (ca. 8 cm above its center, **Figure 1C**). The target was ensonified from different angles, thus allowing echo recordings from all around the object. Several dozen echoes were collected for each angle. The wings of the targets (or the cutout) were spread perpendicular to the direction of ensonification to ensure a good estimation of the maximum target strength of a specific object. This echo was later used for the analysis (**Figure 1D**, see Target Strength Data Analysis). In the visual experiments, the wings were spread similarly to ensure a comparable cross section. The incident signal was measured by placing the microphone at the target's position and recording the signal. Emission and recording gains were adjusted by a known amount to ensure maximum signal-noise-ratio (SNR, while avoiding saturation). The fiber was ensonified without an object to ensure that it did not contribute any addition to the echo. No echo could be detected from the fiber alone (in time or spectral domain). This is not surprising when taking into account that the frequency equivalent to a wavelength of 250 μm is ca. 1.4 MHz.

Target strength data analysis. The recorded echoes were identified by cross correlation with the emitted signal, and the four strongest echoes were used for the analysis. The following analysis was done in order to avoid any inclusion of undesired echoes or noise: First, the frequency slope of the emitted signal was measured from the spectrogram enabling estimating a time-bin for each frequency band. Next, the mean-squared spectrum of this time-bin was estimated (MSS, Matlab) and the power of the relevant frequency band was extracted from it. The same procedure was repeated for the echo and the incident signal. The difference between emission and reception could now be measured (after correcting for gain adjustments).

Geometric decay measurements. In previous studies it was commonly assumed that an insect can be regarded a point reflector and thus that the geometric decay of its echo is proportional to the inverse of the fourth power of its distance ($1/R^4$). To validate this, we performed the following analysis: A 5-cm-long wing-shaped cardboard cutout (similar in shape to the one above) was glued to the optical fiber and hung from the ceiling of the experimental room. Target strength measurements (see results, **Figure 1H**) showed that when they are spread perpendicularly, the wings are a good approximation for the entire insect. We could not use a smaller object because of the sensitivity of our system, but since this object was larger than all of the objects we measured, if it behaves as a point reflector they would also do so. The speaker and microphone were placed as described above, but this time at increasing distances from the cutout, spanning from 50 to 100 cm. Echo analysis was performed as described above. For each frequency band the intensity decay over distance was plotted and a power function was fitted to the data (**Figure 1I**).

Microphone directionality compensation. In contrast with the expected theoretical results, target strength measurement showed a pronounced drop above 80 kHz. We hypothesized that this was an artifact resulting from the placement of the microphone 8 cm off-axis relative to the reflected echo (**Figure 1C**), a phenomenon that should become more pronounced in the high frequencies. To determine the extent of this effect, we repeated target strength recordings with a relatively large object (3 cm wing shaped paper cutout) placing the microphone at different azimuth angles relative to the reflection's axis (**Figure 1E**). This approach allowed us to estimate the effect of the angle on echo intensity across frequencies (essentially the beam of the reflected echo). We then used this estimation to correct the target-strength for the larger objects (i.e., moth, ant, and lacewing, **Figure 1F**). For the small-sized targets, frequencies above 80 kHz were discarded since the echoes were weak and measurements too noisy. It is important to note that this correction did not affect our detection range estimations since both bat species in the focus of this study call below 80 kHz. It only affected target strength results above 80 kHz.

R. microphyllum call amplitude measurements

Two wild *R. microphyllum* bats in northern Israel were caught in their roost and mounted with a 3.5 gr on-board ultrasonic microphone (Knowles, FG 23329) which recorded bats' echolocation for periods of 5 s every 30 s along one full night. Sampling rate was 94 kHz and the data was stored on an on-board flash memory. The devices were collected after several days by re-capturing the bats in their roost and the recordings were analyzed. Bats' call amplitude was determined by taking the peak or RMS voltages of the calls and converting them to dB SPL at 10 cm according to a calibrated 40 DP ultrasonic microphone (GRAS).

Calibration was performed using playbacks with the same speaker described above which were recorded by the on-board Knowles microphone and the GRAS microphone.

Because the on-board microphone was mounted on the back of the bat—it was glued using surgical glue (Permatype) between the scapula ca. 1 cm behind the head of the bat—we had to compensate for beam directionality in order to estimate the amplitude of the forward beam. Thus, a piston model (which was shown relevant for bats, e.g., Jakobsen et al., 2013) was used to estimate the difference between the peak of the main lobe and the amplitude of the call 180° behind it (Equation 1).

$$R_p(\theta) = \left| \frac{2 \cdot J_1\,(k \times a \times \sin(\theta))}{k \times a \times \sin(\theta)} \right| \tag{1}$$

Where: $R_p(\theta)$—the ratio between pressure on-axis and at an angle θ, J_1—first order Bessel function of the first kind, λ—the wavelength, $k = 2\pi/\lambda$, set to and 0.013 m, and a—the piston radius was set to 0.01 m (the bat is an oral emitter, a denotes the radius of its mouth).

This analysis resulted in a ca. −30 dB decrease at 150°. The piston model is symmetric thus having a peak equal to the main one at 180° which is not the case for the bat. Since we wanted to be sure not to overestimate echolocation detection range we used a safer –20 dB compensation value thus probably underestimating echolocation.

P. kuhlii call amplitude measurements

Wild bats were recorded in a park in Tel-Aviv using a 12 synchronized microphone array (USG1216H 12 channel A/D converter, Avisoft, Knowles microphones FG23329). The array was arranged with 10 microphones in a straight line (equally spread over 1.5 m at a height of 1.5 m above ground), and two additional microphones on a vertical axis, one 27 cm below and one on the ground 1.5 below the central horizontal microphone.

The recordings from 4 of those 12 microphones—the leftmost, middle, rightmost, and lowest ones—were later used to estimate the bat's position and thus reconstruct its flight trajectory. This was done by an in-house code (Matlab), which implemented a Time Difference of Arrival (TDOA) algorithm. This made it possible to estimate the distance of the call's origin from the microphones. Only calls that were part of a flight path heading toward the array (i.e., with their horizontal peak falling within the array) were analyzed. We could not tell if the bat was pointing its beam above the array. Actually this was probably the case because bats were flying above the array so our SPL estimations were therefore probably underestimations of the real emission levels.

The call's amplitude in dB SPL (peak and RMS) was then derived using a calibrated microphone (GRAS, 40 DP) which was calibrated relative to the array's microphones. Geometric attenuation was compensated for, assuming a 6 dB decay for every doubling of the distance. Atmospheric attenuation was accounted for with alpha = 0.3 m^{-1} (according to a temperature of 30°C and a humidity of 70%, taken from a table). Ambient light levels were recorded at the same time (see below).

Maximal echolocation detection range calculation

The maximal echolocation detection range was calculated by numerically solving the RADAR/SONAR equation (Skolnik, 1970) for the distance variable R.

$$\mathrm{P}_r = \frac{P_t \cdot \sigma_{bs} \cdot e^{-2\alpha(R-0.1)}}{\left(\frac{R}{0.1}\right)^4} \tag{2}$$

Where P_r is the power returning back to the bat's ear (per m^2, see below), P_t is the power transmitted by the bat, σ_{bs} is the backscattering cross-section, α is the atmospheric attenuation [alphas were 0.1 m^{-1} for *Rhinopoma* (28 kHz) and 0.3 m^{-1} for *Pipistrellus* (40 kHz) according to a temperature of 30° and a humidity of 70%] and R is the distance of the object from the bat.

The target's cross-section was calculated from the target strength by this formula:

$$TS = 10\log\left(\frac{\sigma_{bs}}{4\pi r^2}\right) \tag{3}$$

Where r is the distance from the target. In our case, the target strength was calculated at a distance of 60 cm, so r was set to this value. The transmitted power used was the maximal call strength measured in the abovementioned experiments, in dB SPL at 10 cm.

Following the debate in the literature about the hearing threshold of bats (Moss and Schnitzler, 1995), two alternative simulations representing the two extreme hypotheses were performed, one with the minimum P_r set to 0 dB (see for instance Kick, 1982), and the other with it set to 20 dB (see for instance Griffin et al., 1960). P_r essentially takes into account the brain's hearing sensitivity but also the ear's gain (or area) and is actually in units of W/m^2. In our opinion the 0 dB threshold is more suitable for our analysis because it represents the maximum hypothetical threshold bats exhibit in the lab while the higher threshold (20 dB) represents the actual sensitivity observed in the field (when noise in present). Since in the visual estimation (see below), we use the maximal hypothetical range estimated in the lab with no noise, the fair comparison would be the 0 dB threshold. Still, we show both results.

Visual experiments

The following measurements (Light Measurements, Contrast Measurements, Reflectivity Measurements) were necessary prerequisites for estimating visual detection range according to the methods which will be described below (Maximal Visual Detection Range Calculation).

Light measurements. Ambient light illuminance levels in the various experiments were captured by a Fourier Education MultiLogPRO data logger with a 0–300 lux light detector. The accuracy of the sensor is ±4% (thus ca. 0.04 lux for the range we were measuring). We define the range between 1 and 10 lux as intermediate light level. This ambient light is typical for the time of the day between dusk and complete darkness when many bats are active and many insects are available. We define darkness as ambient luminance <1 lux.

Contrast measurements. The four targets mentioned above were photographed at the same light conditions against two different backgrounds: sky and vegetation. The photos were taken by a Canon EOS Kiss X5 camera set without flash. Pictures were taken from around the time bats emerge from their roosts until darkness (i.e., 1–10 lux).

The Weber contrast is essential for calculating the detection range in our first method. It represents the contrast between the object and the background and was calculated by measuring the average pixel amplitude of the target and of the background (only for the red sensors).

$$\text{Weber contrast} = \frac{I - I_b}{I_b} \tag{4}$$

Where I is the intensity of the object and I_b is the intensity of the background (i.e., sky or vegetation). We only used the higher contrasts (e.g., with the sky background) thus overestimating visual detection range. We discuss the effect of lower contrast in the discussion.

Reflectivity measurements. Target reflectivity is the proportion of the photons that hit the target returning from it. It was used in the second approach for calculating the visual detection range. To measure target reflectivity the targets described above were taken to a dark room in which the walls are black assuring minimal light reflectance, and hung from the ceiling attached to the optical fiber. They were photographed by a Canon EOS Kiss X5 camera set to a $1/60''$ exposure time and an aperture of $f/4$, with a constant flash burst. The reflectivity was calculated by comparing the target's pixel intensity to that of a white board (100%) while making sure that the white is not bleached (stayed under the saturation level of the camera-sensor).

$$\text{reflectivity} = \frac{I}{I_b} \tag{5}$$

Where I is the intensity of the object and I_b is the intensity of the white paper. The values calculated were: Moth—0.6, Ant—0.3, Lacewing—0.55, and mosquito—0.45.

Maximal visual detection range calculation. Two different approaches were used to estimate the maximal visual detection range for the experiment targets. The first is based on the visual acuity measurements which are a measure of the minimum resolution angle found in previous studies (see Table 2 in Eklöf, 2003, for a summary). Because visual acuity measures the maximal resolution range, and we were interested in the maximal detection range (which might be longer), we had to find a way to translate visual acuity into a detection threshold (or sensitivity).

We relied on the results of Lie (1980) who showed that in the far periphery of the human eye (where photo-receptor composition includes rods-only and should be most similar to the bat's eye) the minimum detection angle is ca. 3.5 times smaller than the minimum resolution angle for contrast levels similar to the ones found in our study. We compared Lie's measurements in the photopic or the scotopic regimes and both generated similar results. For *P. kuhlii* we used an acuity angle (0.8°) smaller than that found for the species that are phylogenetically closest to ours (0.9°, *P. rueppellii* and *P. nanus,* Table 2 in Eklöf, 2003) and the same as the smallest angle measured for any vespertilionid (Suthers and Wallis, 1970). Since there was no estimation for a *Rhinopomatidae* bat, for *R. microphyllum* we took a value that is close to the smallest value found for any bat—0.5° (Table 2 in Eklöf, 2003, e.g., Suthers, 1966; Chase, 1972). It should be emphasized that bat acuity measurements found in the literature for micro-bats vary a lot ranging between 0.3 and 5° (Altringham and Fenton, 2005) and we chose values that are very close to the lower bound to ensure overestimation of the visual detection range. The maximal detection range was then derived following basic geometry:

$$D = \frac{S}{2 \cdot \tan\left(\frac{V}{2 \cdot 3.5}\right)} \tag{6}$$

Where D is the detection range, S is the target's longest dimension, and V is the minimum acuity angle converted into radians. 3.5 is the factor taken from Lie (1980).

It is important to note that the visual acuities of the species that we used (i.e., 0.8 and 0.5°) were estimated for stimuli with much higher contrast than any of our targets (white and black stripes, e.g., Bell and Fenton, 1986), and therefore this compensation of 3.5 is likely an overestimation (see **Figure 1G** to see how acuity depends on contrast). Moreover, Hecht and Mintz (1939) actually showed that visual acuity and visual sensitivity are virtually the same (in humans) as light intensity approaches threshold. In fact, the only study that tested visual range (or sensitivity, Bell and Fenton, 1986) found a value of 1° for *Eptesicus fuscus* which is very similar to *P. kuhlii* in both its echolocation signal and foraging style. This implies a 4 time over estimation in our study ($0.8/3.5 = 0.22°$).

Because the visual measurements above were based on several assumptions, we used a second different approach to validate our estimations. This approach was to directly estimate the photon flux necessary for object detection by a bat. This approach can be thought of as equivalent to estimating the minimal sound pressure level required for sound detection. Here, we relied on the results of Ellins and Masterson (1974) that tested the big brown bat's (*E. fuscus*) discrimination performance of a white vs. a black card under different light conditions.

The photon flux (photons per unit area per second) of a reflecting object at a distance D can be estimated from to the ambient illuminance E (light power per area—lux), the reflectance of the object ρ (measured in percent, %), and the object's area, A (m^2), according to the following proportion (Ryer's, 1997):

$$\text{Flux Intensity} \propto \frac{E \cdot \rho \cdot A}{\pi \cdot D^2} \tag{7}$$

Note that flux depends on the available photons (E) and the object's "visual target strength" ($\rho \cdot A$) and decays according to geometric spreading ($1/D^2$). Ellins and Masterson (1974) found that bats perform at chance level, and thus could not detect the object anymore for a white object positioned at a distance of $D = 0.37$ m with an area of $A = 51.6\,\mathrm{cm}^2$, a reflectance of $\rho = 89.5\%$, at a light level between 0 and 0.00079 lux (we thus used the middle $E = 0.000395$ lux).

When plugging these numbers in equation 5 one reaches a threshold of 4.24e-6 lux/steradian. This is thus an estimation of the minimal photon flux necessary for detection of an object by the big brown bat. The visual acuity reported for this species is 0.7–1° thus similar to the acuities we used above (Bell and Fenton, 1986; Koay et al., 1998). We could now use this threshold along with the reflectivity measurements of the objects in our experiment (ρ, see above) and the targets' surface area (A, measured with an image processing tool—imageJ) to estimate D (Equation 7)—the maximum detection range for the objects in this study under different ambient light levels (5 or 10 lux). A was estimated with the insect wings spread perpendicular to the camera, thus in a posture comparable to the ensonification posture. Notice that our estimations thus assume that detection range increases linearly with illuminance which is very likely an overestimation.

RESULTS

DO BATS USE ECHOLOCATION UNDER INTERMEDIATE LIGHT LEVELS?

Some studies have implied that bats "turn-off" echolocation when light is sufficient to use vision (e.g., Bell, 1985). We therefore first had to prove that the bat species in the focus of this study use echolocation under intermediate light levels. To do this, we monitored changes in calling rate and calling intensity. On-board recordings of *Rhinopoma* during the first hour after sunset show that these bats do not increase call intensity or call rate as light levels decrease (**Figures 2B,C**). Statistical analysis actually showed a significant decrease in calling rate (One-Way ANOVA for each bat, $F_5 > 11$, $P < 10^{-9}$), but we believe this to be a result of bats flying with fewer conspecifics as distance from the roost increases. In the *Pipistrellus* bat we could not quantify call rate, but we can report that all catching maneuvers observed by us were accompanied by feeding buzzes independently of ambient light levels. We found significant changes in call intensity which nevertheless did not reveal any systematic increase or decrease over time in *Rhinopoma*, and no significant changes in *Pipistrellus* as light levels decreased (One-Way ANOVA for each *Rhinopoma*, $F_5 < 2.5$, $P < 0.05$ and One-Way ANOVA for all *Pipistrellus* bats, $F_3 < 2$, $P > 0.05$, **Figures 2A,B**). These results suggest that echolocation is used by these bats irrespective of ambient light levels as long as they are below 10 lux.

ECHOLOCATION DETECTION RANGE

It is very hard to estimate the exact detection range for a small object (e.g., an insect). The RADAR/SONAR equation (Equation 2) is usually used for this purpose, but two of its important parameters, the hearing sensitivity of the bat and the target strength of the object, are difficult to measure. Moreover, one can measure the bat's signal intensity (peak or RMS) but this signal is usually composed of many frequencies while it is not clear how to model the brain's temporal-spectral integration for such a signal. To estimate detection ranges we measured the target strength of five objects with different sizes, we measured bats' emission intensity and estimated the geometric attenuation factor. Bats' emission intensity corresponded with estimations for other species varying around peak levels of 130 dB SPL (Holderied and Von Helversen, 2003; Surlykke and Kalko, 2008). Our target strength measurements (**Figure 1H**) confirm previous findings (Waters et al., 1995; Houston et al., 2004). The measurements also showed the expected relation between target strength and the ratio between the size of the target and the wavelength, i.e., a steady increase for ratios smaller than 1 and saturation thereafter.

Detection ranges were estimated for two hearing thresholds (0 and 20 dB SPL, **Tables 1,2**) and ranged between 2 and 3.5 m in *Pipistrellus kuhlii* and 2 and 6.5 m for Rhinopoma microphyllum for the higher threshold, and between 4 and 7 m (*P. kuhlii*) 5.5 and 14 m (*R. microphyllum*) for the lower threshold when using peak emission levels. Interestingly, our data suggests that *R. microphyllum* performs better for all objects due to its lower

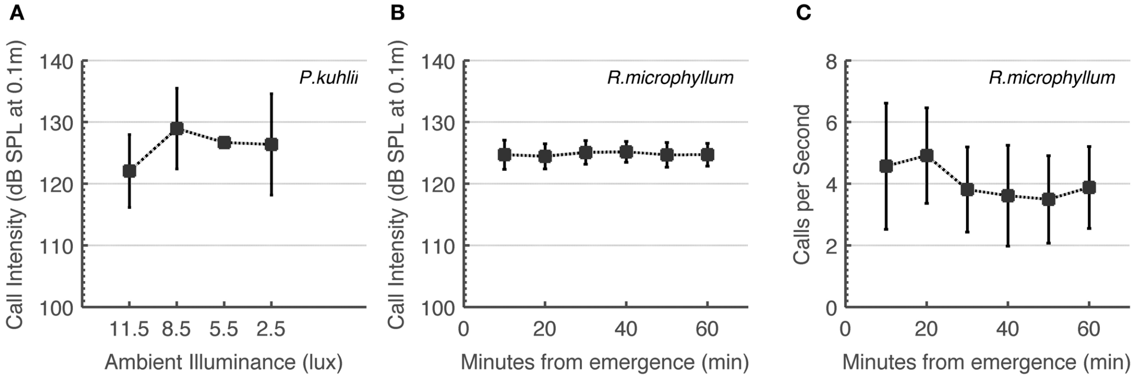

FIGURE 2 | *R. microphyllum* and *P. kuhlii* use echolocation under intermediate light levels to the same extent as in the dark. **(A)** *P. kuhlii* call intensity as a function of ambient light levels. **(B)** *R. microphyllum* call intensity as a function of time after sunset. **(C)** *R. microphyllum* call rate as a function of time after sunset. Both **(B,C)** were measured at the beginning of the month so that moonlight was limited. All panels show means and standard deviations.

Table 1 | Echolocation and visual detection range for *P. kuhlii.*

Object/method	Acoustic (m) (20 dB)	Acoustic (m) (0 dB)	Visual method 1(m)	Visual method 2(m) 10 lux	Visual method 2(m) 5 lux
Moth (18 mm)	4 (3)	7 (5.5)	4.5	6	4
Ant (13 mm)	3.5 (2.5)	7 (5.5)	3.5	3.5	2.5
Lace wing (10 mm)	2.5 (1.5)	5 (4)	2.5	1.5	1
Mosquito (3.5 mm)	2 (1)	4 (3)	1	0.5	0.5
Artificial wing (3 mm)	2 (1)	4 (3)	–	–	–

Echolocation-based detection ranges are shown for four types of prey and one artificial small object. Ranges are shown for two alternative hearing sensitivities (0 or 20 dB SPL) and for either the peak or RMS (in brackets) emission levels. Visual detection range is presented for two different methods, (1) based on visual acuity and (2) based on photon flux. The second method is estimated for two different light levels (5, 10 lux). All ranges are given in meters.

Table 2 | Echolocation and visual detection ranges for *R. microphyllum*.

Object/method	Acoustic (m) (20 dB)	Acoustic (m) (0 dB)	Visual method 1(m)	Visual method 2(m) 10 lux	Visual method 2(m) 5 lux
Moth (18 mm)	6.5 (4.5)	14 (10.5)	7	6	4
Ant (13 mm)	5.5 (3.5)	12.5 (9)	5	3.5	2.5
Lace wing (10 mm)	3 (2)	8 (6)	4	1.5	1
Mosquito (3.5 mm)	2 (1.5)	5.5 (4)	1.5	0.5	0.5
Artificial wing (3 mm)	2 (1.5)	5.5 (4)	–	–	–

*All ranges are in meters. See **Table 1** for details.*

emission frequency which suffers from less atmospheric attenuation. This is true even for the smallest objects for which the higher frequency of *P. kuhlii* results in increased target strength. The artificial wing-like cutout had a target strength (and thus a detection range) which was almost identical to that of the similar sized mosquito, confirming that for such small insects when the wings are spread perpendicular to the axis of ensonification they are the main echo source.

The maximal detection range for small targets while assuming a hearing threshold of 20 dB corresponded well to reaction distances of bats to prey that have been measured for hunting Pipistrelle bats in the field (1–2 m, Kalko and Schnitzler, 1993) while the 0 dB estimations corresponded with detection ranges estimated for *E. fuscus* in the lab (3 m for a 5 mm sphere, Kick, 1982).

A recent paper has proposed to model insect wings as planar reflectors instead of point reflectors to calculate the target strength of insects (Armstrong and Kerry, 2011). We empirically tested these calculations for a large (5 cm long) wing-shaped cutout (see methods) and found that even the largest wing-surfaces bats encounter still behave much more than a point reflector than like a planar reflector (**Figure 1I**).

VISUAL DETECTION RANGE

The exact visual detection range for a small object is a complicated function which depends on the contrast, the spatial frequencies of the object and the transfer functions of the eye. Very little research has tried to assess the behavioral or physiological visual detection range of bats and moreover, the physiology of the bat eye is far from being understood (see Eklöf, 2003, for a summary). We therefore used two alternative approaches to estimate the range from which the bats studied here can detect four real insects.

In the first approach, we used the visual acuity (or maximum resolution) which represents the minimum separable angle for two nearby objects and which was estimated for several bats (e.g., Bell and Fenton, 1986; Eklöf, 2003). We translated visual acuity into detection range (see methods). In the second approach we relied on behavioral experiments performed in *E. fuscus* (Ellins and Masterson, 1974) and tried to estimate the minimum photon flux a bat can detect. Importantly, both methods provided similar ranges, strengthening our confidence in the estimations. Estimations ranged between 0.5 and 7 m depending on object size and were consistently lower than the equivalent echolocation based detection range (**Tables 1**, **2**). Notice that the second method gives different estimations depending on the illuminance.

COMPARING VISION WITH ECHOLOCATION

In the analysis above we systematically chose parameters that overestimate visual detection range and underestimate echolocation detection range. This was to ensure that any advantage found for echolocation is real and might even be more salient in reality. In brief (see methods for full details), the decisions taken to overestimate vision include: (1) using the higher contrast among the two measured (sky vs. vegetation). (2) The assumption that sensitivity is 3.5 higher than acuity. (3) Using the smallest visual acuity measurements reported in the literature. (4) In the second approach—assuming that range increases linearly with illumination. In echolocation we probably underestimated the emitted sound pressure level (by several dB at least).

Despite using this conservative approach, we found that for detecting small objects, echolocation is advantageous over vision under the light conditions examined (intermediate to low light levels; **Figure 3**). Statistical analysis confirms that the differences between echolocation and visual detection ranges are significant for both species (t-test, $P < 0.05$ for *P. kuhlii* and $P < 0.01$ for *R. microphyllum*). When comparing the two modalities statistically, we used the average of the two visual estimations (using the 10 lux condition for the second approach) and averaging the two peak acoustic estimations (acquired for two hearing sensitivities). For each species, we then subtracted the echolocation range from the visual range and ran a t-test to check that the difference is significantly higher than zero. Moreover, even if we were to use the 20 dB worse estimation (which we find unsuitable, see methods) vision would become slightly beneficial over echolocation (0.5 m) only for one case of detecting a moth by *P. kuhlii*.

DISCUSSION

ECHOLOCATION IS ADVANTAGEOUS FOR FINDING SMALL PREY

Apart from being a superior navigational sense under extremely dark circumstances (e.g., Griffin, 1958), we find that echolocation is also superior to vision for detecting and tracking small insects even at intermediate light levels (1–10 lux). This seems to be the case for both species we examined even though they use different signal designs and hunt for different sized prey. **Figure 3** even suggests that the "sonar advantage" is most pronounced at the typical insect size each bat species eats: flying ants (*Rhinopoma*), mosquitos (*Pipistrellus*). Estimating the (visual or echolocation) detection range requires several assumptions. In our analysis however, we systematically made assumptions that *over*estimate visual detection range and *under*estimate echolocation detection range. This guarantees that the advantage we found for echolocation is likely to be real. The advantage of echolocation over vision has in fact been suggested once before (Fenton et al., 1998; Altringham and Fenton, 2005) suggesting that for a 19 mm sphere echolocation detection range is five times larger than the visual range in dim light (exact light levels not stated). The fact that we find a smaller advantage (up to 2 folds) is probably a result of our conservative approach.

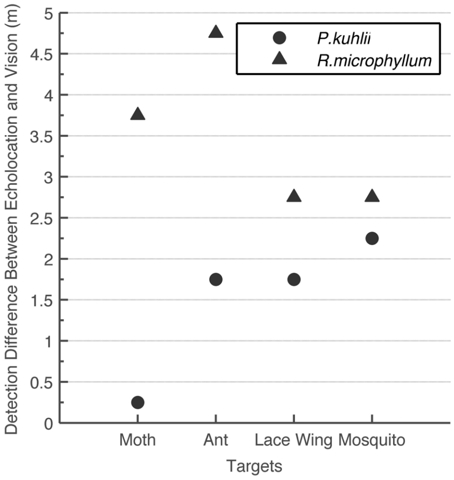

FIGURE 3 | Comparison between visual and echolocation detection ranges. The difference (in meters) between echolocation detection range and the visual detection range for two bat species and four insects. In all cases echolocation detection range was higher than visual detection range.

Echolocation provides several additional advantages over vision. One such advantage is that it tends to provide more continuous tracking, losing the object only when it disappears behind a background. In vision on the other hand, even if the target stays in front of any object its contrast might change dramatically depending on the background, causing it to disappear frequently. We found that a vegetation background, as opposed to the sky led to a 3–5 fold decrease in contrast (**Table 3**) which would result in a 3–5 fold decrease in the visual detection ranges reported above.

In addition, echolocation also provides much more accurate estimations of the distance of an object, its velocity (calculated by integrating several echoes) and sometimes even the distance of the background behind it (Aytekin et al., 2010; Melcón et al., 2011).

Despite these advantages of echolocation over vision, we cannot rule out the possibility that in some species or in some situations (especially when contrast is high) visual cues could assist in prey detection (e.g., Bell and Fenton, 1986; Eklöf et al., 2002). Vision has some advantages such as not suffering from sensory interference that might arise when conspecifics forage together while using similar frequencies (Ulanovsky et al., 2004; Chiu et al., 2008; Bates et al., 2010). We conclude that much more behavioral and physiological research is necessary to understand the extent to which echolocating bats rely on vision.

ECHOLOCATION AND VISUAL DETECTIONS RANGE FOR LARGE OBJECTS

Large landscape objects such as forest edges have recently been estimated to have a maximal echolocation detection distance by bats of about 50 m (Stilz and Schnitzler, 2012). Other studies have estimated even longer ranges (e.g., 90 m in Holderied and Von Helversen, 2003) but the order of magnitude is similar. The main reason for this limited distance is the strong atmospheric attenuation of ultrasound. The visual detection range for large objects is undoubtedly several orders of magnitude larger because sound attenuates much faster than light (e.g., Altringham and Fenton, 2005). For instance, when using visual acuity estimations with an

Table 3 | Insect contrast against different backgrounds and under different light levels.

Object/background	Sky		Ground/vegetation	
	10 lux	0.5 lux	10 lux	0.5 lux
Moth	0.93	0.93	0.33	0.31
Ant	0.94	0.91	0.46	0.37
Lace wing	0.82	0.70	0.33	0.12
Mosquito	0.75	0.66	0.24	0.08

acuity angle of 0.5°, a detection range of 2 km is reached for a sphere of 5 m diameter.

ECHOLOCATING BATS INTEGRATE VISUAL AND SONAR BASED INFORMATION TO PERCEIVE THE WORLD

We find that both bat species tested here rely on echolocation even when light levels are high enough to allow good vision. Since the detection range of even large objects using echolocation is short (no more than 100 m, Holderied and Von Helversen, 2003; Stilz and Schnitzler, 2012) we hypothesize that in intermediate light levels characteristic of dusk, many bats use bimodal sensing. On the one hand, bats predominantly rely on vision for orientation, navigation and avoiding large background obstacles (e.g., Williams and Williams, 1967; Chase, 1981; Mistry, 1990), while on the other hand they mainly rely on echolocation when searching for small prey (**Figure 4**). Clearly, these two are not mutually exclusive behaviors. A *P. kuhlii* bat which uses echolocation to search for insects probably uses vision at the same time to keep track of nearby background targets such as trees and buildings. A *R. microphyllum* bat will search for queen ants in open space using echolocation while visually following the distant terrain to monitor its location relative to the roost. The brains of these two bats must therefore constantly integrate two streams of information acquired by two different modalities into a single image of the world.

THE EVOLUTION OF ECHOLOCATION

Many previous discussions on the evolution of echolocation in bats have focused on whether echolocation or flight evolved first (Speakman, 2001; Denzinger et al., 2004; Simmons et al., 2008). One important question that has not been sufficiently addressed in our opinion is how echolocation could evolve from a rudimentary- (as in echolocating birds) and probably complementary sensory system into the highly complex sonar system observed in bats today. Our results show that echolocation improves the ability of bats to detect small objects even when there is sufficient light for using vision to orient and avoid large obstacles. If we follow the evolutionary scenario proposed by Simmons and Geisler (1998) of flying bats first using vision only (Simmons et al., 2008), echolocation could improve gradually for the detection of increasingly small targets in parallel to using vision for orientation and navigation. In fact, the selective advantage of evolving echolocation is still given, even if the detection range it allows is similar to vision (and not better than vision). This is because the integration of multiple sensory information leads to a more robust percept (Deneve and Pouget, 2004).

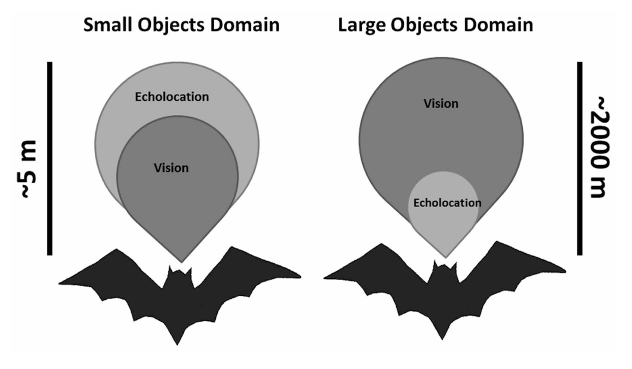

FIGURE 4 | Sketch depicting the two sensory domains used by echolocating bats that are flying in intermediate light levels. Left—echolocation is slightly advantageous when searching for small prey. Right—Vision is strongly advantageous when avoiding large obstacles. Scale bars depict the approximated detection distances, but are qualitative and not quantitative (especially for the large objects domain).

In this evolutionary discussion we focus on the gains of certain sensory abilities and not on their costs. We hope that future studies can shed light on the additional maintainance costs of evolving specialized nocturnal eyes in comparison to the additional costs of emitting frequently in ultrasound (Speakman and Racey, 1991).

Our finding that the ability to detect insect-like (small-) targets is the main advantage of echolocation raises two interesting questions: How advantageous is the detection of small targets in terms of food intake, and if it is advantageous why didn't echolocation evolve for insect detection in the other group of flying vertebrates–birds?

ADVANTAGES OF SMALL TARGET DETECTION IN TERMS OF FOOD INTAKE

Several studies have shown that in aquatic-, or water rich habitats including desert stream habitats chironomids (mosquitos) make up 53–94% of the emerging aquatic biomass, with 90% of them being insects less than 7 mm length (Jackson and Fisher, 1986; Gray, 1993; King and Wrubleski, 1998; Lynch et al., 2002). Many of these insects have a peak of activity around dusk when many bats start foraging (Racey and Swift, 1985; Rydell et al., 1996). Furthermore, there are many chironomid species, some of which are active even during the winter months of harsh continental zones (Krasheninnikov, 2012) so that the availability of Chironomidae as prey is nearly all year round. This is in contrast to moths whose seasonal occurrence is very peaked (Yela and Herrera, 1993). Bats in temperate zones, do predominantly feed on small Diptera (Vaughan, 1997; Dietz et al., 2007) which can be as small as 3 mm wing-length (Houston et al., 2004) and a recent molecular diet analysis of two African molossids also showed diets to be largely composed of dipteran prey (Bohmann et al., 2011). It seems therefore that the ability of bats to detect small prey in intermediate light levels has opened up for them a new and significant niche.

WHY DIDN'T BIRDS EVOLVE ECHOLOCATION FOR INSECT DETECTION?

Our data show that the use of high frequencies (ultrasound) is essential for the detection of small targets (**Figure 1H**). Ultrasonic hearing is common in mammals even among non-echolocating mammals such as tarsiers (Ramsier et al., 2012), tree shrews (Heffner et al., 1969), rats, and mice (Heffner and Heffner, 1985), whereas in birds ultrasonic hearing has probably never evolved (Necker, 2000). Manley (2012) details the essential evolutionary steps mammals went through to obtain ultrasonic hearing: about 230 million years ago a middle ear consisting of three ossicles instead of one, and 100 million years later a tuned basilar membrane, specialized prestines and a coiled cochlea. Since birds only had one ossicle at their disposal and lacked the other adaptations, evolution to receive ultrasound was less probable and has not (yet) evolved. Another reason why ultrasonic hearing did not evolve in birds might be that since their hearing canals are coupled even birds with small heads can estimate the direction of a sound source with high precision. In small mammals however, since the ears are uncoupled, only in high frequencies would wavelengths be small enough to allow precise directional hearing (Heffner and Heffner, 2008; Christensen-Dalsgaard, 2011). This ability to hear and locate the rustling (highly ultrasonic) noises of an approaching predator would provide a selective advantage to small mammals and thus would be probably passed on quickly.

The inability of birds to operate in ultrasound has not prevented them from using audible echolocation (probably 25 species; Brinkløv et al., 2013), nor from being nocturnal. We hypothesize that the lack of ultrasound reception and hence the ability to detect small (insect-) targets has kept birds out of the niche of insectivorous bats. Of the 10,000 bird species inhabiting our planet none are likely to be able to detect small targets (Griffin and Suthers, 1970; Griffin and Thompson, 1982) by using echolocation, whereas more than 1000 species of bats are. Of the purely visually orienting birds there are only about 80 species of birds (nightjars) which exclusively feed on insects at night and these are limited in the following ways: (1) Dietary studies suggest that nightjars rely on catching large (13 × 6 mm) insects (mainly Coleoptera/Lepidoptera, very few Diptera) for their survival (Taylor and Jackson, 2003). (2) They are active in twilight rather than at night and other than in bats they require a minimum light level of 0.03 mW/m^2 ∼1/30 lux to be active (Jetz et al., 2003). They usually forage by perching on the ground and detecting insects against the sky. In nightjars we do not (or only rarely) see specializations such as trawling, gleaning, or foraging in extreme clutter or extreme open space (Holyoak, 2001).

At the same time echolocation has allowed bats to specialize on alternative detection modes, such as flutter detection using Doppler shifts (Schnitzler, 1970), or gleaning prey from vegetation (Neuweiler and Fenton, 1988), which, in turn, might have pushed their radiation into different climatic zones on earth and into many different niches.

In conclusion, we hypothesize that the ability to hear ultrasound has provided mammals with the unique potential to detect small prey items by means of sonar. Bats have probably exploited this potential to an extreme degree and have capitalized on the vast biomass of small flying insects active around dusk. Here, we bring strong evidence that they could use echolocation and vision in a complimentary fashion which would enable a gradual evolution of echolocation.

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Ultrasonic predator–prey interactions in water–convergent evolution with insects and bats in air?

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Toothed whales and bats have independently evolved biosonar systems to navigate and locate and catch prey. Such active sensing allows them to operate in darkness, but with the potential cost of warning prey by the emission of intense ultrasonic signals. At least six orders of nocturnal insects have independently evolved ears sensitive to ultrasound and exhibit evasive maneuvers when exposed to bat calls. Among aquatic prey on the other hand, the ability to detect and avoid ultrasound emitting predators seems to be limited to only one subfamily of Clupeidae: the Alosinae (shad and menhaden). These differences are likely rooted in the different physical properties of air and water where cuticular mechanoreceptors have been adapted to serve as ultrasound sensitive ears, whereas ultrasound detection in water have called for sensory cells mechanically connected to highly specialized gas volumes that can oscillate at high frequencies. In addition, there are most likely differences in the risk of predation between insects and fish from echolocating predators. The selection pressure among insects for evolving ultrasound sensitive ears is high, because essentially all nocturnal predation on flying insects stems from echolocating bats. In the interaction between toothed whales and their prey the selection pressure seems weaker, because toothed whales are by no means the only marine predators placing a selection pressure on their prey to evolve specific means to detect and avoid them. Toothed whales can generate extremely intense sound pressure levels, and it has been suggested that they may use these to debilitate prey. Recent experiments, however, show that neither fish with swim bladders, nor squid are debilitated by such signals. This strongly suggests that the production of high amplitude ultrasonic clicks serve the function of improving the detection range of the toothed whale biosonar system rather than debilitation of prey.

Keywords: predator–prey interaction, echolocation, ultrasound, toothed whale, Alosinae, bat, moth, evasivemaneuvers

INTRODUCTION

Predation is one of the major driving forces in the evolution of the morphology and behavior of organisms (Dawkins and Krebs, 1987; Vermeij, 2002). In tight predator–prey interactions, the natural selection pressure for evolving abilities to detect and catch, or detect and avoid, the other part can be strong and lead to an evolutionary arms race, where adaptations in one species lead to counter adaptations in the other (Dawkins and Krebs, 1987; Dielt and Kelly, 2002).

A classic neuroethological example of how predation and the sensory means of predators have affected the life and sensory systems of prey organisms is the interaction between echolocating bats and ultrasound detecting nocturnal insects, in particular moths. Bats emit intense ultrasonic calls and use the echoes reflected off objects to search for and capture prey (Griffin, 1958; Schnitzler et al., 2003; Schnitzler and Kalko, 2008; Moss and Surlykke, 2010). Bats are important nocturnal predators and therefore place a strong selection pressure on their prey to evolve means to detect and avoid them (Kalka et al., 2008). This selection pressure has driven the evolution of ears sensitive to ultrasonic bat calls in eight moth families (Miller and Surlykke, 2001). Kenneth Roeder, a pioneer in the research of ultrasonic hearing in insects, conducted in the fifties and sixties behavioral experiments where he exposed moths to ultrasonic signals mimicking bat echolocation calls. He found that moths exhibit a complex pattern of anti-predator responses depending on the repetition rate as well as the intensity of the echolocation signals impinging on them (Roeder, 1964, 1967; Miller and Surlykke, 2001). When moths are exposed to low-intensity ultrasonic bat calls, they exhibit negative phonotactic behavior, where they turn and fly directly away from the sound source with increased flying speed. If moths are exposed to high-intensity ultrasonic calls mimicking a bat just before a prey-capture attempt, they will exhibit an erratic evasive response with unpredictable flight patterns that often ends in a power dive or passive drop toward the ground (Fullard, 1998; Fullard et al., 2008; Jacobs et al., 2008). Thus, not only do moths react when exposed to bat calls, they also exhibit an anti-predator response that is correlated with the strength of the predation risk.

It is not only moths that have evolved ears sensitive to ultrasound. Bats feed on a variety of nocturnal insects (Fullard,

1998) and it is generally accepted that the heavy predation pressure from echolocating bats has led to convergent evolution of ears sensitive to ultrasonic signals in at least six orders of insects; Lepidoptera (8 families of nocturnal moths), Neuroptera (lacewings), Coleoptera (beetles), Dictyoptera Mantodea (praying mantids), Orthoptera (katydids, crickets and grasshoppers), and Diptera (parasitic fly species) (Yack and Fullard, 1993; Hoy and Robert, 1996; Yack and Fullard, 2000; Conner and Corcoran, 2012). The ultrasound sensitive ears in combination with sudden evasive maneuvers mitigate predation risk from echolocating bats, increasing the insect's chance of survival by at least 40% (Surlykke et al., 1999). Some bats have lowered the intensity of their calls by 20–40 dB, apparently as a counterstrategy against the ultrasound sensitive ears (Goerlitz et al., 2010). While other bats echolocate at frequencies outside the best hearing range of moths (Fullard, 1998; Fullard et al., 2008; ter Hofstede et al., in press). Both strategies appear to serve the same purpose of rendering the signals difficult to detect by insect prey (Fullard, 1998). Some bats may also broaden their echolocation beam in the last phase of pursuit to keep the insect within their "acoustic field of view" in spite of evasive maneuvers (Jakobsen and Surlykke, 2010). Thus, in the predator–prey interactions of bats and insects there are examples of both strategies and counterstrategies by prey and predator.

Like bats, echolocating toothed whales use a highly advanced biosonar system to detect and catch prey. It has therefore been suggested that despite the very different physical environments of air and water a similar acoustic predator–prey arms race should exist between echolocating toothed whales and their prey (Mann et al., 1998; Astrup, 1999). During the last 15 years several studies have focused on toothed whales and their prey and in the light of the new results we here seek to address and discuss the possible convergent evolution in the acoustic interactions between bat–insect and toothed whale–prey interactions. We do that by providing a brief overview of differences and similarities of echolocation in bats and toothed whales and discuss the implications for biosonar behavior in the two mammalian groups. Then we compare the defense strategies in marine prey with defense strategies in nocturnal insects and discuss the functional basis for developing sensory systems to detect ultrasonic echolocation signals emitted by toothed whales and bats.

ECHOLOCATION IN BATS AND TOOTHED WHALES

Echolocation is an active sensory process where the echolocating animal emits the sound energy which it subsequently hears as echoes reflected off objects ahead of it. Information is then extracted from the environment by the acoustic features of the returning echo and by the delay from sound emission to echo detection. The approximate echo level (EL) returning to the echolocating animal can be estimated using the active sonar equation that includes the target strength (TS), the source level of the emitted sound pulse (SL) and the transmission loss (TL) (all in dB) (Urick, 1983):

$$\mathrm{EL} = \mathrm{SL} + \mathrm{TS} - 2 \times \mathrm{TL} \tag{1}$$

Detection of a returning echo is possible when the EL is higher than the hearing threshold of the echolocating animal or higher than the ambient noise or clutter levels if they surpass the hearing threshold. To forage successfully with sound, echolocating animals in both air and water engage in the phases of search, approach and capture of prey as defined by Griffin (1958). However, air and water are physically two very different types of media and therefore offer very different conditions for the production, transmission, and reflection of sound (Madsen and Surlykke, 2013). The sound speed and density in air are considerably lower than in water which results in very different acoustic impedances of the two media.

Bat echolocation calls can reach SLs of up to 140 dB re 20 μPa (pp) at 0.1 m in air (Surlykke and Kalko, 2008), whereas most toothed whales generate SLs up to 225 dB re 1 μPa (pp) at 1 m in water (Au, 1993; Madsen et al., 2004; Wahlberg and Surlykke, 2013). However, source levels should not be compared directly across the water–air interface. First, the source levels in air and water are given with different reference values and different reference distances. Secondly, the acoustic impedance, given by the ratio of the acoustic pressure and particle motion of an acoustic wave, is much lower in air than in water. This makes it more difficult to generate high-intensity acoustic signals in air than in water. Actually, the sound levels emitted by bats are close to the upper limit of efficient sound production in air. Bats apparently compensate for this restriction by emitting pulses that are relatively long, up to 30–1000 times longer than toothed whale echolocation clicks. This means that the bat sound pulses will carry more energy for a given sound pressure level. When we take these different durations of the signals and the different impedances of the medium into account, a 2 ms bat call in air with a SL of 140 dB re 20 μPa (pp) at 0.1 m has an energy flux density of around 5×10^{-5} J/m^2 and a 50 μs long toothed whale click with a source level of 225 dB re 1 μPa (pp) at 1m has an energy flux density of 4×10^{-2} J/m^2. Thus, bat calls in air are emitted with an energy content about 3 orders of magnitude below those of toothed whale clicks (Madsen and Surlykke, 2013). Both signals are, however, among the highest biologically produced sound intensities found in either media.

Another important difference between air and water is that the sound speeds vary by almost a factor of five between the two media. The wavelength at a given frequency will therefore be almost five times longer in water compared to air. Wavelengths are important for biosonar operation in two ways: (1) for the generation of directional sound beams to increase the SL and decrease clutter levels, and (2) to ensure geometric backscatter from targets of interest, and to extract information of the physical properties of the target by detecting interference patterns generated by multiple reflections at different parts of the target. Geometric backscatter for most prey sizes of interest for bats and toothed whales, will occur when their biosonars operate at frequencies higher than 5–15 kHz depending on prey size (Madsen and Surlykke, 2013). However, many species of both bats and toothed whales produce sound for echolocation at much higher frequencies scaled inversely to their body size. To achieve high directionality of the transmitting beam, an echolocating animal must produce sounds at short wavelengths relative to the size of their transmitting aperture. Small animals must hence use higher frequencies to produce the same directionality as larger specimen. The directionality of sound production can be quantified using the directionality index, DI. This is the source level difference (in decibels) between the directional source in question and an omnidirectional source emitting the same power (Au and Hastings, 2008). Bats seem to operate their biosonars with directionality indices between 11 and 18 dB (Jakobsen et al., 2013), while toothed whales operate their sound beams with DIs from 24 to 32 dB (Wahlberg and Surlykke, 2013). The price to pay for using higher frequencies in small echolocating species is that the frequency dependent absorption is high. The effect is much more dramatic in air which is likely explaining why bats operate at lower frequencies compared to their size than toothed whales (Madsen and Surlykke, 2013). Consequently, most bats and toothed whales emit sonar pulses in a similar frequency range from 15 to 150 kHz.

From the above-mentioned source levels of bats and whales the estimated prey detection ranges of bats are 3–10 m (Holderied and Helversen, 2003; Jung et al., 2007) whereas the estimated prey detection ranges of toothed whales are 15–325 m (Au et al., 2007; Madsen et al., 2007). The huge difference in detection ranges between bats and whales is mainly caused by whales using much higher source levels and the sound absorption being much lower in water. It might therefore be expected that toothed whales would produce sonar pulses at slower rates than bats because the two way travel ranges to their prey targets are much longer. However, because of the almost five times faster speed of sound in water compared to in air, toothed whales have two-way travel times that are almost five times shorter than bats for a certain target range. This results in surprisingly similar biosonar sampling rates for most species of bats and toothed whales (Madsen and Surlykke, 2013).

Both bats and toothed whales employ various versions of the Griffin model of search, approach and capture, where the interpulse intervals and output levels are reduced with range to the prey (Griffin, 1958; Au and Benoit-Bird, 2003; Jensen et al., 2009). Not all species reduce output levels and ICI's in the approach phase (Madsen et al., 2005), but all studied echolocating bats and toothed whales in the wild employ fast repetition rates in the so-called buzz during prey capture attempts, when hunting for moving prey (DeRuiter et al., 2009; Madsen and Surlykke, 2013; Ratcliffe et al., 2013).

Thus despite the vast differences in size of bats and toothed whales and the very different media in which they operate their biosonars, echolocation necessitates the exposure of prey items to high ultrasonic sound levels at high pulse rates. It follows that these predators loudly announce their presence to prey and predators equipped with sensory means to detect them.

BIG BANG—OR NOT?

Toothed whales can generate very intense sound pressure levels up to 225 dB re 1 μPa (pp) (Au, 1993), in the case of the sperm whale even up to 240 dB re 1 μPa (pp) (Møhl et al., 2003); the highest known sound pressure generated by any animal. The reason why toothed whales produce such high sound pressure levels has been lively debated. It clearly enables the animal to detect prey items at longer ranges, or prey items with low target strengths (Equation 1). However, it has also been speculated that the intense ultrasonic clicks not only play a role in echolocation but also helps the whale to catch prey by acoustic debilitation (Berzin, 1971; Norris and Møhl, 1983). Such a dramatic use of sound is known from another aquatic predator–prey interaction between snapping shrimps and their prey. Snapping shrimps make broadband clicks by an extremely rapid closure of the specialized snapper claw, (Herberholz and Schmitz, 1999). The clicks are produced by the collapse of cavitation bubbles generated in a fast flowing water jet during claw closure (Versluis et al., 2000). The clicks can give rise to sound levels of 220 dB re 1 μPa (pp) at close range. A single snap from the claw seems to be sufficient to stun the prey (reviewed by Herberholz and Schmitz, 1999). It is therefore tempting to speculate that toothed whales may use sound in a similar manner. A major difference between snapping shrimps and whales is, however, that the prey of snapping shrimp is exposed to a water jet with particle accelerations much higher than what even a free field pressure of 240 dB re 1 μPa (pp) would predict. It is not known whether it is the sound pressure or particle acceleration that debilitates the prey. Therefore, the fact that snapping shrimps may be able to debilitate prey does not necessarily mean it is possible for the toothed whale to do the same, even though the emitted pressure levels for the toothed whale can be higher than for the snapping shrimp.

Nevertheless, several early experiments did lend support to this so-called biological big bang hypothesis by demonstrating that high exposure levels could disorient fish (Zagaeski, 1987; Mackay and Pegg, 1988; Marten and Norris, 1988). However, many of these experiments used stimuli with very little spectral and temporal resemblance to toothed whale echolocation clicks (Zagaeski, 1987; Mackay and Pegg, 1988; Marten and Norris, 1988). Zagaeski (1987) successfully debilitated guppies with an exposure level of more than 230 dB re 1 μPa (pp), generated with a spark generator. Norris and Møhl (1983) fired small blasting caps in the vicinity of several species of small cephalopods with little evidence of debilitation. In both these experiments the spectral content of the stimuli had a low frequency emphasis and the rise time of the signals was much faster compared to a toothed whale echolocation click. In addition, the source was very close to the animal. The fast rise time of the stimuli and the close proximity between the animal and the source may both induce a large excess particle motion, which can cause damage to the fish tissue that would not be observed using more realistic signals and ranges.

During the last 15 years, our knowledge of toothed whale echolocation signals has increased along with the capability to reproduce them in the laboratory. Experiments using simulated echolocation signals at ultrasonic frequencies with exposure levels up to 226 dB re 1 μPa (pp) and repetition rates of up to 200 clicks/s, show that neither squid (Wilson et al., 2007) nor fish with swim bladders (Benoit-Bird et al., 2006; Schack et al., 2008) are debilitated by intense ultrasonic pulse trains. The obvious question is whether the exposure levels in these controlled debilitation trials are representative of the levels evoked by echolocating toothed whales in the wild. Deployments of sound recording tags on foraging toothed whales have shed light on that issue and shown that toothed whales consistently reduce their source level 20 dB or more when they initiate the buzz phase about a body length from their prey (Madsen et al., 2002, 2005; DeRuiter et al., 2009) (**Figure 1**). Therefore, echolocating toothed whales do not

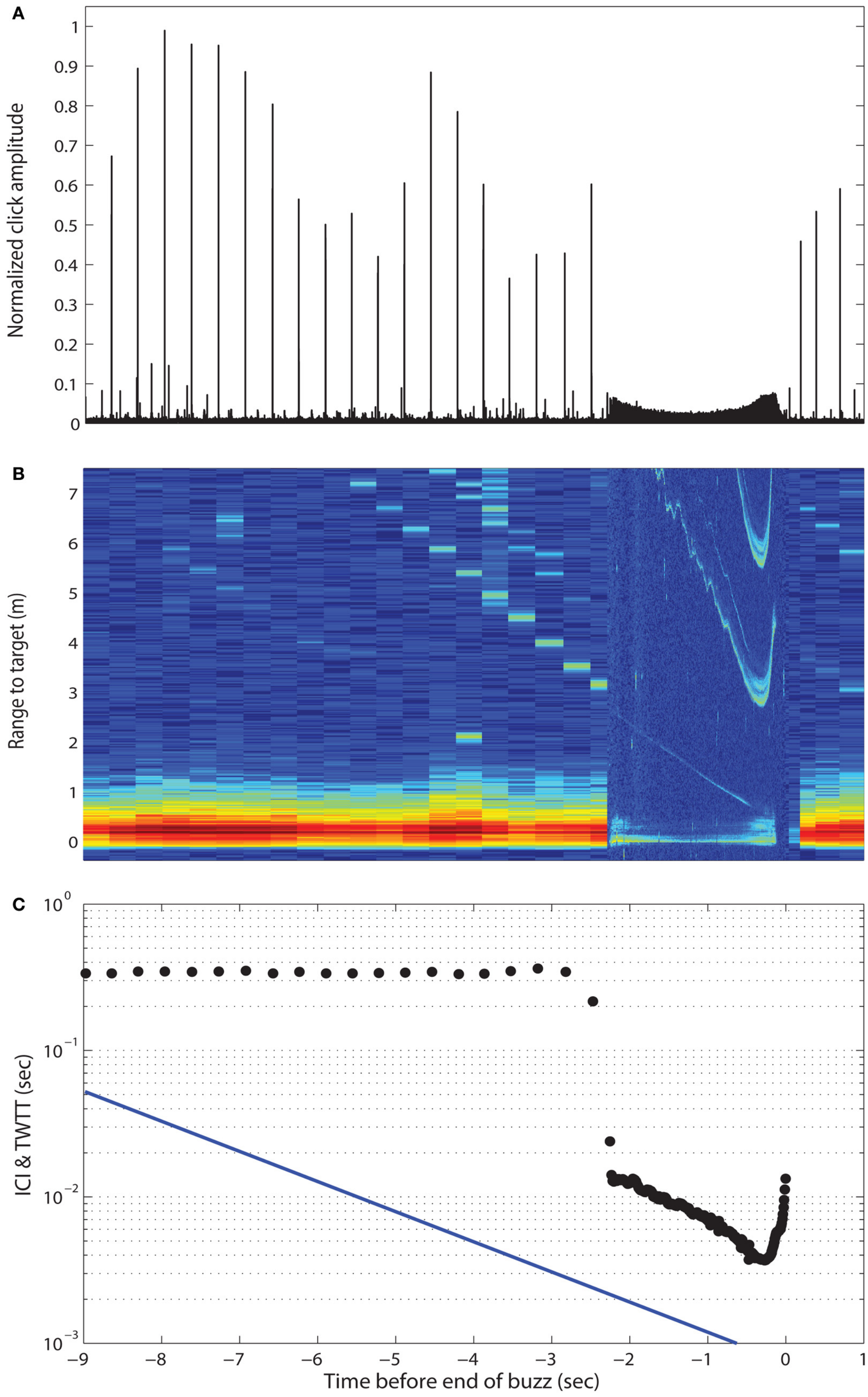

FIGURE 1 | Approach and buzz phases of an echolocating Blainville's beaked whale. (A) Envelopes of the emitted clicks as time from end of buzz. Note the dramatic change in click amplitudes during buzzing. **(B)** Echogram of the emitted clicks and echoes from the approached prey. **(C)** Interclick interval (ICI) and two-way travel time (TWTT). Adapted with permission from Madsen et al. (2013).

maximize the impinging sound pressure level on their prey, as would be expected if they were attempting to debilitate it. In fact, none of the estimated received sound pressure levels prior to or during buzzing exceed those of the exposures mentioned above, failing to debilitate prey in the laboratory. Further, many toothed whales show evidence of chasing their prey over considerable distances (de Soto et al., 2008; Aoki et al., 2012); another observation that is inconsistent with the debilitation hypothesis. Thus, we conclude that whales do not debilitate prey with intense ultrasound, but use their high-amplitude clicks for locating and tracking their low target strength, mobile prey targets at long ranges.

DEFENSE STRATEGIES

To reduce or avoid predation by echolocating bats and toothed whales, prey can follow a variety of defense strategies (Brodie and Brodie, 1999). One of the primary defense mechanisms is to avoid being detected by the predator in the first place. In the case of an echolocating predator with acute hearing this can be achieved by acoustic crypsis, where the potential prey reduces the detection range of the echolocating toothed whale, either passively or actively.

The detection of the target prey can be impeded by a reduction in target strength or an increase in noise or clutter. Prey may thus reduce the detection range by minimizing the echo to noise/clutter ratio. Aquatic prey can accomplish this by seeking refuge among other echoic targets such as other organisms, the sea floor or rocks to hide acoustically between clutter or reverberation by which the echo of the prey is masked by other stronger echoes. This has also been seen in moths flying close to vegetation causing a reduction in the prey capture success of echolocating bats (Rydell, 1998). Prey can also have a small target strength and thereby decrease the echoes reflected back to the echolocating predator. Some toothed whales feed on deep water cephalopods, including members of *Histioteuthidae* and *Cranchiidae* (Clarke, 1996). These ammoniacal cephalopods have very little muscle mass and one of the consequences is a low target strength. They therefore produce a small echo compared to more muscular cephalopod species making them a more difficult target to detect (Madsen et al., 2007).

Some fish species are soniferous, which give the toothed whales the opportunity to eavesdrop on these sounds and use them as homing signals. Gulf toad fish have been shown to reduce or stop sound production when exposed to low-frequency dolphin sounds (Remage-Healey et al., 2006). This situation resembles that of potential bat prey using sound for their own intraspecific sexual communication, e.g., calling frogs (Tuttle and Ryan, 1981) or stridulating orthopterans (Belwood and Morris, 1987). Also here does the prey face the dilemma whether to keep on producing sounds to attract mates, at the risk of being eaten by the bat or to go silent at the risk of losing a mating (Belwood and Morris, 1987; Akre et al., 2011; Jones et al., 2011).

If a prey is detected, secondary defence mechanisms, such as startle behaviors and evasive manoeuvres function to reduce the risk of capture. In bat–insect interactions we find several examples of insects that are able to detect ultrasonic bat calls and exhibit evasive manoeuvres (Miller and Surlykke, 2001). Some moths from the family Arctiidae, tiger moths, have taken the defence strategies even further by emitting ultrasonic pulses when exposed to echolocation signals of bats. These anti-bat signals serve different purposes in different species of tiger moths; in some species they advertise moth toxicity, in others they startle the bat. It has recently been shown that anti-bat signals emitted by some tiger moths can also directly jam the bat biosonar (for a detailed review, see Conner and Corcoran, 2012). Similar examples of secondary defence strategies to toothed whale echolocation signals have not been found in marine prey species. The reason for this may be linked to the fact that secondary defence strategies require that the prey can detect the echolocation signals of the approaching predator; an ability that has evolved several times in insects, but seems to be quite rare in marine prey species as we shall see below.

ULTRASOUND DETECTION IN MARINE PREY

In contrast to overwhelming evidence of acoustic interactions between echolocating bats and their prey, our knowledge about toothed whales and their prey is sparse. Analysis of stomach contents show that toothed whales feed on a variety of different fish and cephalopod species (Simila et al., 1996; Santos et al., 2001a,b,c). However, only few studies have addressed if fish and cephalopods can detect the intense ultrasonic cues provided by echolocating toothed whales. Longfin squid (*Loligo pealeii*) do not show any detectable behavioral or neurophysiological responses when exposed to very intense ultrasound (Wilson et al., 2007; Mooney et al., 2010) and most fish species studied so far can only detect sounds up to some 500 Hz (Hawkins, 1981). Some fish species have specialized gas-filled structures in mechanical connection with their inner ears. These structures improve hearing sensitivity and extend the functional bandwidth up to frequencies between 3 and 5 kHz given by the resonance frequency of the gas-filled structures (Hawkins, 1981; Popper et al., 2003).

Despite this, recent experiments have shown that a few fish species can detect frequencies significantly higher than the resonance frequency of their swim bladder or other gas-filled structures in connection with their inner ears. Astrup and Møhl (1993) showed that conditioned cod would exhibit bradycardia when exposed to long ultrasonic pulses of 38 kHz above 203 dB re 1 μPa (pp). The authors suggested that these conditioned cardiac responses to ultrasound serve as evidence that cod can detect ultrasonic clicks emitted by echolocating toothed whales and might use the ability to reduce the risk of predation (Astrup and Møhl, 1993; Astrup, 1999). However, Schack et al. (2008) shed serious doubt on the findings of Astrup and Møhl (1993) by demonstrating that unconditioned cod do not exhibit any behavioral or cardiac responses when exposed to intense ultrasound. Schack et al. (2008) suggested that cod in the study of Astrup and Møhl (1993) were conditioned to low frequency or electrical artifacts rather than to the ultrasonic component of the exposure, and concluded that cod under natural conditions either fail to detect ultrasound or do not connote it with predation risk from toothed whales. Neither scenario would result in any reduction in the predation risks from ultrasound emitting toothed whales.

There are only few other studies reporting ultrasound detection in fish, and they are all based on Clupeiform fish species belonging to the subfamily Alosinae (Popper et al., 2004; Wilson et al., 2008). Kynard and O'Leary (1990) discovered that high frequency sonar at 160 kHz caused behavioral responses in migrating American shad (*Alosa sapidissima*). Subsequent studies, conducted in the search for an efficient way of keeping fish away from power plant water intakes, found that high frequency sounds at 110–160 kHz (180 dB re 1 μPa) were very effective in deterring Blueback herring (*Alosa aestivalis*) (Nestler et al., 1992) and alewives (*Alosa pseudoharengus*) (Dunning et al., 1992). However, it was debated if the fish actually detected the ultrasound or whether they detected low frequency byproducts of ultrasound emission. A few years later Mann et al. (1997, 1998) measured the first audiogram of an Alosinae, the American shad and showed that this species could detect ultrasound up to 180 kHz with a best sensitivity in the ultrasonic frequency range at around a frequency of 38 kHz and with a threshold of 146 dB re 1 μPa (pp) (**Figure 2**). Subsequent studies showed that other species belonging to the Alosinae, including the Gulf menhaden (*Brevoortia patronus*), allis shad (*Alosa alosa*) and twaite shad (*Alosa fallax*), can detect ultrasound (Mann et al., 2001; Gregory et al., 2007; Wilson et al., 2008, 2011). A few other Clupeiform fishes not belonging to the Alosinae have been tested for ultrasound detection, but with a negative outcome (Mann et al., 2001, 2005). From our current knowledge, the ability to detect ultrasound thus seems to be limited to only the 16 species of the subfamily of Alosinae, out of a total of more than 30,000 species of fish. Future studies will hopefully test for this by providing audiograms for more fish orders.

EVASIVE REACTIONS OF ALOSINAE

Nestler et al. (1992) and Mann et al. (1998) speculated with inspiration drawn from studies on the acoustic interaction in air between bats and their prey (Roeder, 1962, 1967) that ultrasound detection in Alosinae serves as a defense against echolocating toothed whales. Behavioral studies conducted on American shad and allis shad in test tanks support this hypothesis: When shad are exposed to ultrasonic signals in the forms of either ultrasonic tones (Plachta and Popper, 2003; Wilson et al., 2008) or ultrasonic clicks mimicking the echolocation signals emitted by toothed whales (Wilson et al., 2011), they exhibit an escape response that is highly correlated with the intensity of the emitted signals. Wilson et al. (2011) exposed allis shad to ultrasonic click trains played with constant sound pressure levels, but with varying energy levels per time unit, generated by different repetition rates thereby mimicking a toothed whale at different phases of biosonar-based approach and capture. By keeping the sound pressure level constant and changing the click repetition rate, it was shown that the ultrasound detector in allis shad operates as an energy detector with a response threshold of 151 ± 6dB re 1 μPa^{2}s. Furthermore when shad were exposed to ultrasonic click trains with high repetition rates, mimicking the buzz phase of a prey capture attempt of a toothed whale, the fish would exhibit a very strong response with high swimming speeds and faster reaction times. In contrast, when the repetition rate was decreased, mimicking a toothed whale at longer ranges, the response would be weaker and slower. The shad would, independent of the repetition rate and pre-exposure orientation, almost always turn away from the directional sound source at an angle of 180° (**Figure 3**). This behavior not only increases the distance to the toothed whale, but also make the shad a more difficult target to detect

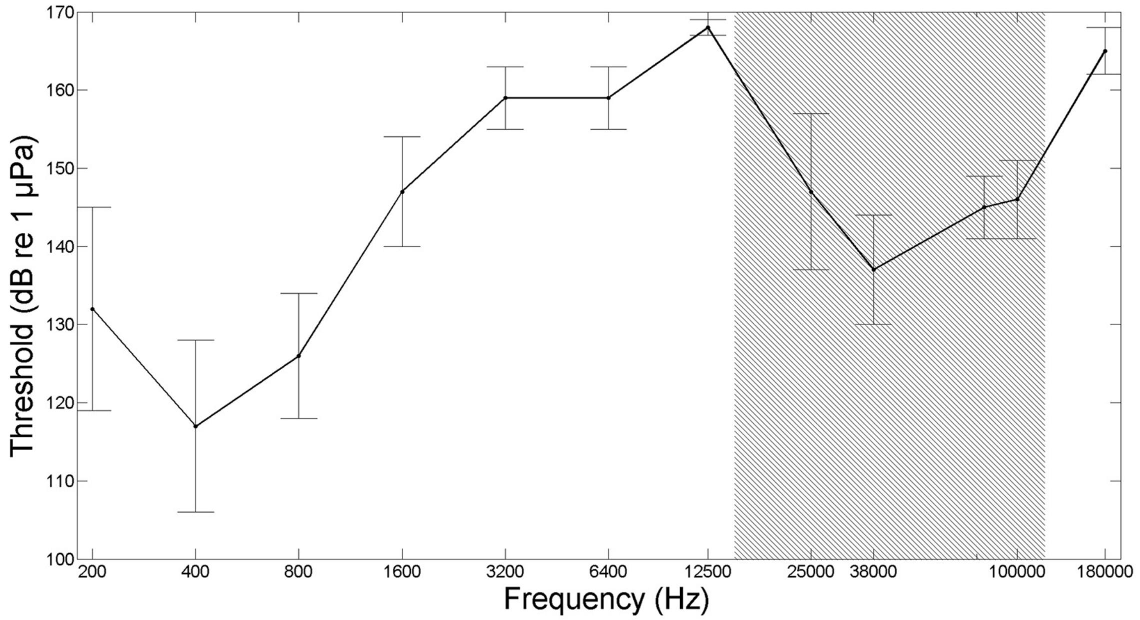

FIGURE 2 | Audiogram from American shad (*Alosa sapidissima*), based on conditioned cardiac responses in five fish. Gray shaded area marks the frequency range of toothed whale echolocation signals (modified from Mann et al., 1997, 1998).

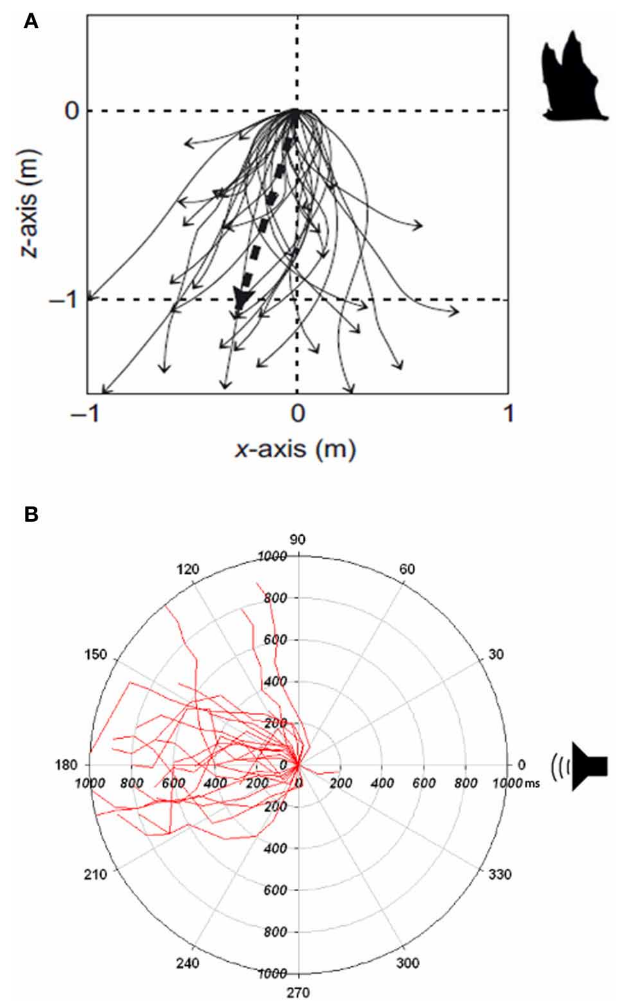

FIGURE 3 | (A) Evasive dive profile of Grote's tiger moths (*Bertholdia trigona*) exposed to echolocating bats in a natural setting. The start position of the tiger moth is at 0,0 m when it starts a dive toward the ground (Adapted with permission from Corcoran and Conner, 2012). **(B)** Directional evasive maneuvers from allis shads (*Alosa alosa*) exposed to intense ultrasonic clicks played at a repetition rate of 250 clicks per second, mimicking echolocation signals from an approaching toothed whale in the buzz phase. The plot gives the angle between the sound source and the fish. At 0° the shad are facing the sound source (Adapted from Wilson et al., 2011).

with sonar, as the target strength of a fish from the tail aspect can be reduced by up to 14 dB compared to a broad side aspect (Au et al., 2007). Swimming directly away results in a reduction of the detection range by 50% for the toothed whale (following Equation 1) (Wilson et al., 2011).

The response thresholds in allis shad are high, just like the response threshold in ultrasound sensitive moths (Surlykke et al., 1999). Such high response thresholds may reflect a trade-off between being caught by the predator, and the costs associated with unnecessary, but costly escape maneuvers. Despite the high detection threshold in ultrasound sensitive moths, Surlykke et al. (1999) estimated that a moth would be able to detect a bat at a range 10 times the range over which a bat would be able to detect the moth. A similar calculation for shad and a dolphin show that the shad would be able to detect a bottlenose dolphin at a distance of between 10–190 m (Mann et al., 1998; Wilson et al., 2011). In contrast to the moth–bat interaction, it is therefore likely that the bottlenose dolphin can detect a school of shad before the shad can detect the dolphin (Au et al., 2007). Still, the shad would be able to detect a bottlenose dolphin in the approach phase, well before it enters the final prey capture phase and most likely have sufficient time to take evasive actions.

Based on the behavioral experiments conducted in test tanks, the reaction of shad to ultrasound is consistent with it being an anti-predatory response against echolocating toothed whales. Like ultrasound sensitive insects, shads exhibit evasive maneuvers that are highly correlated in strength with the magnitude of the acoustically conveyed predation risk (**Figures 3A,B**).

ULTRASOUND DETECTORS

How Alosinae detect ultrasound has been an enigma since the ultrasonic sensitivity of these fish was discovered more than 15 years ago. Much more is known about the ultrasound sensitive ears of nocturnal insects that use mechanoreceptors as sound receivers in conjunction with tympanic membranes made of their cuticle at different positions on the body e.g., thorax (Notuidae), abdomen (e.g., Pyralidae) and mouthparts (e.g., Sphingidae) (Miller and Surlykke, 2001; Conner and Corcoran, 2012). In moths belonging to the family of Noctuidae, the anatomy of the ear is relatively simple: It consists of a thin tympanic membrane in a recess below the hind wing on the metathorax. A relatively large air sac, an expanded part of the respiratory system, is located behind the membrane. Mechanically coupled to the membrane are two mechanoreceptors, so called scolopidia, distinguished into A1 and A2. They attach to the same part of the tympanic membrane and are very similar in terms of their morphology and overall shape of their hearing threshold curves, but their sensitivity differ with A1 being approximately 20 dB more sensitive than A2 (Roeder, 1967; Fullard, 1998). When an ultrasonic sound wave impinges on the insect body, the membrane starts vibrating; this excites the sensory cells to increase their firing rate of action potentials (Roeder, 1967).

To evolve an ultrasonic pressure detector in water seems to be more challenging, perhaps because of the very different physical properties of air and water. Detection of ultrasound in water requires a gas-filled structure with wall properties that permit oscillations at ultrasonic frequencies. In addition, the gas-filled structure needs to be connected to a sensory receptor that can transduce the oscillatory motions into a neuro-electrical signal.

In all clupeiform fish, gas-filled structures (extensions from the swim bladder) are mechanically connected to two groups of mechanoreceptor hair cells, the lateral line and inner ear. The anterior part of the swim bladder has two gas-filled tubes that extend to the two inner ears, where they expand to gas-filled bullae encapsulated in bony structures (O'Connell, 1955; Retzius, 1881). The gas-filled bullae have a highly advanced structure (Wilson et al., 2009) and in most clupeiform fish, each bulla can be divided into a prootic bulla and a pterotic bulla (O'Connell, 1955). The function of the pterotic bulla is unknown, but the prootic bulla is believed to be an auditory specialization since it is connected to the utricle of the inner ear (O'Connell, 1955). The lateral line is also coupled to the prootic bulla. The perilymph of the prootic bulla and the sea water in the lateral line canals are only separated by the thin lateral recess membrane found in the back of the lateral recess, wherefrom the primary branches of the lateral line radiates (O'Connell, 1955; Denton and Blaxter, 1976; Hoss and Blaxter, 1982).

Enger (1967) suggested that the gas-filled bullae with mechanical connection to the utricle act as a pressure-to-displacement converter in Clupeidae. This makes Clupeidae sensitive to both the pressure and particle motion component of the sound field. The ability to detect the pressure component makes these fish capable of detecting higher frequencies and provides them with a more sensitive hearing (Hawkins, 1981). However, most clupeids can only detect sound below 10 kHz (Enger, 1967; Mann et al., 2001, 2005). Since the gas-filled bullae in addition are mechanically connected to the lateral line, it has been suggested that vibrations of the bullae also generate fluid motions in the cephalic lateral line canals, and thereby cause a deflection of the hair cells in the neuromasts of the lateral line (Denton and Blaxter, 1976; Denton and Gray, 1983; Gray, 1984).

The mechanical connections between the lateral line, the inner ear and the gas-filled bullae in clupeids are unique. It is therefore tempting to hypothesize that the ultrasound detector in Alosinae is associated with the unique bullae complex, and that the gas-filled bullae are acting as a transducing element that translate the ultrasonic pressure wave into a local particle motion stimulating the sensory receptor (Higgs et al., 2004). The gas-filled bullae are indeed involved in ultrasound detection in the Alosinae: Wilson et al. (2009) showed that the gas-filled bullae in Gulf menhaden pulsate when placed in an ultrasonic sound field, and furthermore that replacement of gas in the bullae with fluid eliminates the ability to detect ultrasound. Since the bullae are connected to both the inner ear and the lateral line, it is possible that the sensory receptor is part of either the lateral line or the inner ear.

Mann et al. (1998) suggested that the utricle of the inner ear is where the ultrasound sensory receptor in Alosinae is located because of the highly advanced anatomy. Higgs et al. (2004) found morphological differences in the sensory epithelium of the utricle between Alosinae and other clupeids. The sensory epithelium of the utricle in Clupeidae is divided into three parts; the anterior, posterior and middle (Popper and Platt, 1979). However, the anatomical support for the middle section of the sensory epithelium in Alosinae is thinner and therefore more loosely connected to the rest of the maculae compared to other clupeids. Higgs et al. (2004) speculated that the looser connection could make the utricle sensitive to high frequency vibrations induced by the gas-filled prootic bullae and the elastic thread. Despite of this, there is no experimental evidence to support that the utricle mediates ultrasound detection.

Another theory suggests that the ultrasound sensory receptor is associated with the lateral line (Nestler et al., 1992; Wilson et al., 2009). This theory is supported by the observation that the neural response to ultrasound disappears by mechanical manipulation of part of the lateral line overlying the base of the lateral line, i.e., the lateral recess. This manipulation does not damage neither the gas-filled bullae nor the inner ear, as evidenced by the ability to detect a 600 Hz tone after this manipulation. Therefore the lateral line plays an important role in ultrasound detection and the most parsimonious explanation is that the sensory receptor is either to be found in the lateral line or in association with the lateral line (Wilson et al., 2009) (**Figure 4**).

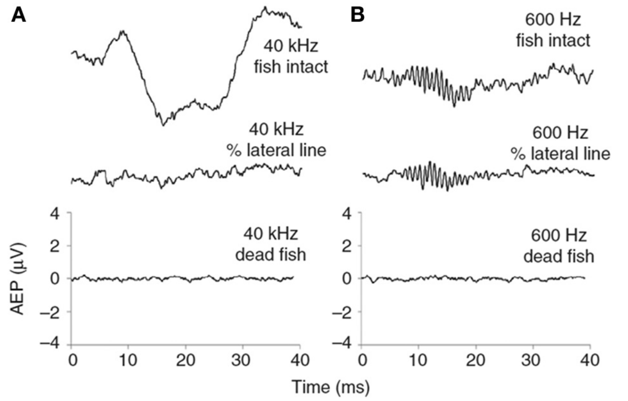

FIGURE 4 | Neural responses of a Gulf menhaden measures as evoked potentials to (A) a 40 kHz and (B) a 600 Hz 20 ms long tone before and after mechanical manipulation of the lateral line. Adapted from Wilson et al., 2009.

WHY IS ULTRASOUND DETECTION SO RARE IN AQUATIC PREY COMPARED TO IN NOCTURNAL INSECTS?

In water ultrasound detectors have as far as we know only evolved in very few fish species, the Alosinae, whereas most fish and cephalopods, and most likely also crustaceans, cannot detect intense ultrasound. This is in contrast to the situation for insects in air, where ears sensitive to ultrasound have evolved in many orders of nocturnal insects independently (Miller and Surlykke, 2001; Conner and Corcoran, 2012). Despite a remarkable evolutionary convergence in the biosonar behavior and frequency range of echolocation signals from bats and toothed whales, the evolution of ultrasound detection in prey is much rarer in water than in air.

This may be due to the fact that the two groups of echolocating predators have evolved in two very different media and therefore the functional starting points for the evolution of ultrasound detection in their prey is very different. Ultrasound reception requires detection of the pressure component of a sound field. That in turn calls for receptors with structures having an impedance difference compared to the surrounding medium. In simple insect ears the large impedance difference between the surrounding air and the insect body in combination with the air sac behind the tympanic membrane, generates vibrations of the membrane relative to the rest of the insect body when insects are exposed to ultrasound (Roeder, 1967). In fact, non-differentiated mechanoreceptors attached to the cuticle are sensitive to airborne sound with a best frequency of around 2 kHz at sound pressures above ca. 80 dB SPL (Yack and Fullard, 1990). Thus, the precursor for an ear is readily available in insects. In water, the situation is quite different: A fish or cephalopod without gas-filled structures in the body is almost acoustically transparent, since their bodies have impedance close to the impedance of the surrounding water. Fish and cephalopod have evolved a low frequency hearing system, where deflections of hair cells are caused by differential motion of dense ear stones with respect to the hair cells and the rest of the body (Sand and Karlsen, 2000). However, this accelerometer ear is in most species sensitive only up to a few hundreds of Hz (Kalmijn, 1989). Several fish species have, in addition to the accelerometer ear, gas-filled structures, such as the swim bladder, mechanically connected to their inner ears. These gas-filled structures render the fish sensitive to the pressure component of the sound field and hence capable of detecting frequencies higher than the resonance frequency of their otolith organs. Still, even though some fish have a strong mechanical connections between their ears and the gas-filled structures, they can only hear up to 3–5 kHz (see review by Hawkins, 1981; Popper et al., 2003). The only exception found so far is the subfamily Alosinae that are capable of detecting intense ultrasound with their gas-filled bullae complex. Thus, evolving an ultrasound detector in fish and cephalopod seems to require challenging anatomically adaptations compared to insects, and this might be one of the reasons why ultrasound detection in marine species seems limited to Alosinae.

Another and perhaps even more important difference might be found in different selection pressures working in the two acoustic interactions. The selection pressure for evolving ultrasound detectors is presumably very high for the nocturnal insects, since bats are the only nocturnal insectivores that hunt prey on the wing. In contrast, toothed whale prey is also targeted by a plethora of other marine predators that employ a range of sensory and locomotory means to subdue their prey. Therefore toothed whales are not an exclusive group of predators placing a one-sided evolutionary selection pressure to evolve means to detect and evade them. In addition to the ultrasonic echolocation signals, toothed whales also produce another acoustic cue that is shared with all other aquatic predators: Low frequency hydrodynamic water movements are consistently generated during aquatic feeding by both swimming motions, the head wake of the approaching predator and the subsequent suction and raptorial feeding motions during prey acquisition (Hanke and Bleckmann, 2004; Fish and Lauder, 2006; Werth, 2006) (**Figure 5**). Therefore toothed whales and other aquatic predators provide the prey with strong low frequency cues and the selection pressure for evolving means to detect such cues will presumably be much stronger than the selection pressure to evolve means to detect the ultrasonic cues, because of the universality of this low frequency cue (Vermeij, 2002). The ability to detect infrasonic cues is likely found in most water living metazoans, including copepods (Heuch and Karlsen, 1997), cephalopods (Packard et al., 1990) and bony fish (Sand and Karlsen, 1986; Karlsen, 1992a,b; Karlsen et al., 2004). One of the major driving forces for evolving an acute infrasonic hearing sensitivity might be the necessity for detection of predators (Sand and Karlsen, 2000). Playback studies testing behavioral escape responses of fish when exposed to infrasound mimicking an approaching predator supports this hypothesis, since different fish species exhibit a strong spontaneous avoidance response when they are exposed to infrasound with no or little sign of habituation (Knudsen et al., 1994; Sand et al., 2001; Karlsen et al., 2004).

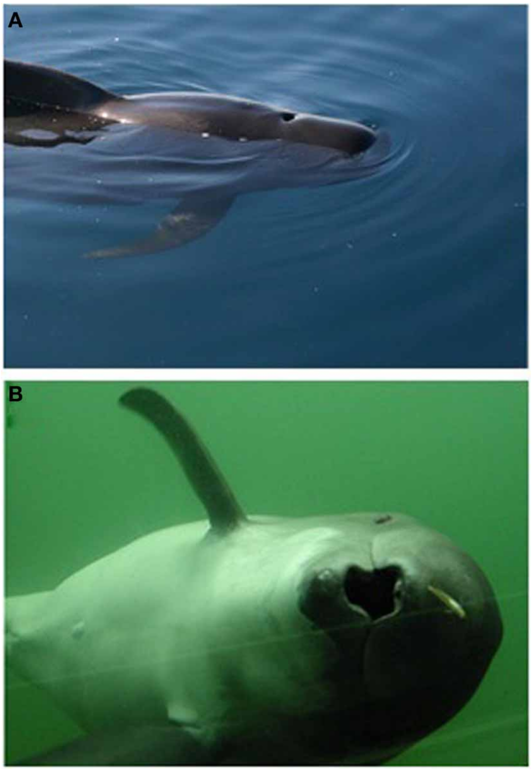

FIGURE 5 | (A) A short-finned pilot whale (*Globicephala macrorhynchus*) swimming at the surface showing the hydrodynamic disturbances generated by the forward motion of the whale (photo: Frants H. Jensen). **(B)** A suction-feeding harbor porpoise (*Phocoena phocoena*) (photo: Fjord and Bælt, Kerteminde, Denmark).

CONCLUSION

Despite the very different physical environments, the evolution of echolocation in bats and toothed whales seem surprisingly convergent in terms of spectral and temporal acoustic features of the sonar signals: Toothed whales and bats operate their biosonar in the same frequency range and with overlapping sample rates. In both scenarios a prey capture involves characteristic changes in particular of time but also intensity parameters to subdivide the pursuit into three phases; search, approach, and finally the buzz phase with extremely high pulse rate just before the prey is caught. The acoustic interaction between bats and their prey, the nocturnal insects, has become a textbook example of an evolutionary arms race between a predator and its prey. Currently available data does not indicate that a similar ultrasonic interaction exists between toothed whales and the majority of their prey.

Predation defense in terms of ultrasonic detection of echolocators seem far from as common among fish and cephalopod prey of toothed whale compared to the insect prey of bats. Still, there are some clear similarities in the anti-predator responses of one group of prey fish, the Alosinae (shad and menhaden), to those of eared nocturnal insects like e.g., moths. The strength of the evasive maneuvers is highly correlated with the magnitude of the acoustical signals conveying a predation risk. If a moth or a shad is exposed to weak echolocation signals mimicking a bat or a toothed whale at a distance, the evasive maneuver consists of a directional response away from the source. However, if the sound exposure is mimicking a bat or a toothed whale nearby, the evasive maneuver is stronger and unpredictable. The behavioral response thresholds for both moths and Alosinae are relatively high, but possibly low enough to provide enough time to successfully escape the predator, while high enough to reduce the number of false, and hence expensive, alarms.

While the ability to detect ultrasound has evolved in many insect families, it has so far only been described in a few fish species belonging to the subfamily Alosinae. In the bat–insect interaction the selection pressure among insects for evolving means to detect and avoid the bat is high, because essentially all nocturnal predation on flying insects stems from these predators. In the interaction between toothed whales and their prey the selection pressure seems much weaker, most likely because toothed whales are by no means the only marine predators placing a selection pressure on their prey to evolve specific means to detect and avoid them. Toothed whales, like all other aquatic predators, produce an omnipresent low frequency sensory cue that can be detected by fish and cephalopods. The selection pressure is presumably stronger to evolve means to detect the low frequency cues, than to develop ultrasound detectors. This is supported by the fact that in all fish and cephalopod species studied up to date we find a high sensitivity to low frequency particle acceleration that may represent an interface for an acoustic arms race between not only toothed whales, but all aquatic predators and their prey.

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Echolocation by the harbor porpoise: life in coastal waters

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The harbor porpoise is one of the smallest and most widely spread of all toothed whales. They are found abundantly in coastal waters all around the northern hemisphere. They are among the 11 species known to use high frequency sonar of relative narrow bandwidth. Their narrow biosonar beam helps isolate echoes from prey among those from unwanted items and noise. Obtaining echoes from small objects like net mesh, net floats, and small prey is facilitated by the very high peak frequency around 130 kHz with a wavelength of about 12 mm. We argue that such echolocation signals and narrow band auditory filters give the harbor porpoise a selective advantage in a coastal environment. Predation by killer whales and a minimum noise region in the ocean around 130 kHz may have provided selection pressures for using narrow bandwidth high frequency biosonar signals.

Keywords: echolocation, biosonar, hearing, harbor porpoise, *Phocoena phocoena*, noise, clutter, coastal waters

INTRODUCTION

The harbor porpoise, *Phocoena phocoena*, is a small whale about 1.5 m long and weighing about 65 kg. The species has a large distribution and ranges as far south as Mauretania and as far north as western Greenland and northern Alaska (Culik, 2011). Harbor porpoises seem to prefer coastal waters, even though they are sometimes seen in the middle of the ocean (Haug et al., 2003; MW, personal observation).

Like other toothed whales, harbor porpoises use echolocation to hunt for their prey, such as fish and squid. They emit intense ultrasonic signals in a narrow sound beam and listen for echoes (Busnel and Dziedzic, 1967; Møhl and Andersen, 1973; Miller, 2010; Koblitz et al., 2012). Their signals are narrow in bandwidth and high in frequency (NBHF; Au, 1997). They share this type of signal with at least three of the other six species in the porpoise family Phocoenidae, the four species of *Cephalorhynchus* dolphins, two species of southern ocean *Lagenorhynchus* dolphins, and the Franciscana dolphin, *Pontoporia blainvillei* (Morisaka and Connor, 2007; Kyhn et al., 2009, 2010; Tougaard and Kyhn, 2010; Melcón et al., 2012). All of the species listed are found in coastal habitats, but also pelagic. The only truly pelagic species of toothed whales known to use NBHF clicks is the pygmy sperm whale, *Kogia breviceps* (Madsen et al., 2005).

From phylogeny (Steeman et al., 2009), one would expect the broadband click to be the ancestral odontocete biosonar signal. What selective pressures caused the appearance of NBHF signals in a few primarily coastal odontocetes? Previously suggested answers to this question have focused on acoustic mechanisms like extracting an echo from noise and antipredator behavior (Andersen and Amundin, 1976; Madsen et al., 2005; Morisaka and Connor, 2007). Here we review such mechanisms in light of new data gathered on noise sources and the acoustic behavior, hearing and sound production of harbor porpoises.

ECHOLOCATION BEHAVIOR OF HARBOR PORPOISES

Harbor porpoise clicks are centered between 130 and 140 kHz with a bandwidth of 6–26 kHz (Dubrovskij et al., 1971; Møhl and Andersen, 1973; Villadsgaard et al., 2007; **Figure 1B**). The duration of the click is around 44–113 μs (Villadsgaard et al., 2007; **Figure 1A**). The signals are produced in the nasal passages just below the blowhole and emitted through the melon in a narrow 11–13° beam (Koblitz et al., 2012; Kyhn et al. submitted, see acknowledgments). The phonic lips, air sacs, and the melon are all involved in sound production (Madsen et al., 2010; Miller, 2010).

Like other toothed whales, harbor porpoises adjust the inter-click intervals of their sound emissions so that the echo does not overlap with the next click emission (Akamatsu et al., 2007; Verfuss et al., 2009; Wisniewska et al., 2012). While searching for prey the normal inter-click interval is around 30–100 ms. As the animal approaches the prey, the inter-click intervals become progressively shorter and ends in a "buzz," with click intervals of about 1.5 ms when the porpoise is about a meter or two from the prey (Verfuss et al., 2009). The porpoise reduces the amplitude of its clicks by approximately 6 dB per halved distance to the target (Atém et al., 2009; Linnenschmidt et al., 2012a,b; Wisniewska et al., 2012).

The audiogram of the harbor porpoise has one of the widest bandwidths of any animal. The best sensitivity is found between about 80 and 140 kHz (Kastelein et al., 2002, 2010). Harbor porpoises can adjust their hearing when listening for echoes at various distances. That is, when the test target is moved toward the animal, the hearing sensitivity and the level of its biosonar signal are progressively decreasing so that the neural response of the echo stays at about the same level (Linnenschmidt et al., 2012a). In this way, the perceived echo level can be adjusted to a convenient amplitude within the dynamic range of the neuro-auditory system.

After transmitting the intense, ultrasonic pulses, harbor porpoises listen for the faint echoes returning from fish and other

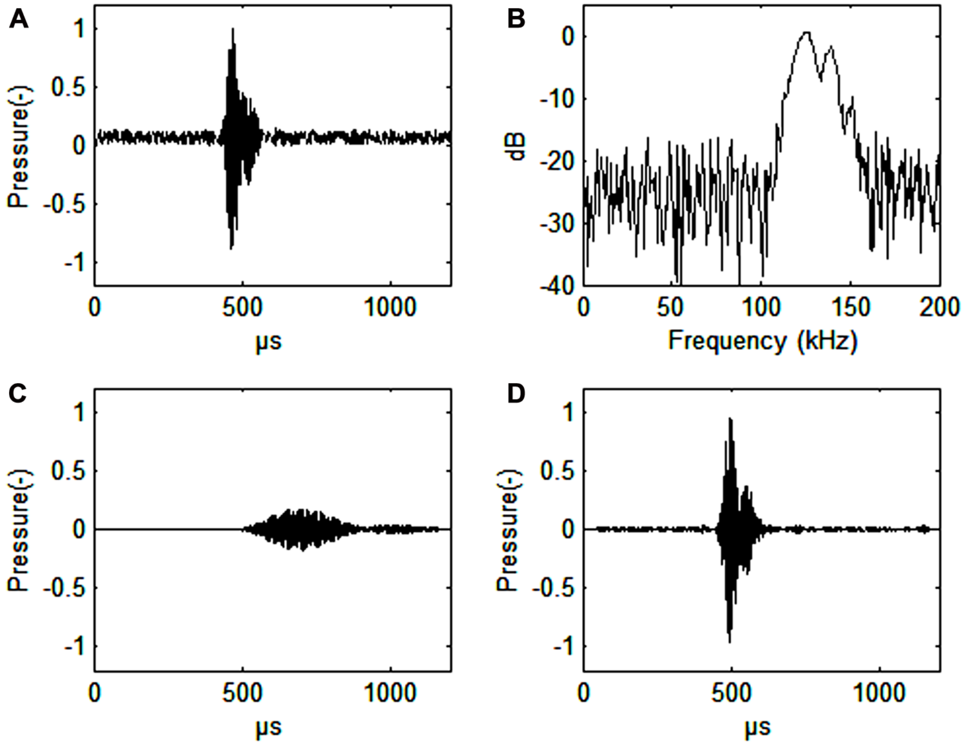

FIGURE 1 | (A) Harbor porpoise click. Signal-to noise ratio 16 dB. **(B)** Spectrum of a harbor porpois click. **(C)** Harbor porpoise click filtered with a band pass filter (128–132 kHz, 4th order Butterworth filter), or about the width of the frequency auditory filter (as estimated by Popov et al., 2006). The axes for pressure are in relative, but comparable values. The dB values are also relative. **(D)** Harbor porpoise click filtered through a simulated third-octave band filter (center frequency 130 kHz, bandwidth 30 kHz, 4th order Butterworth filter). Note the vastly improved time resolution that is only about 10 μs delayed relative to the timing of the original signal **(A)**. Also note the improved signal to noise ratio (34 dB) in C relative to the third-octave filtered signal (30 dB) in D.

items in the water. Besides receiving the signal, ambient noise is also picked up by the hearing system. The porpoise has several ways to reduce the amount of received noise. First, the hearing system is directional, so that most energy is picked up in a cone 22° wide in front of the animal (Kastelein et al., 2005). Thus, the directionality of the receiving system is about twice as wide as that of the transmission system (22° rel. 11–13°). Secondly, when listening to an echo only noise within a restricted bandwidth will disturb perception of that echo. In humans, this bandwidth, called the critical bandwidth, is approximately 23% of the center frequency in question for higher frequencies. These are the so-called third octave bands that form a series of constant Q (quality) filters. Third octave bands are also known to approximately describe some of the critical bands in the auditory systems of dolphins and other odontocetes at higher frequencies (Au and Moore, 1990; Au, 1993).

Popov et al. (2006) used tonal masking to describe the auditory filter functions of a harbor porpoise and Kastelein et al. (2009) measured the critical ratio (which is an estimate of the critical band) of two harbor porpoises using a psychophysical paradigm. Popov et al. (2006) found that the critical bands in the frequency range of echolocation are 3–4 kHz wide. On the other hand, Kastelein et al. (2009) measured critical ratios of 34 and 37 dB at these frequencies indicating a bandwidth of 2.5–5.0 kHz using Fletcher's assumption (Fletcher, 1940). Differences in experimental design could explain the discrepancy between these measurements, as one study used a tonal masker and auditory brainstem recordings and the other used psychophysics for tonal detection in narrow-band noise.

An interesting feature of both of these estimates is that the critical bands do *not* always seem to be a linear function of the center frequency at the frequency band of echolocation, which is the most common feature of critical bands for almost all other vertebrates (Fay, 1988). Instead, porpoises seem to have rather constant auditory filter bandwidths at echolocation frequencies. This has recently been supported by data from the bottlenose dolphin, *Tursiops truncatus*, (Lemonds et al., 2012). The frequency bands measured from both species are narrower than the actual bandwidth of the echolocation signals. There are currently no data available to understand why such filters are advantageous during echolocation.

A narrow band auditory filter gives poor time resolution (**Figure 1C**), which an odontocete needs for determining distance to prey. From observations on blindfolded individuals it is quite obvious, however, that the harbor porpoise knows exactly were the fish is during prey capture (Miller, 2010). Wider filters will improve time resolution (**Figure 1D**). We predict that the harbor porpoise has narrow band and wide band auditory filters running in parallel to effectively extract echoes from noise without losing time resolution. This seems to be the case for the bottlenose dolphin, where wide band auditory filters (constant Q) are found up to 100 kHz in parallel with constant bandwidth filters (about 10 kHz) at auditory frequencies from 60 to 100 kHz (Lemonds et al., 2012). The wide band auditory filters provide good temporal resolution while the narrow band auditory filters may be better for discriminating between echoes of various origins.

NOISE IN THE COASTAL ENVIRONMENT

Wenz (1962) is the standard reference for noise in the open-ocean and coastal areas. There are still surprisingly few studies of coastal water acoustics and all but one deal with lower frequencies outside the NBHF echolocation signals used by the harbor porpoise (see for example Wilson et al., 1985; Piggott, 1964). Recently, however, the noise profile in Fehmarn Belt (coastal waters in the German Baltic) was determined for March, 2012 (**Figure 2A**). The mean noise profile (upper red curve) includes natural and anthropogenic sources while the black curve is a 20 min measurement of noise during rain at sea state (SS) 2. The noise levels in **Figure 2A** follow the general trend in that the levels are about 10 dB higher for frequencies above 1 kHz in Fehmarn Belt relative to those in open ocean waters. Rain contributes to high frequency noise and this is maximum at about 15 kHz at a level of about 88 dB re 1 μPa (1/3 octave band). Harbor porpoises are common in the Fehmarn Belt and the hearing threshold (Kastelein et al., 2002) for the lower frequencies of the audiogram is plotted in **Figure 2A**. It is obvious that harbor porpoises can easily hear noise above about 500 Hz. Rain noise is apparently quite irritating since the animals in the Fjord&Bælt facility at Kerteminde begin to swim rapidly, breaking the water surface ("porpoising") while doing so, for an extended time during rainfall. The same has been observed for several harbor porpoises in facilities in the Netherlands (R. Kastelein, personal communication). This shows that sound outside the frequency range of porpoise biosonar may cause the animal to abort any prevailing behaviors like foraging.

CLUTTER IN THE COASTAL ENVIRONMENT

In biosonar, we define clutter as unwanted echoes from objects near the target of interest. Odontocetes emit their biosonar in directional beams. The beams are shaped like cones having a width in degrees defined by an arbitrary number of dB down from the central axis of the beam, often −3 or −10 dB (Au, 1993). The further the harbor porpoise is from the target of interest, a fish for example, the greater is the ensonified area. If the porpoise can perceive an echo from the prey then it can also perceive clutter echoes from other objects in the biosonar beam having similar echo strengths, which presents problems for detecting prey.

ACOUSTIC ADAPTATIONS FOR NOISE AND CLUTTER

Even though the coastal environment offers abundant and varied prey, finding and capturing it presents several challenges for an odontocete. How does it deal with the general increase in noise level of the coastal environment? What about the plethora of uninteresting clutter echoes from for example bottom structures in relatively shallow water? How is the predator avoiding becoming prey to e.g., the killer whale (*Orcinus orca*)?

Almost all echolocating animals use ultrasonic signals. Ultrasound is needed to get echoes from small objects. Harbor porpoise echolocation signals have a wavelength slightly larger than 1 cm and can be used to obtain good echoes from prey items of this or even smaller size, in other words very small fish. The harbor porpoise NBHF signals have a more than 20 dB lower intensity than most other Odontocetes, but the signals are significantly longer in duration. Thus, the returning echoes will have a lower intensity, a narrower bandwidth and a longer duration as compared to signals emitted by most dolphins. A series of narrow-band auditory filters seems to improve the ability for the harbor porpoise to extract an echo from broad-band noise (**Figures 1C,D**).

There are basically three ways the harbor porpoise can deal with clutter echoes. One is to reduce the amplitude of its biosonar signals so it can perceive echoes from the target but cannot hear clutter echoes from objects having lower target strengths than that of the target. The harbor porpoise does reduce the amplitude of its biosonar as it approaches a prey item (Atém et al., 2009; Miller, 2010). A second way to reduce clutter echoes is to have a narrow sound beam or better yet to be able to change the width of the beam. Being small, like the harbor porpoise, means that it is difficult to maintain high signal directionality. Directionality is mainly governed by the frequency content of the signal and the size of the transducer. In addition, air sacs, cranial structures, and variations of the speed of sound within the melon help to improve directionality (Au, 1993). Having a high frequency signal (approximately 130 kHz) is an advantage since directionality is proportional to frequency for the same emitter size. Using even higher frequencies to get more directionality would be a disadvantage because of increased sound attenuation. Hearing sensitivity would have to follow suit, but this decreases rapidly above 140 kHz (Kastelein et al., 2002, 2010). So having a narrower sonar beam would improve the echo to clutter ratio, but too narrow a beam would be problematic. Naturally the porpoise can steer the beam by moving its head, like visual gazing (Verfuss et al., 2009; Wisniewska et al., 2012). Being able to adjust the beam width would be a great advantage for clutter rejection, but if it can do this, as some bats can (Jakobsen et al., 2013), is unknown. Thirdly, to reject clutter from objects farther than that of interest, the harbor porpoise could use an auditory temporal window, similar to that found in certain bats (Miller, 1991), which would allow processing of echoes in a restricted range. If the porpoise can do this is unknown.

SELECTION PRESSURES FOR ADOPTING A NARROW BAND HIGH FREQUENCY BIOSONAR SIGNAL

Killer whales prey upon harbor porpoises and other marine mammals. Killer whale hearing is best at 20 kHz and one animal showed behavioral responses at 120 kHz. By extrapolation, the behavioral hearing threshold near 130 kHz would be about 90 dB re 1 μPa RMS for 2 s tone bursts (Szymanski et al., 1999). This means that a killer whale should be able to hear the biosonar of a harbor porpoise at up to about 0.5 km (assuming spherical spreading loss, a sound absorption of 40 dB/km and a short auditory time constant of the killer whale) since a wild harbor porpoise can have a source level of about 190 dB re 1 μPa pp (Villadsgaard et al., 2007). This could be a cue for killer whales, which are known to take both harbor

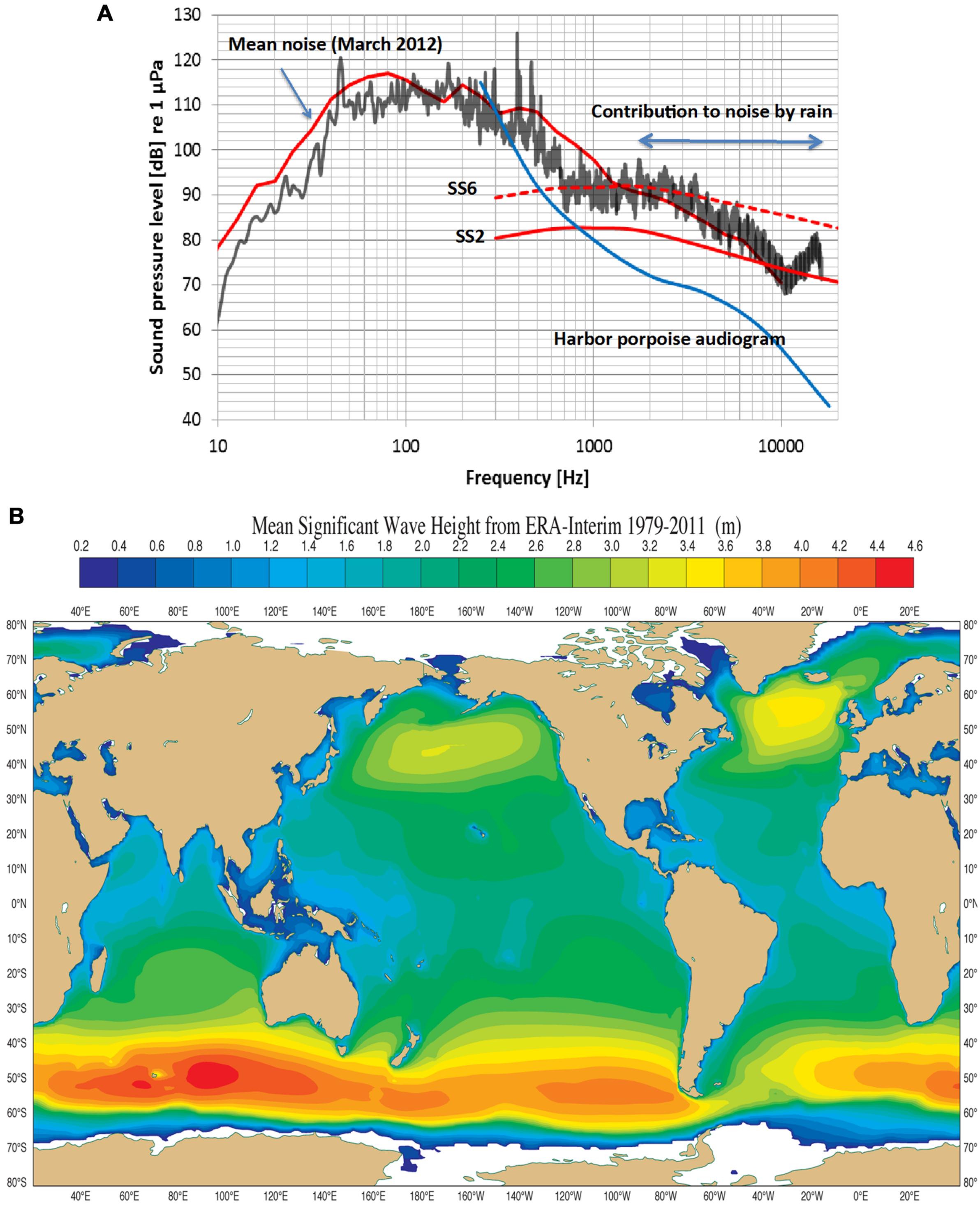

FIGURE 2 | (A) An example of noise measurements (third octave sound pressure levels) in the coastal waters of Fehmarn Belt, German Baltic. Sea state 2 and sea state 6 (ss2, ss6) are taken from Wenz, 1962. The mean curve during the month of March 2012 includes ship noise, which contributes mostly below 1 kHz. The black curve is a 20 min measurement of noise during rain (30–40 mm/h) during sea state 2, raising the noise level from 5 to 10 dB. The "contribution to noise by rain" begins to die out above 16 kHz. The blue curve is the lower portion of a harbor porpoise audiogram (Kastelein et al., 2002). Note that the harbor porpoise can hear all sources of noise above 1 kHz. (Courtesy of Dr. Dietrich Wittekind, DW-ShipConsult, Schwentinental, Germany and funded by the German Federal Agency of Nature Conservation). **(B)** The mean significant wave height (H_S) from 1979 to 2011 in most of the World's oceans lies between 1 and 3 m corresponding to mean Sea States between 3 and 5. Noise from these Sea States meets thermal noise at about 130 kHz, the biosonar frequency of the harbor porpoise (Dee et al., 2011; and courtesy of Dr. Jean Bidlot, The European Centre for Medium range Weather Forecasts, Reading, UK).

and Dall's porpoises (*Phocoenoides dalli*; Matkin et al., 2007). Still, the special characteristics of harbor porpoise biosonar signals certainly make it difficult for killer whales to detect them and may be the selection pressure that drove the harbor porpoise signal to higher frequencies (Andersen and Amundin, 1976; Madsen et al., 2005; Morisaka and Connor, 2007).

There could be another selection pressure driving harbor porpoise biosonar, and that of other odontocetes using NBHF signals, upward into a narrow band of frequencies around 130 kHz. There is a direct relationship between noise level, wind velocity, wave height (H_s) and SS (Wenz, 1962). In addition, as frequency increases from about 10 kHz and upward so does thermal noise by a factor -15 dB + 20 log *f* where *f* is frequency in kHz (Urick, 1983). SS noise at levels of SS2 to SS4 meet thermal noise at about 130 kHz (Wenz, 1962), forming a minimum of combined SS and thermal noise at a level of about 60 dB re 1 μPa rms (assuming a 4 kHz auditory filter bandwidth of the harbor porpoise (Popov et al., 2006). An analysis of mean significant wave H_s in the world's oceans over 33 years shows wave H_s are mostly around 2.4–2.6 m except for smaller areas in the North Pacific and North Atlantic, and the Southern Oceans (**Figure 2B**; Dee et al., 2011). This corresponds to a SS of 4. Measurements in the Mid-Atlantic off Florida and the Pacific off of Baja California gave similar SS values (NASA, 2000). This means that the harbor porpoise listening at 130 kHz cannot hear SS noise below about three because thermal noise dominates, but it can easily hear SS noise of four and above since these dominate when listening at 130 kHz (Kastelein et al., 2002, 2010). If sea states over geological time were at levels 3 and 4 and thermal noise was as it is today then these combined noise sources have a minimum at about 130 kHz. Thus, we hypothesize that the minimum level of sea noise and thermal noise at 130 kHz was a strong selective factor in the evolution of NBHF biosonar in some odontocetes.

Support for the above can be derived from the diversity of species using NBHF biosonar and cranial morphometrics (Galatius et al., 2011). Dall's porpoise has a substantially larger skull than that of the harbor porpoise; larger by ca. 23% in a comparison of both sexes of Californian *Phocoenoides dalli* and *Phocoena phocoena* from the inner Danish waters. In the same comparison, the skeletal structures surrounding the sound producing apparatus were relatively larger in *Phocoenoides dalli* (Galatius et al., 2011). Thus, judging from skull morphometrics the peak frequencies of Dall's porpoise biosonar clicks should differ significantly from those of harbor porpoises, but they do not. The peak frequency of both species, and others, is nearly the same at about 130 kHz (Møhl and Andersen, 1973; Au, 1997). Thus, in the near coastal NBHF species, selection has been for a similar size of the sound generating apparatus (phonic lips etc.) that can produce approximately130 kHz biosonar and not for cranial size.

CONCLUSION

We conclude that over time selective pressure from predation by killer whales may have pushed biosonar up in frequency while the meeting point of SS noise and thermal noise formed a minimum at about 130 kHz providing a convenient end point for narrow band high frequency biosonar. Harbor porpoises can effectively extract echoes from the extra noise in coastal water using their narrow band auditory filters. We propose they also listen with broadband filters to improve temporal resolution.

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Echolocation in Oilbirds and swiftlets

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The discovery of ultrasonic bat echolocation prompted a wide search for other animal biosonar systems, which yielded, among few others, two avian groups. One, the South American Oilbird (*Steatornis caripensis*: Caprimulgiformes), is nocturnal and eats fruit. The other is a selection of diurnal, insect-eating swiftlets (species in the genera *Aerodramus* and *Collocalia*: Apodidae) from across the Indo-Pacific. Bird echolocation is restricted to lower frequencies audible to humans, implying a system of poorer resolution than the ultrasonic (>20 kHz) biosonar of most bats and toothed whales. As such, bird echolocation has been labeled crude or rudimentary. Yet, echolocation is found in at least 16 extant bird species and has evolved several times in avian lineages. Birds use their syringes to produce broadband click-type biosonar signals that allow them to nest in dark caves and tunnels, probably with less predation pressure. There are ongoing discrepancies about several details of bird echolocation, from signal design to the question about whether echolocation is used during foraging. It remains to be seen if bird echolocation is as sophisticated as that of tongue-clicking rousette bats. Bird echolocation performance appears to be superior to that of blind humans using signals of notable similarity. However, no apparent specializations have been found so far in the birds' auditory system (from middle ear to higher processing centers). The advent of light-weight recording equipment and custom software for examining signals and reconstructing flight paths now provides the potential to study the echolocation behavior of birds in more detail and resolve such issues.

Keywords: Oilbird, *Steatornis caripensis*, swiftlets, *Aerodramus*, *Collocalia*, echolocation, biosonar, click

INTRODUCTION

In 1794, Lazzaro Spallanzani reported that blinded bats oriented in complete darkness, and, except for the fluttering of their wings, did so silently. Almost 20 years later, Alexander von Humboldt entered a cave in Venezuela and heard resident Oilbirds (*Steatornis caripensis*, von Humboldt, 1817) clicking noisily as they flew around in the cave that served as the birds' day roost. Had the two men corresponded, the behavior of von Humboldt's Oilbirds might have provided Spallanzani with the clue required to solve his famous bat puzzle, and brought ahead the study of animal sonar (echolocation) by about 135 years. We now know that Spallanzani's "silent" bats and von Humboldt's clicking birds use the same sensory mechanism, negotiating their surroundings via echo-feedback from self-emitted sounds. One key difference being that most echolocating bats operate using ultrasonic frequencies above the human hearing range (>20 kHz) and undetectable by eighteenth and nineteenth century technology. Since Griffin's discovery of biosonar using ultrasonic sound above the range of human hearing [reviewed in Griffin (1958)], it has become evident that toothed whales also use echolocation to negotiate their underwater habitat and detect and track their prey (Kellogg and Kohler, 1952; Norris et al., 1961).

Animal sonar is not, however, synonymous with ultrasound. Echolocation signals of several bat and odontocete species include frequencies well below the 20 kHz limit of human hearing (Leonard and Fenton, 1984; Rydell and Arlettaz, 1994; Møhl et al., 2003). Echolocation based in part or entirely on audible signals has also been demonstrated in three species of Old World fruit bats (*Rousettus aegyptiacus, R. leschenaulti, and R. amplexicaudatus)* within the otherwise non-echolocating family Pteropodidae (Möhres and Kulzer, 1956; Novick, 1958). Certain tenrecs (Tenrecidae) from Madagascar (Gould, 1965), several species of shrew (Soricidae) (Gould et al., 1964; Buchler, 1976; Tomasi, 1979; Forsman and Malmquist, 1988; Siemers et al., 2009) and some blind people (Supa et al., 1944; Griffin, 1958; Thaler et al., 2011) also echolocate with signals of frequencies below 20 kHz.

The only non-mammalian echolocators discovered to date are two groups of birds (**Figure 1**), the Oilbird (Steatornithidae, Caprimulgiformes) and several species of swiftlets (Apodiformes, Apodidae, Collocalliini, *Aerodramus* spp. and *Collocalia troglodytes*). Given the benefits of biosonar under conditions of poor visibility, seals and owls had been proposed as possible echolocators (e.g., Poulter, 1963; Renoulf and Davies, 1982) but neither echolocate (Crafford and Ferguson, 1999; Schusterman et al., 2000). Why echolocation has evolved in some disparately related groups, but not in others, remains a tantalizing question, suggesting that ecological factors play a greater role in its evolution than physiological constraints and opportunities.

Echolocation research over the last 25 years has focused on the biosonar systems of bats and odontocetes. The few published

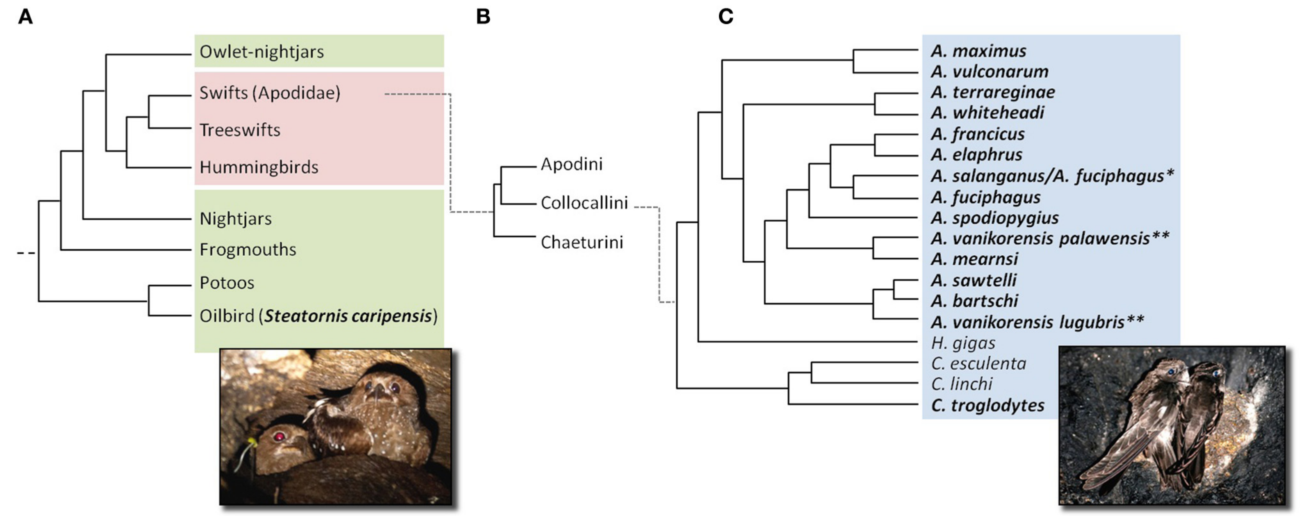

FIGURE 1 | Composite phylogeny based on three separate studies showing relationships between (A) Apodiformes (hummingbirds and swifts—purple) and Caprimulgiformes (nightjars and allies—green) (Hackett et al., 2008), (B) swifts (Apodidae) (Päckert et al., 2012), and (C) swiftlets (Collocallini, blue) (Thomassen et al., 2005). Swiftlets are monophyletic and comprise three genera: *Aerodramus* spp., *Hydrochous gigas*, and *Collocalia* spp. (Thomassen et al., 2005). Twenty-six swiftlet species are currently recognized (Chantler et al., 1999; Thomassen, 2005). Nine species (*A. brevirostris*, *A. hirundinaceus*, *A. infuscatus*, *A. inquietus*, *A. leucophaeus*, *A. nuditarsus*, *A. orientalis*, *A. papuensis*, and *A. unicolor*) were not included in the shown phylogeny and the placement of *A. fuciphagus** and *A. vanikorensis*** was ambiguous. Echolocating species appear in bold. Echolocation has been confirmed for 16 swiftlet species; *H. gigas*, *C. esculenta* and *C. linchi* do not echolocate. Echolocation abilities of remaining species are uncertain. Photographs by Signe Brinkløv: **(A)** Oilbirds (*Steatornis caripensis*) photographed on nest at Dunstan's Cave, Asa Wright Nature Centre, Trinidad (2012), **(C)** Indian Swiftlets (*Aerodramus unicolor*) photographed on nest in a railway tunnel near Pattipola, Sri Lanka (2012).

studies of bird echolocation provide important neuroethological insight and background (Griffin and Suthers, 1970; Fenton, 1975; Konishi and Knudsen, 1979; Griffin and Thompson, 1982; Thompson and Suthers, 1983; Coles et al., 1987; Thomassen et al., 2004; Thomassen and Povel, 2006) but also emphasize that there are many unresolved questions. We suggest that bird echolocation, while almost certainly not as specialized as that of bats and whales, holds the untapped potential for basic research on echolocation using sounds audible to humans, as well as for practical applications such as acoustic monitoring for conservation and management of these often vulnerable birds. Light-weight, state-of-the art field technology now available for the study of bat sonar should be readily applicable to the study of bird echolocation and should help to overcome the challenge of working in remote settings.

Here we review the sensory ecology of echolocating birds, emphasizing several outstanding questions. We consider the design of the birds' echolocation signals, their hearing, and their foraging and roosting behavior. We also speculate about the function and evolution of echolocation in birds and compare it to its use in bats and toothed whales. We further consider why most groups of echolocators, including the birds, use click-type signals rather than the frequency-modulated, often multi-harmonic, signals used by today's laryngeal echolocating bats.

ECOLOGY OF ECHOLOCATING BIRDS

OILBIRD ECOLOGY

Oilbirds (**Figure 1**) roost in natural caves, primarily in tropical forest across NW South America and Trinidad from sea level to 3400 m (Thomas, 1999). Most other caprimulgids (e.g., night hawks and nightjars) are predominantly insectivorous, crepuscular foragers relying on vision to detect and track prey. Oilbirds are nocturnal fruit-eaters, preferentially eating fruits of palms (Palmaceae), laurels (Lauraceae), and incense (Burseraceae). They swallow the fruits whole (up to 6 × 3 cm), digest the pericarp, and regurgitate the seeds (Snow, 1961, 1962; Bosque et al., 1995). A recent GPS-tracking study from Caripe in Venezuela reported that the birds often spend the day outside their roosting cave, sitting quietly in trees (Holland et al., 2009). Detailed accounts of Oilbird ecology are found in Snow (1961, 1962) and Roca (1994).

Briefly, Oilbirds are large (ca. 400 g, body length 45 cm beak-tip of tail, wing span up to 1 m) and capable of slow, maneuverable flight, with estimated flight speeds of 0.5–7 m/s, and of hovering in narrow spaces (Snow, 1961). Like other caprimulgids, Oilbirds have large eyes relative to their head size (**Figure 1**) but smaller than those of owls (Warrant, 2008). Oilbirds and owls have similar, low F-numbers (ratio of focal length to pupil diameter) indicating good visual sensitivity (Warrant, 2008). Remarkably, Oilbirds possess a banked retina with rod receptors arranged in a 3-layered structure, conferring a much higher rod to cone ratio than in owls (Warrant, 2008) with higher rod density ($\sim$1,000,000 mm^{-2}) than any other vertebrate (Martin et al., 2004). This may confer Oilbirds greater visual sensitivity in low-light conditions than owls. Whether this highly sensitive vision trades off spatial resolution remains to be determined (Warrant, 2008). Oilbirds appear to depend primarily on vision whenever possible as evidenced by observations that the incidence of sonar click emissions declines on brightly moonlit nights or in the presence of artificial light sources (Griffin, 1953; Konishi and Knudsen, 1979; Signe Brinkløv and John M. Ratcliffe, pers. obs.). Tapeta lucida occur in the eyes of some caprimulgids (Nicol and Arnott, 1974) but apparently not in Oilbirds (Martin et al., 2004). Oilbirds have large, heavily innervated olfactory organs, suggesting that sense of smell plays an important role in foraging. The birds' own musty odor may play a role in individual recognition (Snow, 1961). Like other caprimulgids, Oilbirds have long rictal bristles around the beak, which may have a close-range tactile function (Snow, 1961).

SWIFTLET ECOLOGY

Swiftlets are monophyletic (Thomassen et al., 2003, 2005; Price et al., 2004; Hackett et al., 2008) comprising approximately 26 species (Apodiformes, Apodidae). Swiftlets are found across the Indo-Pacific region, from the Seychelles and Mascarenes in the Indian Ocean to Tahiti, Mo'orea and the Marquesas in the South Pacific (Chantler et al., 1999; Thomassen, 2005). Numerous subspecies have been identified but swiftlet phylogenetic relationships are not fully resolved (Thomassen et al., 2005). This reflects a lack of distinguishing morphological and nest characteristics as well as incomplete phylogenetic sampling (Chantler et al., 1999). An attempt to use echolocation as a discriminative character to split swiftlets into echolocating (*Aerodramus*) and non-echolocating (*Collocalia* and *Hydrochous*) genera (Brooke, 1970, 1972; Medway and Pye, 1977) was refuted because Pygmy Swiftlets (*C. troglodytes*) also echolocate (Price et al., 2004). Only further research will determine whether or not the *Aerodramus* and *Collocalia* genera are justified and will be maintained (Thomassen et al., 2005).

Swiftlets are much smaller (∼10 g) than Oilbirds and all species have long, narrow wings (Chantler et al., 1999), characteristic of the typical fast flight of other apodids (Lack, 1956; Videler et al., 2004). Swiftlets are mainly diurnal foragers and hunt small insects on the wing (Chantler et al., 1999; Fullard et al., 2010). At night they typically roost in nests located on the walls of natural caves or mines and tunnels, but intriguingly, there are some published observations of nocturnal activity, including feeding, by some swiftlet species outside their cave roosts (Fullard et al., 1993; Chantler et al., 1999; Price et al., 2005). Swiftlet nests are constructed and glued in place with the birds' own saliva and nests of several species are collected for "birds' nest soup," a billion dollar industry fueled by human demand (Chantler et al., 1999).

Similar to the situation for bats within the *Rousettus* genus (Giannini and Simmons, 2003), not all swiftlets echolocate. Echolocation has been confirmed in some species, dismissed in others, and for some species we simply do not know. While *Hydrochous gigas*, *Collocalia esculenta*, and *C. linchi* (**Figure 1**) do not echolocate (Cranbrook and Medway, 1965; Medway and Wells, 1969; Fenton, 1975), at least 16 other swiftlet species do (*C. troglodytes*, *Aerodramus elaphrus*, *A. francicus*, *A. salanganus*, *A. bartschi* (Price et al., 2004); *A. vanikorensis*, (Griffin and Suthers, 1970); *A. brevirostris*, *A. fuciphagus*, *A. maximus*, *A. vulcanorum*, *A. terrareginae* (Thomassen et al., 2004); *A. sawtelli* (Fullard et al., 1993); *A. spodiopygius* (Griffin and Thompson, 1982); *A. papuensis* (Price et al., 2005); *A. hirundinaceus*, *A. unicolor* (Chantler et al., 1999; Signe Brinkløv, pers. obs.). Echolocation abilities of additional species (*A. nuditarsus*, *A. inquietus*, *A. leucophaeus*, *A. whiteheadi*, *A. pelewensis*, *A. orientalis*, *A. mearnsi,* and *A. infuscatus*) are assumed, but remain unconfirmed (Chantler et al., 1999). Swiftlets have relatively large eyes for their body size and they appear to use vision even in low-light conditions (Thomassen, 2005). We were unable to find quantitative data on the visual acuity of swiftlets.

BIOSONAR SOUND PRODUCTION PHYSIOLOGY IN ECHOLOCATING BIRDS

Birds produce their echolocation signals in the syrinx, the vocal organ specific to birds and found near to where the trachea forks into the lungs. The production mechanism for echolocation signals has been studied in one species of swiftlet with a tracheobronchial syrinx (Suthers and Hector, 1982; Thomassen, 2005), and in the Oilbird, which has a bronchial and bilaterally asymmetric syrinx (Griffin, 1944; Suthers and Hector, 1985). No direct observations have been made of the syringes of either Oilbirds or swiftlets, and the following description may need revision in light of more recent work on bird vocal production physiology (Goller and Larsen, 1997; Elemans et al., 2004; Thomassen, 2005).

With these caveats in mind, phonation (clicks and other acoustic signals) in both groups is driven by subsyringeal pressure, initiated during expiration, and controlled by two antagonistic muscle pairs. Contraction of an extrinsic muscle pair (*mm. sternotrachealis*) folds the external tympaniform membranes into the syrinx (or the two half-syringes in Oilbirds) lumen toward the internal tympaniform membranes. The membranes are then set into vibration by the expiratory airflow. In Oilbirds, clicks are actively terminated by contraction of the single pair of intrinsic syringeal muscles (*mm. broncholateralis*). In contrast, the social vocalizations of Oilbirds are terminated passively by relaxation of the sternotrachealis muscles (Suthers and Hector, 1985). Swiftlets lack intrinsic syringeal muscles and terminate their clicks by contraction of extrinsic tracheolateralis muscles (Suthers and Hector, 1982; Thomassen, 2005). Most species of echolocating swiftlet produce single clicks as well as double clicks (two single clicks in quick succession, as described below). The pause between two clicks within a click-pair may be caused by a brief blocking of airflow through the syrinx as the external and internal tympaniform membranes touch. Single clicks appear to arise when the membranes are pulled together before the expiratory airflow generates enough pressure to initiate vibration of the membranes (Suthers and Hector, 1982). Both sides of the swiftlet syrinx appear able to contribute to each member of a click-pair; that is, birds can still emit double clicks even if one side of the syrinx is plugged (Suthers and Hector, 1982).

BIOSONAR SIGNAL DESIGN IN ECHOLOCATING BIRDS

Echolocation behavior involves the same operating principles across animal groups, namely extracting information about the immediate surroundings from returning echoes of one's own signals. However, vocal physiology, mechanisms of sound production, and signal design differ notably among echolocators. The term click is loosely used to describe acoustic signals that are short and do not exhibit any structured changes in frequency over time. Birds, odontocetes, shrews, tenrecs, and echolocating rousette bats use click-type biosonar signals. Contrarily, laryngeal echolocating bats produce acoustic signals characterized by structured changes in frequency over time, such as downward sweeps (**Figure 2**). In our discussion of bird echolocation signals, we will follow Pye's definition of clicks as "broadband impulse sounds with no clearly defined coherent 'carrier' frequency, no evidence of frequency modulation and an amplitude pattern that is rapid and transient" (Pye, 1980). We will use "click" to define the basic signal unit of bird echolocation and "click burst" to describe two or more clicks produced in rapid succession.

ECHOLOCATION SIGNAL DESIGN IN OILBIRDS

The first description of Oilbird sonar emissions was based on field recordings of naturally behaving birds flying within a cave (90 m from entrance) at Caripe, Venezuela (Griffin, 1953). Signals from sequences where only one bird was detected on the microphone were described as stereotyped and readily audible to humans at a distance up to 180 m from the bird. Each click consisted of only a few sound waves, and thus was of very brief duration (ca. 1 ms), with most energy between 6 and 10 kHz (**Table 1**). Notably, clicks were not emitted at a regular rate, but in bursts of 2–6+ clicks, with nearly constant within-burst click intervals of 2.6 ms and little within-burst variation (Griffin, 1953).

Konishi and Knudsen (1979) reported that Oilbird signal energy was unevenly distributed from 1 to 15 kHz, with most energy from 1.5 to 2.5 kHz, coincident with the birds' most sensitive area of hearing (Konishi and Knudsen, 1979). The auditory threshold curve, derived from cochlear evoked potentials, showed maximum sensitivity at 2 kHz, with a roughly 20 dB decline per octave for higher frequencies, indicating that Oilbirds should be deaf, or at least largely insensitive, to sounds above 6 kHz (**Figure 3**). Konishi and Knudsen (1979) included obstacle avoidance experiments revealing that Oilbirds successfully detect and avoid disks of ≥20 cm diameter but may have failed to detect disks ≤10 cm diameter. However, discs with diameters ≤20 cm were presented in an array where individual disks were spaced at 5 times the chosen disc diameter. This means that trials with discs ≤10 cm likely affected the ability of the Oilbirds to negotiate

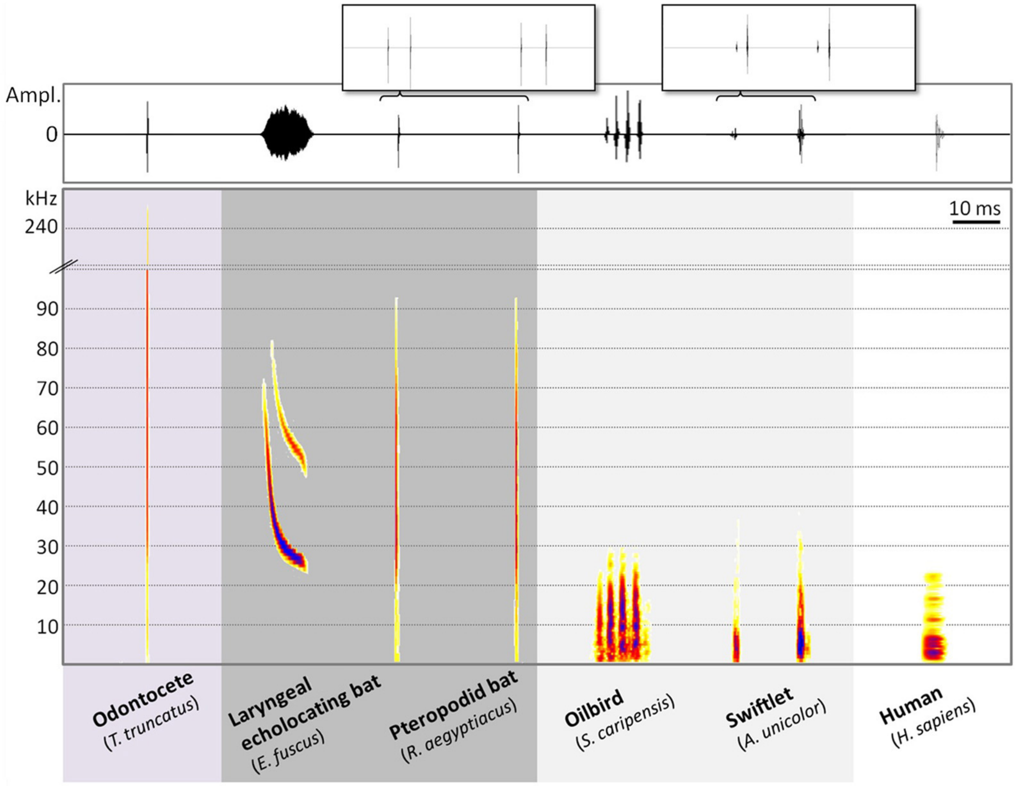

FIGURE 2 | Composite waveform (top) and spectrogram (bottom) of echolocation signals from 6 vertebrate species: common bottlenose dolphin (*Tursiops truncatus*), sample rate (*fs*) = 500 kHz; laryngeal echolocating bat (*Eptesicus fuscus*), *fs* = 250 kHz; tongue-clicking pteropodid bat (*Rousettus aegyptiacus*), *fs* = 250 kHz; Oilbird (*Steatornis caripensis*), *fs* = 75 kHz; swiftlet (*Aerodramus unicolor*), *fs* = 250 kHz and echolocating blind human subject (*Homo sapiens*), *fs* = 48 kHz. Top inserts both have total time scales of 300 ms and illustrate the double clicks often emitted by echolocating *Rousettus* spp. and most echolocating swiftlet species. Bat and bird recordings made by Signe Brinkløv, dolphin recording courtesy of Magnus Wahlberg, human recording courtesy of Cynthia Moss. Spectrograms were created in BatSound v. 4 using an FFT size of 256, except for those from *R. aegyptiacus* and *S. caripensis*, for which an FFT size of 128 was used. All spectrograms were made using 98% overlap. Colors indicates relative amplitude going from low (light color) to high (darker color). Note the interrupted frequency scale between 100 and 230 kHz. Waveform amplitudes have all been normalized to the same level.

Table 1 | Summary of Oilbird (*Steathornis caripensis*) echolocation click parameters described in previous literature.

References	Click parameters		Recording site	Recording condition	System frequency response
	Duration (ms)	**Frequency (kHz)**			
Griffin, 1953	1	6–10	Field Venezuela	Inside cave	Within ±6 dB 50–15,000 Hz
Konishi and Knudsen, 1979	>20	1.5–2.5	Aviary Trinidad	Birds hovering	Flat 50–20,000 Hz
Suthers and Hector, 1985	40–50	No data	Laboratory Trinidad	Handheld birds, blindfolded	Flat 100–40,000 Hz

Despite similar frequency responses across recording systems, data for click duration and frequencies with most energy are noticeably different. Griffin (1953) reported that a 2 kHz high pass filter was used in the analysis of some recordings but that such a filter was implemented only after verification that the unfiltered recordings had no appreciable energy components below 2 kHz.

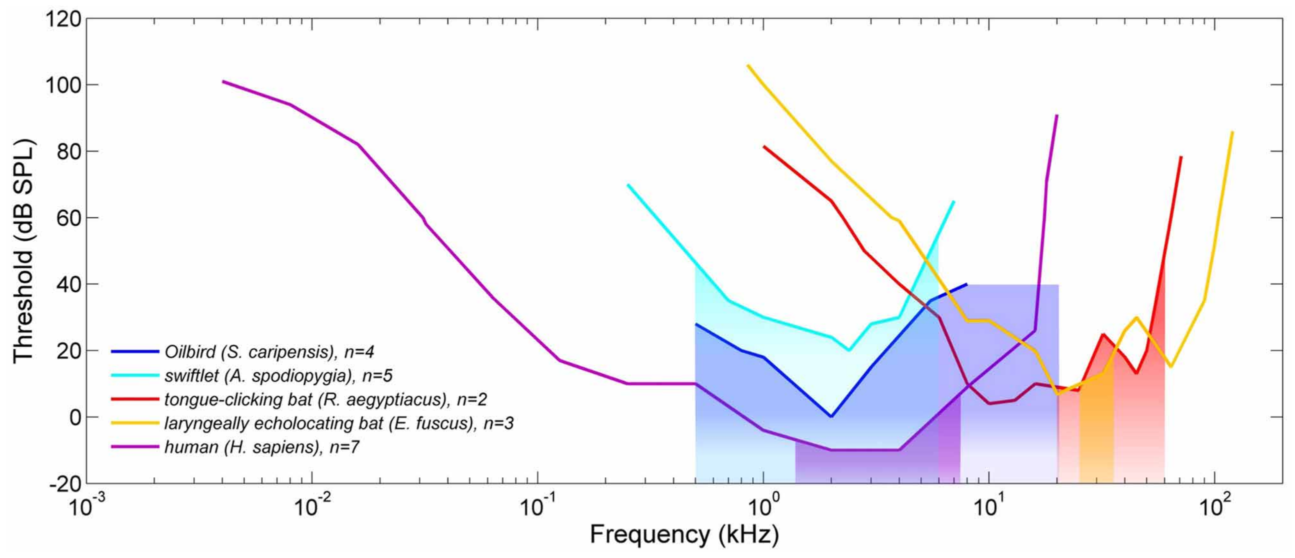

FIGURE 3 | Comparative audiograms for 5 vertebrates, all of which are capable of some form of echolocation. Audiograms shown are visually estimated averages derived from previous experiments with Oilbirds (*Steatornis caripensis*) (Konishi and Knudsen, 1979), one swiftlet species (*Aerodramus spodiopygia*) (Coles et al., 1987), one tongue-clicking pteropodid bat species (*Rousettus aegyptiacus*) (Koay et al., 1998), one laryngeal echolocating bat species (*Eptesicus fuscus*) (Koay et al., 1997) and humans (*Homo sapiens*) (Jackson et al., 1999). Audiograms of *R. aegyptiacus*, *E. fuscus* and *H. sapiens* were obtained from behavioral experiments, whereas thresholds from *S. caripensis* and *A. spodiopygia* were based on neurophysiological data from anaesthized birds. Note that relative threshold differences should not be directly compared due to differences in experimental conditions, e.g., different ambient noise levels. Colored blocks correspond to the frequency range where echolocation signals of each group have most energy (measured as −15 dB bandwidth—frequency range 15 dB down from either side of the spectrum peak—of a single click per species), for example the red block is the −15 dB bandwidth of a *R. aegyptiacus* click. The recording used for bandwidth measurements of human echolocation clicks was provided by C. Moss and the −15 dB bandwidth of *A. spodiopygia* was estimated from Figure 3B in Coles et al. (1987). Remaining bandwidths were measured from recordings made by Signe Brinkløv.

a course through such an array, as the inter-disc spaces (≤50 cm) were only half of the birds' wingspan. As in bats and whales, an increase in signal repetition rate was noted prior to avoidance manoeuvres (Konishi and Knudsen, 1979).

Suthers and Hector (1983) reported that Oilbirds acoustically detected obstacles as small as 3.2 cm in diameter using signals with most energy at 0.5–3.0 kHz. They suggested that the birds used either continuous pulsatile signals (durations of 40–80 ms) or, occasionally, much shorter pulses emitted at repetition rates ranging from only a few every second to 12 s^{-1} (Suthers and Hector, 1985). From handheld birds, Suthers and Hector (1983) estimated signal intensity as ~100 dB SPL rms at 20 cm to 1 m distance. There are no published quantitative estimates of signal intensity in free-flying Oilbirds and we do not know if the birds can adjust the intensity of their signals, as do laryngeal echolocating bats and toothed whales.

ECHOLOCATION SIGNAL DESIGN IN SWIFTLETS

Echolocation has been confirmed in 16 species of swiftlets (Chantler et al., 1999) and existing descriptions of swiftlet echolocation signals are more congruent, even across recording conditions, than those for Oilbirds (**Table 2**). Swiftlet clicks are composed of frequencies completely within the human auditory range, with most energy between 1 and 10 kHz.

With notable exceptions, most swiftlet species emit both single and double clicks (Thomassen et al., 2004). Double clicks, or click-pairs, are emitted more frequently than single clicks (up to 75% of the time) and so close together that they, as the

Table 2 | Summary of swiftlet echolocation click parameters as described in the literature.

References	Species	Click parameters						Recordingsite	Recording condition	System frequency response
		Click duration (ms)		Intra-pair interval (ms)	Inter-pair interval (ms)	Click frequency (kHz)				
		1st	2nd			1st	2nd			
Griffin and Suthers, 1970	*A. vanikorensis*	Few	4–8	Several	48–358	5–8	5–8	Dark chamber Papua New Guinea	Flying birds	within ±3 dB 700–12,000 Hz
Fenton, 1975	*A. hirundinacea*	–	4–8	–	48–250	4–8	4–8	Cave Papua New Guinea	Free-flying birds	Within +2/−6 dB 70–13,000 Hz
Suthers and Hector, 1982	*A. spodiopygia*	3	>3	18–25	–	2–8	2–8	Dark chamber Australia	Hovering birds	Flat 100–37,000 Hz or 100–75,000 Hz
Coles et al., 1987	*A. spodiopygia*	3–8	4–6	18	> 40	3–8	4–6	Dark chamber Australia	Tethered bird attempting flight	Not described
Fullard et al., 1993	*A. sawtelli*	1–3	–	–	107–186	6–8		Cave Atiu	Free-flying birds	within ±2 dB 500–15,000 Hz
Thomassen and Povel, 2006	*A. brevirostris*	2	3	12	–	3–5	3–6	Various localities and contexts		–
	A. elaphrus	2	3	12		2–5	2–6			
	A. fuciphagus	3	3	12		3–6	3–7			
	A. maximus	6	7	11		3–6	2–7			
	A. salanganus	4	5	14		3–6	3–7			
	A. terrareginae	2	3	18		3–6	2–7			
	A. vanikorensis	2	3	17		2–4	2–5			
	A. vulcanorum	2	4	14		2–7	1–10			

All click parameters are rounded to nearest whole number. (–) indicates that data was not described in the literature source or, in the case of A. sawtelli, that no values exist because this species only produces single clicks.

click-bursts of Oilbirds, sound like a single sound to human ears (Griffin and Suthers, 1970). Each click within a pair lasts 1–8 ms, with the second often of higher amplitude (Griffin and Suthers, 1970; Suthers and Hector, 1982; Coles et al., 1987). Clicks in a pair are separated by 11–25 ms (**Table 2**).

Swiftlet clicks have been described as highly stereotyped, varying little in design regardless of situation (Thomassen and Povel, 2006). However, swiftlets increase click repetition rate when facing complex challenges, such as approaching obstacles (Griffin and Suthers, 1970; Coles et al., 1987) or their nests (Signe Brinkløv, pers. obs. of *A. unicolor* in railway tunnels). Fullard et al. (1993) found that birds emitted higher repetition rates when entering caves than when exiting caves or flying from closed to more open space. Meanwhile, no context-dependent changes were found in signal frequency (Fullard et al., 1993), as compared to the adaptive, context-dependent changes in signal frequency found in many laryngeal echolocating bats.

CURRENT KNOWLEDGE OF THE ECHOLOCATION ABILITIES OF BIRDS

ECHOLOCATION AND HEARING ABILITIES OF OILBIRDS

Oilbirds have only a single middle ear bone in each ear (as opposed to the three found in mammals), a simple cochlea (Martin, 1990), and thus, like other birds, are expected to be insensitive to frequencies above 10 kHz (Dooling et al., 2000). As noted above, Oilbirds emit conspicuous echolocation signals at frequencies well within the human hearing range and little to no energy above 20 kHz. However, it remains unclear whether most frequency content falls below 5 kHz (Konishi and Knudsen, 1979), or above 5 kHz as described in the earlier field study (Griffin, 1953). Konishi and Knudsen (1979) argued that main frequency content at 6–10 kHz, as reported by Griffin (1953), would result in a mismatch between emitter and receiver. However, Konishi and Knudsen (1979) displayed data points on Oilbird auditory sensitivity up to but not beyond 8 kHz. None of the studies described above seem limited by the frequency range of the recording systems used (**Table 1**) and so the upper limit of sound frequencies tested by Konishi and Knudsen (1979) was apparently based on the reasonable assumption that Oilbirds do not hear frequencies above 8 kHz. Konishi and Knudsen (1979) also suggest that Oilbirds exhibit little or no directional hearing at frequencies up to 4 kHz and beyond, as predicted by the size of the birds' heads and lack of any external ear structures. While Griffin's (1953) work was done in the field, Konishi and Knudsen's (1979) descriptions are from captive animals. If Oilbirds can change the frequency content of their clicks by shifting signal energy to higher frequencies in the presence of loud ambient low frequency noise, this might occur more often in the field than in captivity.

Existing descriptions of echolocation signal parameters from Oilbirds also reveal discrepancies concerning signal duration (**Table 1**) and raise questions about how clicks in general are defined by bioacousticians. Griffin (1953) described Oilbird biosonar signals as having a minimum duration of 1 ms, thus referring to a click as the smallest subunit within a burst of sonar emissions. Konishi and Knudsen (1979) used "click' to describe each >20 ms burst of pulses, reasoning based on their recordings that each burst comprises a complex waveform with pulsatile elements rather than a series of discrete pulses. They noted increases in repetition rate between rather than within burst units as birds approached a variety of obstacles. They also argued that because each burst, rather than each burst subunit (i.e., click), is registered as a single, coherent unit by the human ear, by extension they would be registered as a single sound at the bird's more simple ear. Suthers and Hector (1985; their Figure 5) also referred to each click as a burst of several amplitude peaks rather than the subunits within each burst. The number of subunits within a burst varies (Griffin, 1953; Signe Brinkløv, pers. obs.), but whether this variation is of any functional significance to the birds is unknown. The well rounded, if conflicting, data set on Oilbird echolocation makes this species especially attractive for future integrative lab and field-based studies.

ECHOLOCATION AND HEARING ABILITIES OF SWIFTLETS

Swiftlet clicks appear to have most energy over a 1–10 kHz frequency range. Based on rule of thumb calculations, the birds should only detect objects ≥34 mm diameter, but can apparently detect objects as small as 6.3 mm diameter (metal rods) at levels above chance (Griffin and Suthers, 1970; Griffin and Thompson, 1982). Corroborating this, Smyth and Roberts (1983) reported a detection threshold of 10–20 mm, while Fenton (1975) found that *A. hirundinacea* detected vertical rods down to 10 mm diameter and potentially even smaller. These data suggest that swiftlets receive useful echo information via the higher frequency portions of their clicks, even though these components contain less energy. However, for this to be plausible the birds must hear, at least to some extent, higher frequencies. This is not supported by data from single neuron recordings from the midbrain auditory nucleus of *Collocalia spodiopygia*, which indicate best frequency thresholds from 0.8 to 4.7 kHz (Coles et al., 1987).

Whatever the ultimate size limit of object detection by swiftlet biosonar, observations of increased click repetition rates from birds approaching their nests in the wild (Fullard et al., 1993; Signe Brinkløv, pers. obs.) suggest that swiftlets use echolocation to locate their nests. And, because swiftlet nests are 50–100 mm in diameter (Coles et al., 1987; Chantler et al., 1999), even a conservative detection size threshold would indicate that the nest itself should be readily detectable by swiftlet echolocation.

SINGLE AND DOUBLE SWIFTLET BIOSONAR CLICKS: A WEST-EAST TRANSITION?

A. sawtelli, endemic to Atiu, one of the Cook Islands, only emits single clicks, giving rise to the hypothesis of an evolutionary West-East transition from double clicks to the obligate emission of single echolocation clicks (Fullard et al., 1993, 2010). However, Thomassen et al. (2004) reported that several relatively western species of swiftlets can also emit single clicks. Conversely, *A. vanikorensis* in the more centrally located Phillipines and New Guinea appears to emit only double clicks (Thomassen et al., 2004).

Whether single and double clicks serve specific, even separate functions that are correlated to certain behaviors is also unknown, as is whether swiftlets can actively control which type is emitted. Interestingly, although assumed to echolocate, we are unaware of scientific accounts of echolocation in the

Polynesian Swiftlet, *A. leucophaeus*, at the far eastern geographic distribution of swiftlets. *A. leucophaeus* is missing from recent attempts to resolve the controversial swiftlet phylogeny but ostensibly includes three subspecies found on Tahiti, Mo'orea, and the Marquesas in French Polynesia (Chantler et al., 1999). More knowledge about the genetic relationship between *A. leucophaeus* and the geographically close single click emitter *A. sawtelli*, along with information about the nature of *A. leucophaeus* echolocation clicks, could help elucidate why some swiftlets only emit single clicks and possibly the underlying functional reasons for the use of single and double clicks.

Egyptian rousettes (*R. aegyptiacus*, Pteropodidae) use double clicks to point their sound beam to the right and left of a target to trade localization over detection (Yovel et al., 2010). Rousette bats echolocate using tongue clicks and this means of echolocating contrasts with the situation in laryngeal echolocating bats, which direct their sonar beam with high precision directly at the target (Jakobsen and Surlykke, 2010). It would be interesting to see whether the double clicks of swiftlets function like those of *Rousettus*.

ECHOLOCATION FOR ORIENTATION, ECHOLOCATION FOR FOOD DETECTION?

Echolocating birds use clicks dominated by low frequencies (Konishi and Knudsen, 1979; Coles et al., 1987), limiting their ability to detect small targets. A target reflects echoes only if its cross section is at least roughly one-third as large as the wavelengths impinging on it (Pye, 1980; Jakobsen et al., 2013). Therefore, bird echolocation clicks are not suited for detection of smaller objects such as insect prey <2–3 cm in diameter. Although echolocating birds appear to lack the highly specialized and flexible echolocation abilities of laryngeal echolocating bats and toothed whales they are clearly adept at maneuvering and locating their nests within the dark interior of their cave roosts.

Several anecdotal observations suggest that Oilbirds occasionally echolocate outside caves and around fruiting palm trees (Konishi and Knudsen, 1979; Suthers and Hector, 1985). Snow (1961) reported that he never heard clicks from Oilbirds feeding at night. Staff at the Asa Wright Nature Center in Trinidad provided us with contradictory reports indicating that Oilbirds do click while flying around fruiting palms (Signe Brinkløv, pers. comm.). As Oilbirds eat fruit that is considerably larger than the insect prey of swiftlets (Snow, 1961; Bosque et al., 1995) and often visit trees with a conspicuous shape (e.g., palms), the use of echolocation to find food remains an enticing possibility.

One of us (M. Brock Fenton) has spent considerable time listening for echolocation clicks from swiftlets on Papua New Guinea (*A. hirundinacea*) and in Australia (*A. spodiopygia*) and never heard clicks from night-flying birds except as they returned to their roosts. Notably, however, Atiu Swiftlets (*A. sawtelli*) and Papuan Swiftlets (*A. papuensis*) click not only in their caves but also outside at night, apparently while hunting insect prey in low light (Fullard et al., 1993; Chantler et al., 1999; Price et al., 2005). In swiftlets, echolocation may thus be more advanced in some species than others, but this is highly speculative. If so, the relationship between two click/one click flexibility and the use of echolocation outside the cave would be one area to explore. Oilbirds and swiftlets both orient visually when ambient light conditions are sufficient, as indicated by the absence of echolocation sounds altogether under such conditions and suggested by their oversize eyes relative to other birds. However, the absence of data on light levels taken concurrently with acoustic recordings make it unclear under exactly what conditions the birds should be expected to rely on echolocation over vision.

ECHOLOCATION IN A SOCIAL CONTEXT

Inside their roosts, echolocating Oilbirds and swiftlets must deal with a host of reverberations from cave surfaces as well as a cacophony of clicks from conspecifics. Besides orientation, bird echolocation signals may serve a role in communication. Laryngeal echolocating bats react to the feeding buzzes emitted by con- and hetero-specifics moments before contact with an airborne insect (Gillam et al., 2007; Übernickel et al., 2013), and change their echolocation behavior when flying in groups as opposed to alone (Obrist, 1995; Ratcliffe et al., 2004; Brinkløv et al., 2009).

In addition to echolocation clicks, Oilbirds and swiftlets produce a range of more tonal signals (Suthers and Hector, 1985; Thomassen and Povel, 2006). For example, Oilbird social squawks resemble a prolonged click burst, including up to 20+ subunits, and are often emitted as several birds fly together (Suthers and Hector, 1985). Such signals likely serve a communicative function to birds flying in close proximity (e.g., as agonistic "honks" to prevent collision, Signe Brinkløv, pers. obs.), analogous to social functions suggested for bat buzzes (i.e., call rates >100calls/s) emitted outside the context of prey-capture (Bayefsky-Anand et al., 2008). Moreover, both Oilbirds and swiftlets appear to forage socially, as indicated by observations of birds arriving at feeding locations and returning to caves in groups of 2 or more individuals (Snow, 1961; Signe Brinkløv, pers. obs.). Swiftlets should be able to maintain visual contact during their daytime foraging bouts, but for nocturnal Oilbirds, biosonar signals may facilitate social cohesion in flight.

There is enough inter-specific variation in swiftlet biosonar clicks to render them species-specific, primarily based on inter-specific variation of maximum click frequency (Thomassen and Povel, 2006). It is plausible then that swiftlet echolocation clicks could be used in conspecific recognition, potentially of relevance where several species have overlapping geographical distributions and may either share or compete for access to caves. However, the social signals of swiftlets are also species-specific (Thomassen and Povel, 2006) and may serve equally well or better for this and other purposes. On a similar note, the morphological asymmetry of the Oilbird syrinx may allow for individual recognition during vocal communication. Individual differences in vocal tract asymmetry have been suggested as a means for Oilbirds to distinguish echoes originating from their own echolocation signals from those clicks and echoes originating from their roostmates (Suthers and Hector, 1988).

WHY CLICK?

Many species of non-echolocating swiftlets and swifts (Apodidae) are acoustically conspicuous to human observers. Two examples are the "screaming" parties of Common Swifts on the wing

(*Apus apus*; Lack, 1956) and the conspicuous flight chirps of Chimney Swifts (*Chaetura pelagica;* Bouchard, 2005). Indeed, the syringes of most non-Oscine birds (e.g., Oilbirds and swiftlets) are well-suited to producing a wide range of acoustic signals (Suthers and Hector, 1985). Why then, do Oilbirds and swiftlets use clicks for echolocation? As Buchler and Mitz (1980) noted, there is no obvious reason why two signals with the same power spectra, one a click, the other a frequency-modulated signal, should differ in their basic utility in echolocation. If anything, single-sweep, frequency-modulated signals may be advantageous, allowing the echolocator to produce a longer signal, with more overall energy, in which a particular frequency is essentially time-stamped (Simmons and Stein, 1980).

We propose that echolocating birds use click-type signals for echolocation because they are short in duration, permitting detection of objects even at very short distances (i.e., with no overlap between signal and echo). At the same time click-type signals do not require the laryngeal specializations observed in bats necessary to produce a sufficiently short frequency-modulated signal. In the non-echolocating Chimney Swifts, none of the frequency-modulated and/or harmonic signals reported by Bouchard (2005) would be short enough to serve as an effective echolocation signal in a cave roost. Additionally or alternatively, clicks may be more effective biosonar signals for detection of objects at greater distances because they may be (i) less energetically expensive to produce using the syrinx and (ii) louder than other signal designs using the same energy input. We note that despite several attempts to uncover any morphological and neurological specializations, none have yet been found in the syringeal morphology, hearing abilities, middle ear morphology or higher processing centers (auditory nuclei) of Oilbirds or echolocating swiftlets that set them apart from non-echolocating birds (Konishi and Knudsen, 1979; Thomassen, 2005; Iwaniuk et al., 2006).

EVOLUTION OF BIRD ECHOLOCATION

A recent phylogenomic study of the birds embeds swiftlets within what appears to be the paraphyletic Caprimulgiformes, the avian order that includes Oilbirds (Hackett et al., 2008). Nevertheless, the most parsimonious evolutionary scenario consists of three independent originations of syringeal echolocation in birds, once in the precursor to Oilbirds and twice within the swiftlets (**Figure 1**). Both groups use echolocation to gain access to roosting sites and nests in caves and deep gorges, where they may be protected from some predators. This common ecological variable may have provided evolutionary impetus for the multiple appearances of echolocation within the clade. An analogous connection between cave-dwelling and use of echolocation seems to be present in rousette bats (Giannini and Simmons, 2003). One avenue of future research would be investigations of the species-specific relationships between the visual systems, presence or absence of echolocation, and preferred light-level of the cave roost within an evolutionary context using the comparative method. Information about the ontogeny of echolocation is at present also completely unknown.

Echolocation almost certainly originated independently in Apodiformes and Caprimulgiformes and likely evolved independently within two distinct lineages of swiftlets (Price et al., 2005; Thomassen et al., 2005). The inaccessibility of many species of swiftlets and resulting lack of genetic and acoustic data means that the evolutionary pathways of swiftlet echolocation remain to be unravelled. Increased molecular sampling and systematic documentation of swiftlet echolocation abilities will be necessary to further resolve their phylogenetic history. Such research would help to clarify species limits, answer questions about the evolution of obligate single click emitting species and address the predominance of those species that produce both double and single biosonar clicks.

Most echolocating bats forage only at night (Neuweiler, 1984), spending the day resting in their roosts. Echolocating swiftlets, like the vast majority of birds, are diurnal foragers (Chantler et al., 1999). Thus, despite their use of cave roosts and similarities in feeding ecology (i.e., the capture of flying insects on the wing) (Fenton, 1975), swiftlets and similar-sized insect-eating bats are not likely to compete with one another directly, due to temporal separation of foraging activities. Similarly, there is no evidence that either echolocating bats or swiftlets feed on one another. Oilbirds and rousette bats exploit a similar niche, albeit on different continents. Interestingly, both Oilbirds and rousette bats are nocturnal frugivores, and both use click-type echolocation and dark roosts during the day (Griffin et al., 1958; Snow, 1961). In the New-World tropics, where Oilbirds and a number of smaller frugivorous New World leaf-nosed bats (Phyllostomidae) overlap both spatially and temporally when foraging, there appears to be very little overlap in fruit preference between these groups. Oilbirds consume large fruits, often with large seeds that are later regurgitated (Snow, 1962), while phyllostomid bats are much smaller and feed preferentially on fruits with small seeds that are chewed or expelled while eating (Wendeln et al., 2000; Mello et al., 2011).

FUTURE RESEARCH STEPS

Further studies of the echolocation systems of birds will be valuable additions to the ever-expanding and progressive field of bat and toothed whale echolocation research. State-of-the-art lightweight field equipment (e.g., multi-microphone arrays) and custom-designed computational software should provide better quality recordings of biosonar signals from Oilbirds and swiftlets. Experiments could be designed to compare signals of birds flying in different contexts, for example, field versus captivity, open space versus cave interior and multiple versus single birds, to help resolve current uncertainties about signal design. Further, such recordings should help identify *who* says *what*, *when,* and *where* even in complex situations where several birds are flying together and provide useful clues about echolocation in a social context.

The highly specialized echolocation systems of toothed whales and laryngeal echolocating bats have provided and continue to provide fascinating insights into the mammalian auditory system and active sensory processes in animals across taxa. By comparison, echolocation in birds has received almost no attention. This is perhaps because we have implicitly regarded bird biosonar as unsophisticated and, thus, less interesting. Perhaps, less cynically, it is simply because bats are found everywhere, save past the tree-line and on a few isolated Oceanic islands, while echolocating birds are far less wide-spread and in general more difficult to gain access to than are bats.

Deployment of portable tags with hydrophones and accelerometers has contributed greatly to the understanding of toothed whale acoustic behavior in deep waters where the animals roam beyond visual inspection (Madsen et al., 2005; Johnson et al., 2007; Jensen et al., 2011). Corresponding on-board archival microphone tags would be ideal to assess the level of any active and adaptive control over sonar signal characteristics in birds, clarify the potential role of bird echolocation in the context of in-flight social interactions and allow us to determine if Oilbirds echolocate while foraging. In-flight GPS recorders have already been used to track movements of Oilbirds in the field (Holland et al., 2009) and their large size makes Oilbirds ideal subjects for the first acoustic tagging study of echolocating birds. Further, direct endoscopic visualization of syringeal mechanisms is now possible (Goller and Larsen, 1997), as are *in vitro* neuromuscular preparations to study the biomechanic mechanisms involved in avian and mammalian sound production (Elemans et al., 2004, 2011). Such techniques could be put to use in better understanding biosonar click production in Oilbirds and swiftlets.

The tongue-clicking pteropodid bat *R. aegyptiacus* uses echolocation to detect and discriminate objects better than previously suspected (Yovel et al., 2011). Echolocation in birds may be similarly underappreciated. Moreover, a deeper understanding of echolocation in birds, rousette bats, and shrews and tenrecs would have its own rewards. Echolocation by blind people is now more common and better understood, and comparisons to non-human echolocators using similar click-type signals may help us learn more about and improve human biosonar. In a broader sense, understanding animal biosonar across taxa will undoubtedly reveal similarities and differences across different groups of animals that have independently evolved biosonar systems with respect to all aspects of their biology, from ecology and evolution, to the neurophysiology and biomechanics of sound production and echo processing.

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Echolocation may have real-life advantages for blind people: an analysis of survey data

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Some people can echolocate by making sonar emissions (e.g., mouth-clicks, finger snaps, feet shuffling, humming, cane tapping, etc.) and listening to the returning echoes. To date there are no statistics available about how many blind people use echolocation, but anecdotal reports in the literature suggest that perhaps between 20 and 30% of totally blind people may use it, suggesting that echolocation affords broad functional benefits. Consistent with the notion that blind individuals benefit from the use of echolocation, previous research conducted under controlled experimental conditions has shown that echolocation improves blind people's spatial sensing ability. The current study investigated if there is also evidence for functional benefits of echolocation in real life. To address this question the current study conducted an online survey. Thirty-seven blind people participated. Linear regression analyses of survey data revealed that, while statistically controlling for participants' gender, age, level of visual function, general health, employment status, level of education, Braille skill, and use of other mobility means, people who use echolocation have higher salary, and higher mobility in unfamiliar places, than people who do not use echolocation. The majority of our participants (34 out of 37) use the long cane, and all participants who reported to echolocate, also reported to use the long cane. This suggests that the benefit of echolocation that we found might be conditional upon the long cane being used as well. The investigation was correlational in nature, and thus cannot be used to determine causality. In addition, the sample was small ($N = 37$), and one should be cautious when generalizing the current results to the population. The data, however, are consistent with the idea that echolocation offers real-life advantages for blind people, and that echolocation may be involved in peoples' successful adaptation to vision loss.

Keywords: vision loss, blindness, adaptation, mobility, correlation, regression

INTRODUCTION

Some people, just like certain echolocating bats and marine mammals, can echolocate by making sonar emissions (e.g., mouth-clicks, finger snaps, feet shuffling, humming, cane tapping, etc.) and listening to the returning echoes (Stoffregen and Pittenger, 1995; Schenkman and Nilsson, 2010; Teng and Whitney, 2011). Echolocation can be learned by both blind and sighted people with normal hearing (Worchel and Mauney, 1951; Ammons et al., 1953; Teng and Whitney, 2011). Blind people are typically better at echolocation than sighted people, yet some sighted people can approach the accuracy of blind echolocation experts (Teng and Whitney, 2011). Recent research suggests that even the sighted human brain has cortical areas devoted to the processing of echoes (Thaler et al., under review). It is possible that the blind human brain capitalizes on these "pre-mordial" echolocation areas when acquiring echolocation skills (Thaler et al., 2011). To date there are no statistics available about how many blind people use echolocation, but anecdotal reports in the literature suggest that perhaps between 20 and 30% of totally blind people may do so (Wölfflin, 1909; Lamarque, 1929; Villey-Desmeserets, 1930).

The question arises what functional benefits people experience through the use of echolocation. In the context of bats it has been suggested that echolocation skills may have been naturally selected for, because they offer functional advantages, such as improved spatial orientation and/or acquisition of food (e.g., Schnitzler et al., 2003). Following this line of reasoning, we might hypothesize that blind people echolocate, because it offers broad functional benefits for them as well. Echolocation abilities in certain bats are the result of millions of years of evolution (Neuweiler, 2003; Denzinger et al., 2004). In contrast, a person's visual impairment and echolocation ability arise during that person's lifespan. I want to emphasize, therefore that here I am using the comparison to bats to emphasize potential analogies in terms of functional benefits, not to emphasize potential analogies in terms of evolutionary mechanisms. From the hypothesis that blind people use echolocation, because it offers functional benefits follows that there should be measurable functional benefits for blind people who echolocate, as compared to blind people who do not echolocate. Consistent with the hypothesis that echolocation offers functional benefits, previous research conducted under controlled experimental conditions has shown that echolocation improves blind people's spatial sensing ability, in that it improves their ability to determine distance, location, motion, size, shape or material of surfaces (for reviews see for example Stoffregen and Pittenger, 1995; Schenkman and Nilsson, 2010). Blind people can use echolocation for example to determine if a distant object is made out of denim, wood or metal (Kellogg, 1962), if a distant object is concave or flat (Thaler et al., 2011), to detect if there is a gap as small as 0.02 m between two objects placed 1 m away (Teng et al., 2012), to distinguish moving from stationary surfaces (Thaler et al., 2011) or to determine when a collision with an approaching wall is imminent (e.g., Supa et al., 1944; Cotzin and Dallenbach, 1950). It is an open question, however, to what degree functional improvements measured under controlled experimental conditions translate into benefits in real life [see for example Lane et al. (2008) for a discussion of this issue in the context of rehabilitative interventions]. Thus, the current study investigated if there is also evidence for functional benefits of echolocation in real life.

To address this question, the current study conducted a survey that was available on the internet and that was directed at blind people. Thirty-seven people participated in the survey. The survey solicited demographic information from participants, information about vision loss, general health and mobility. As indicators of participants' functional abilities I analysed data about participants' mobility, salary and relationship status. Mobility was defined by Long (1990) as "the ability to move about in the home and community" and by Foulke (1971) as "the ability to travel safely, comfortably, gracefully and independently through the environment." Mobility was used as indicator for blind people's functional abilities, because vision loss has a negative impact on mobility (Brabyn, 1982; Brown and Brabyn, 1987; Long, 1990; Long et al., 1990; Deiaune, 1992; Salive et al., 1994; Roentgen et al., 2009). Based on this previous research, we also decided to assess mobility separately with regard to familiar and unfamiliar environments. Furthermore, because vision loss can be associated with a negative effect on salary (Tielsch et al., 1990, 1991; Houtenville, 2003) and the formation of romantic relationships (Van Hasselt, 1983; Huurre and Aro, 1998), these were also chosen as functional indicators. In short, if echolocation benefits blind people in real life it should be associated with a positive difference in any of these measures.

I used regression analyses to assess the role played by participants' use of echolocation while statistically controlling for their gender, age, level of visual function, general health, employment status, level of education, Braille skill, and use of other mobility means. I found that echolocation made a unique positive contribution to salary, and mobility in unfamiliar places, such that people who use echolocation had higher salary and higher mobility in unfamiliar places than people who did not use echolocation. The investigation was correlational in nature, and thus cannot be used to determine causality. In addition, caution must be exercised when generalizing results obtained with a small sample (sample size in current study was 37) to the population (Anderson and Vingrys, 2001). The data, however, are consistent with the idea that the use of echolocation has real life advantages for blind people.

MATERIALS AND METHODS

All procedures were approved by the Applied Psychology Ethics Board at Durham University.

DATA COLLECTION

The survey was posted together with a letter of information on a publicly available internet page. Information about the survey was spread via word of mouth and by contacting organizations in contact with blind people. Specifically, an e-mail inquiry was sent that asked organizations if they were interested in spreading information about the survey for example by forwarding information about the survey to their members or by including information in a newsletter or online newsfeed. Together with the inquiry, information about the research, including ethical approval and data protection policies, and the address of the website that hosted the survey had been provided. Participants could access the survey and the letter of information by going to this website. The survey itself was a text file that participants downloaded. Blind participants can read web pages and electronic documents using screen-reader software, which converts written into spoken text. The majority of participants (36 out of 37) completed the survey by typing their answers into the text file and e-mailing it to the experimenter (LT). One participant contacted the experimenter (LT) by phone, and submitted answers over the phone. The first survey question solicited participants' informed consent. To assure confidentiality, participant's e-mail and e-mail address were deleted after their answers had been recorded. The survey was available from November 2011 until November 2012.

SURVEY DESIGN

The first question of the survey solicited participant's informed consent. Questions 2–14 solicited information about the participant's gender, age, country of residence, cause of vision loss, age at which vision loss started, level of visual function, general health, employment status, salary, level of education, Braille skill, relationship status, use of mobility means, in that order. Question 8 used a single item response to solicit participant's opinion of their general health. Though single item responses are less detailed than longer measures of a person's health, they can be valid and reliable indicators (Bowling, 2005). Question 10 solicits information about participant's salary using salary categories rather than monetary value to bear on salary differences across countries. Questions 15 and 16 ask about the participant's mobility in familiar and unfamiliar environments, respectively. Previous studies have shown the usefulness of self-reported mobility measures in this form (Turano et al., 1999, 2002). The survey was available in English and German. Survey questions and the answer coding scheme for the English version are provided in the Appendix.

PARTICIPANTS

37 people (18 female) responded to our survey. Respondents came from six different countries (UK: 13, USA: 4, Canada: 4, Germany: 14, Spain: 1, Australia: 1), mean age was 40.6 years ($min = 18$; $max = 67$; $median = 37$; $SD = 14.4$). For the majority of respondents vision loss was present or began at birth ($n = 21$, 56.8%), or it began before 16 years of age ($n = 11$, 29.7%) (see also **Table 1**). Twenty-two (59.2%) participants were

Table 1 | Summary of participants' responses to survey question 6 "When did your vision loss start."

Age in years when vision loss began	Frequency	Percent
0	21	56.8
0.5	1	2.7
1.0	2	5.4
2.0	1	2.7
3.5	1	2.7
4.0	1	2.7
6.0	1	2.7
12.0	2	5.4
14.0	1	2.7
15.0	1	2.7
17.0	1	2.7
18.0	1	2.7
45.0	1	2.7
48.0	1	2.7
53.0	1	2.7
Total	37	100.0

For the majority of participant vision loss was present or started at birth (56.8%).

Table 2 | Summary of participants' responses to survey question 5 "What is the main cause of your vision loss."

Cause of vision loss	Frequency	Percent
Accident	2	5.4
Amaurosis	1	2.7
Blind born, glaucoma	1	2.7
Cone dystrophy	1	2.7
Genetic disorder, macular degeneration	1	2.7
Glaucoma, cataract	2	5.4
Glaucoma, macular degeneration	1	2.7
Leber's congenital amaurosis	4	10.8
Microphthalmia	1	2.7
Optic atrophy	1	2.7
Optic nerve atrophy	2	5.4
Optic nerve damage	1	2.7
Prematurity	4	10.8
Prematurity, glaucoma	1	2.7
Prematurity, retrolental fibroplasya	1	2.7
Retinal degeneration	1	2.7
Retinal detachment	2	5.4
Retinitis pigmentosa	3	8.1
Retinitis pigmentosa, alstrom syndrome	1	2.7
Retinitis pigmentosa, glaucoma	1	2.7
Retinitis pigmentosa, macular degeneration	1	2.7
Retinoblastoma	3	8.1
Virus during pregnancy	1	2.7
Total	37	100.0

Reported cause of vision loss was heterogeneous, but the most commonly reported were Retinitis Pigmentosa ($n = 6$, 16.2%), Prematurity ($n = 6$, 16.2%), and Leber's Congenital Amaurosis ($n = 4$, 10.8%).

totally blind. Reported cause of vision loss was heterogeneous, but the most commonly reported were Retinitis Pigmentosa ($n = 6$, 16.2%), Prematurity ($n = 6$), and Leber's Congenital Amaurosis ($n = 4$, 10.8%) (see also **Table 2**). The majority of participants ($n = 30$, 81.1%) considered themselves to be in generally good health. In terms of mobility means, by far the most commonly used mobility method used was the long cane ($n = 34$, 91.9%). This was followed by use of human guide ($n = 16$, 43.2%), echolocation ($n = 10$, 27%), GPS ($n = 6$, 16.2%), and guide-dog ($n = 5$, 13.5%) (for more details see Appendix, Question 14).

DATA ANALYSIS

I investigated the following variables as markers of participants' functional abilities: salary, mobility in familiar places, mobility in unfamiliar places, and relationship status. To investigate the potential role that echolocation may play for each of these variables I conducted regression analyses for each of these measures separately.

For variables salary, mobility in familiar places and mobility in unfamiliar places I used stepwise linear regression with echolocation as predictor. In addition, I included participant's use of other mobility means, their sex, age, education level, employment status, Braille skill, general health, and their level of visual function as predictor variables. This way, the contribution through echolocation was evaluated while controlling for the contribution of the other variables. To follow up on linear regression results I used non-parametric tests. This was also done considering that the dependent variables were obtained using rating scales, and that therefore results obtained using parametric methods, such as linear regression, should be considered alongside non-parametric methods. As non-parametric measure of effect size I computed probability of superiority (PS) as suggested by Grissom and Kim (2012, pages 292–294), which estimates the probability that a score randomly drawn from one population will be greater than a score randomly drawn from another population.

For the variable relationship status I used binary logistic regression instead of linear regression to bear on the binary form of the response categories for those variables (relationship status: no relationship in past or present vs. relationship either in past or present). I used the forward likelihood ratio method for variable selection.

All statistical analyses were conducted using SPSS v20.0.

RESULTS

Figure 1 provides an overview of the results. **Figure 1** shows effect sizes for those predictors for which both linear regression coefficients and non-parametric tests were significant.

SALARY

The regression showed that predictors echolocation [unstandardized coefficient $B = 0.88$; $t_{(31)} = 2.42$, $p = 0.022$] and employment status [unstandardized coefficient $B = 1.65$, $t_{(31)} = 5.13$, $p < 0.001$] contributed significantly to the overall model [$F_{(2,\ 31)} = 20.121$, $p < 0.001$, $R^2 = 0.562$], whereas none of the other predictors were significant. Consistent with the regression results non-parametric tests for independent samples comparing salary between people who use echolocation and people

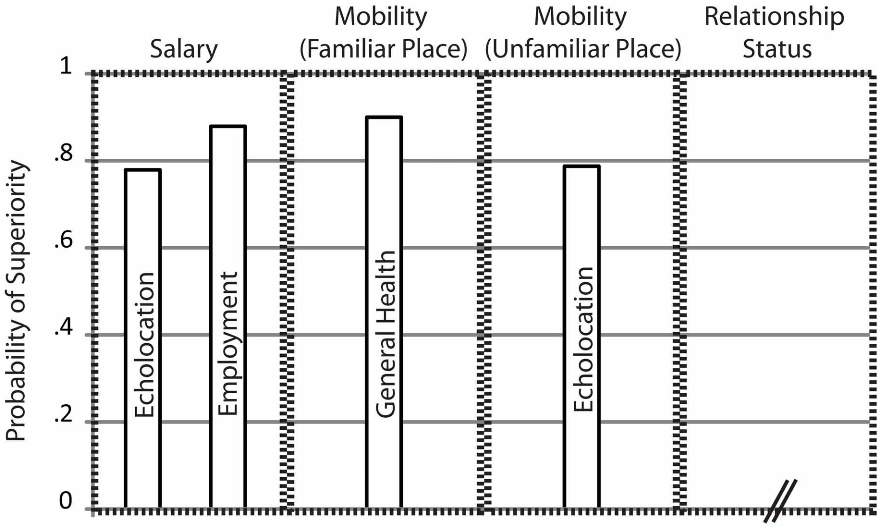

FIGURE 1 | Summary of Results. Bars indicate non-parametric measures of effect-size, i.e., Probability of Superiority as suggested by Grissom and Kim (2012), for those predictors for which both linear regression coefficients and non-parametric tests were significant. Predictors are listed separately for variables "Salary," "Mobility in Familiar Places," "Mobility in Unfamiliar Places," and "Relationship Status." For the variable "Relationship Status" no predictor contributed significantly. For the other variables names of significant predictors are inscribed within each bar. Probability of Superiority estimates the probability that a score randomly drawn from one population will be greater than a score randomly drawn from another population. For example, Probability of Superiority of 0.78 for predictor "Echolocation" for variable "Salary" means that the probability that a randomly drawn salary score from an echolocating population will be greater than a randomly drawn salary score from a non-echolocating population is 0.78.

who do not use echolocation was significant (Mann–Whitney $U = 49$, $p = 0.01$), as was the comparison between people who are employed and people who are not employed (Mann–Whitney $U = 34.5$, $p < 0.001$). Probability of superiority for echolocation ($PS_{\text{Echolocation}}$) was 0.78. This means that the probability that a randomly drawn salary score from an echolocating population will be greater than a randomly drawn salary score from a non-echolocating population is 0.78. $PS_{\text{Employment}}$ was 0.88. Weights for both predictors were positive, and *PS* for both predictors were larger than 0.5. Thus, the data suggest that people who are employed and/or people who use echolocation have higher salary. The finding that blind people who are employed have higher salary than blind people who are not employed makes intuitive sense and is consistent with previous data (e.g., Houtenville, 2003). The finding that the use of echolocation is associated with higher salary is novel.

MOBILITY IN FAMILIAR PLACES

The regression showed that predictors general health [unstandardized coefficient $B = 1.034$, $t_{(33)} = 3.392$; $p = 0.002$], cane use [unstandardized coefficient $B = 1.292$; $t_{(33)} = 3.25$; $p = 0.003$] and education [unstandardized coefficient $B = 0.241$; $t_{(33)} = 2.281$; $p = 0.029$] contributed significantly to the overall model [$F_{(3,\,33)} = 13.816$; $p < 0.001$; $R^2 = 0.557$], whereas none of the other predictors was significant. Subsequent non-parametric tests comparing average mobility in familiar places between participants reporting good health and participants reporting not good health was significant (Mann Whitney $U = 22$; $p < 0.001$). PS_{Health} was 0.9. However, the comparison between cane users and cane non-users was not significant (Mann–Whitney $U = 25.5$, $p = 0.116$). Neither was the comparison across education levels (Kruskal–Wallis $X^2_{(4)} = 7.267$; $p = 0.122$). The unstandardized coefficient for general health was positive, and PS_{Health} was larger than 0.5. Thus, in their entirety, the data suggest that good general health is associated with higher mobility in familiar places. Thus, blind people that report to be in good health also report to find it easier to move around in familiar environments than people that report to not be in good health. This finding is in agreement with previous research showing that better self-reported general health is related to better mobility (e.g., Harada et al., 1999).

MOBILITY IN UNFAMILIAR PLACES

The regression showed that predictors echolocation [unstandardized coefficient $B = 0.755$, $t_{(34)} = 3.026$; $p = 0.005$] and education [unstandardized coefficient $B = 0.239$; $t_{(34)} = 2.43$; $p = 0.021$] contributed significantly to the overall model [$F_{(2,34)} = 9.326$; $p = 0.001$; $R^2 = 0.354$], whereas none of the other predictors were significant. Subsequent non-parametric tests comparing average mobility in unfamiliar places between participants reporting using echolocation and those not using echolocation were significant (Mann–Whitney $U = 57$; $p = 0.003$), and $PS_{\text{Echolocation}}$ was 0.79. The comparison across various levels of reported education level was not significant [Kruskal–Wallis $X^2_{(4)} = 8.626$; $p = 0.071$]. The unstandardized coefficient for echolocation was positive, and $PS_{\text{Echolocation}}$ was larger than 0.5. Thus, in their entirety, the data suggest that the use of echolocation is associated with higher mobility in unfamiliar places. The finding that blind people who use echolocation find it easier to move around in novel places than blind people who do not use echolocation is novel.

RELATIONSHIP STATUS

None of the predictors contributed significantly to the overall model.

DISCUSSION

Based on the hypothesis that blind people use echolocation because it offers functional benefits I would expect that there should be measurable functional benefits for blind people who echolocate, as compared to blind people who do not echolocate. As laid out in the introduction, consistent with this hypothesis previous research under controlled experimental conditions has shown that echolocation improves blind people's spatial sensing abilities [for reviews see Stoffregen and Pittenger (1995) and Schenkman and Nilsson (2010)]. The current study investigated if there is also evidence for functional benefits of echolocation in real life. In an opportunity sample of 37 participants I found that echolocation was associated with higher salary and mobility in unfamiliar places. This finding is consistent with the idea that echolocation may indeed have functional advantages for blind people in real-life.

The sample size was relatively small (37 participants total, 10 echolocators). This has implications both for statistical analyses and sampling veracity.

With regard to statistical analyses low sample sizes may lead to low statistical power, as well as to problems using parametric statistical procedures, i.e., the regression approach. For the current study the use of parametric procedures can also be considered problematic because some dependent measures were obtained via rating scales. One point to consider in this context is that regression results were always followed up using non-parametric tests, confirming that the results hold also when using distribution free testing methods. In addition, despite low sample size reliable effects for echolocation were found for mobility in new places and salary, so lack of statistical power *per se* is not an issue.

With regard to sampling veracity the small sample size implies that one must be cautious when generalizing the current results to the population (Anderson and Vingrys, 2001). The survey was available for 12 months, and participation was solicited through word-of-mouth and advertising through various organizations in contact with blind people. Despite these efforts we received only 37 responses, exemplifying previously reported problems with the soliciting of participation of blind participants for survey research. For example, Turano et al. (1999) sent out 299 questionnaires and after three attempts of mailing the surveys and telephoning each participant to take part via audio response, stressing the difficulty of gathering a large enough population sample, only 145 (under 50%) were returned. Nzegwu and Dooley (2008) managed to collect only 94 responses despite sending letters to 5000 parents and 1500 children and data collection spanning over a year. In sum, the size of our sample necessitates that caution is needed when generalizing the current findings to the population. Similarly, our sample was comparably young and comprised a large number of people who had lost sight early in life. In the overall demographic of blind people, there is a large number of people who lose vision in old age. In fact, in the year 2002 82% of the 314 million visually impaired adults recorded by the World Health Organization were 50 years and older at time of onset of blindness (Resnikoff et al., 2004). It follows that people who lose sight in old age are under-represented in our sample. Older people tend to make less use of the Internet (Kaye, 2000). Thus, the most likely reason for the low number of older people in our sample is that the survey was posted online. Future research is needed to determine if similar results will be obtained in a sample that includes larger numbers of people who lost sight in old age.

Another point to consider is that our study is correlational in nature. As such, we cannot determine if the use of echolocation causes better salary and mobility in unfamiliar environments, or if people who have better mobility in unfamiliar environments and higher salary also echolocate. With regard to mobility in unfamiliar environments, however, previous laboratory research supports the idea that echolocation may actually cause improvements. Specifically, previous research shows that echolocation improves spatial sensing. Blind people can use echolocation for example to detect a 3-degree change in the horizontal position of an object placed 1.5 m away (Thaler et al., 2011) or a gap as small as 0.02 m between two objects placed 1 m away (Teng et al., 2012), or a 4″ displacement in depth at a distance of 90 cm (Kellogg, 1962). This would suggest that the use of echolocation would also lead to improved mobility in unfamiliar environments, where mobility cannot rely on memory, but requires the exploration of a novel spatial layout. With regard to salary, there is no previous laboratory research, but it would seem improbable that the use of echolocation *per se* would lead to an increase in salary. However, it is possible that for example the increased mobility in unfamiliar places as mediated though echolocation may have a positive impact on blind people's professional autonomy and in this way also on their salary.

The majority of our participants use the long cane, and all of our participants who echolocate, also use the long cane. This suggests that the benefit of echolocation we found might be conditional upon the long cane being used as well. It also suggests that echolocation and long cane may have complementary functions. For example, it is possible that the cane is more beneficial to sense the layout of the ground surface, which might be challenging to sense through echolocation because the overall sound reflection of the ground surface may mask more subtle changes in layout, such as a rising curb or a pothole. In contrast, echolocation may be more useful to sense surfaces elevated off the ground around head level, where the cane is inconvenient to apply, and/or may pose risks to other people in the environment.

The design of our survey was deliberate. As such, no investigation was made into matters such as questionnaire validity or reliability, or to what degree the phrasing or ordering of questions may have influenced participants' answers. With regard to the solicitation of the use of mobility means, the different techniques were simply listed and for example no particular definition of echolocation was used (compare Question 14 in the Appendix). One might argue, therefore that only respondents familiar with this technique would respond positively, and that knowledge of echolocation might perhaps be related to educational level.

Our statistical analyses, however, that controlled for educational level, suggest that differences in educational level cannot explain our findings. Finally, most of the questions solicited demographic information, or they were chosen based on the previous literature. In sum, we think that it is unlikely that the results are due to methodological artifacts related to the design of the questionnaire.

As mentioned in the introduction, previous research suggests that up to 30% of blind people may echolocate. Consistent with this, 10 out of the 37 people in the sample reported to use echolocation. Since previous laboratory studies as well as our current data suggest that the use of echolocation may lead to functional benefits for blind people, the question arises, why not more blind people echolocate. One possible explanation is lack of knowledge.

An early description of a blind person avoiding obstacles was given by Diderot in 1749. Initially the mechanisms underlying this "obstacle sense" were unclear, and it was thought that it might be an ability of only a few gifted people. In the 1940's/1950's, however, it became clear that echolocation was auditory in nature, and that anybody with normal hearing can learn it (Supa et al., 1944; Cotzin and Dallenbach, 1950; Worchel and Mauney, 1951; Ammons et al., 1953; Teng and Whitney, 2011). Thus, lack of knowledge might not be the (sole) reason for echolocation not being used more by blind people. Another possibility for the limited popularity of echolocation might be concerns about social stigma. Specifically, echolocation requires people to generate sonar emissions, such as finger snaps, shuffling with their feet, clicking with their tongue, humming, repetitive speaking, etc., and blind people may be reluctant to produce sonar emissions out of concern to appear "odd." In addition, behaviors in blind people that "appear to have no goal directed purpose" and that appear to be out of the norm are considered maladaptive mannerisms, sometimes also referred to as "Blindisms" and they are discouraged from an early age (Eichel, 1978; Molloy and Rowe, 2011). There is the possibility that spontaneously generated sonar emissions might be considered maladaptive mannerisms and therefore be discouraged, and this will affect the degree to which blind people will make use of echolocation.

In summary, our data are consistent with the idea that echolocation offers functional benefits for blind people in real life. This finding, together with previous laboratory research, provides converging evidence for the idea that echolocation may play a role in peoples' successful adaptation to vision loss.

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APPENDIX

This Appendix contains survey questions and response options. The response coding scheme as well as the frequency of responses in the different response categories are shown below each question.

1. Please tell us if you have read the letter of information and consent to participate in this survey
   [Yes:1(37), No: 0(0)]
2. What is your gender
   [male: 0(19), female: 1(18)]
3. What is your date of birth
   (mm/yyyy) (mean = 40.6 years, min = 18; max = 67; median = 37; *SD* = 14.4)
4. Where do you live currently
   (country) (UK: 13, USA: 4, Canada: 4, Germany: 14, Spain: 1, Australia: 1)
5. What is the main cause of your vision loss
   (free text answer) see **Table 2**
6. When did your vision loss start
   (mm/yyyy) see **Table 1**
7. Please describe the level of your visual function
   [total blindness: 0(22), partial vision: 1(15)]
8. Do you consider yourself to be in generally good health?
   [No: 0(7), Yes: 1(30)]
9. What is your current employment status
   [going to school: 0(2), going to university: 0(11), unemployed: 0(4), employed full time: 1(10), employed part time: 1(5), self employed: 1(1), retired: 0(4)]
10. How would you describe your salary
    [none: 0(4), benefits: 1(12), minimum wage: 2(2), above minimum wage: 3(11), high income: 4(5), missing: (3)]
11. How would you describe your level of education
    [none: 0(0), primary/middle school: 1(4), secondary/high school: 2(13), Bachelors degree: 3(7), Masters degree: 4(11), Doctoral degree: 5(2)]
12. What is your skill in reading Braille
    [none: 0(11), little: 1(2), fluent: 2(24)]
13. Please indicate your current relationship status
    [single without previous relationship: 0(7), single with previous relationship: 1(11), currently in a relationship: 1(18), missing: (1)]
14. Which mobility means do you use
    [partial vision, Yes: 1(4), No: 0(33)]
    [long cane, Yes: 1(34), No: 0(3)]
    [guide dog, Yes: 1(5), No: 0(32)]
    [human guide, Yes: 1(16), No: 0(21)]
    [echolocation, Yes: 1(10), No: 0(27)]
    [auditory sensory substitution device, Yes: 1(2), No: 0(35)]
    [tactile sensory substitution device, Yes: 1(0), No: 0(37)]
    [GPS, Yes: 1(6), No: 0(31)]
    [Other, Yes: 1(2), No: 0(35), monocular, compass]
15. When you are on your own in a familiar place, how difficult do you find it to find your way around
    [very difficult: 0(3), somewhat difficult: 1(3), somewhat easy: 2(11), very easy: 3(20)]
16. When you are on your own in a new place, how difficult do you find it to find your way around
    [very difficult: 0(6), somewhat difficult: 1(22), somewhat easy: 2(6), very easy: 3(3)]