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RECEIVED 13 November 2025
REVISED 02 February 2026
ACCEPTED 16 February 2026
PUBLISHED 20 March 2026

CITATION

Zheng X, Wang D, Zhai L, Declercq AM,
Loor A, Dong H and Zhang J (2026)
Litsea citrata essential oil provides
insights into the modulation of heat
stress-associated transcriptomic
responses in *Artemia franciscana*.
Front. Mar. Sci. 13:1745901.
doi: 10.3389/fmars.2026.1745901

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Litsea citrata essential oil provides insights into the modulation of heat stress– associated transcriptomic responses in *Artemia franciscana*

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Litsea citrata essential oil (LC-EO) has been proposed as a potential natural additive for mitigating heat stress in aquaculture species. This study evaluated the association between LC-EO exposure, survival performance, and transcriptomic responses of *Artemia franciscana* under normal (28°C) and heat-stress (34°C) conditions. Supplementation with 0.005% LC-EO was associated with significantly improved survival under heat stress. Transcriptomic analysis identified 318 differentially expressed genes (DEGs) under normal conditions and 764 DEGs under heat stress. Functional enrichment analyses indicated that LC-EO exposure was associated with altered expression of genes involved in detoxification, antioxidant-related processes, immune regulation, nucleotide metabolism, and cellular maintenance, with enriched pathways including lysosome, glutathione metabolism, cytochrome P450, purine and pyrimidine metabolism, and RNA polymerase. Quantitative RT-PCR validation of eight selected DEGs showed strong concordance with RNA-Seq data ($R = 0.83–0.94$, $P < 0.001$), supporting the reliability of the transcriptomic dataset. Collectively, these results suggest that LC-EO exposure is associated with transcriptional modulation of stress-responsive pathways under heat stress, generating testable hypotheses regarding its potential role in enhancing thermal tolerance in *A. franciscana*. Functional validation will be required to determine whether these transcriptomic changes translate into causal physiological protection.

KEYWORDS

Artemia franciscana, heat stress, immune response, *Litsea citrata*, RNA-seq

1 Introduction

Aquaculture is one of the fastest-growing food production sectors worldwide, providing essential animal protein and supporting the livelihoods of billions of people (Nabi et al., 2025). However, its sustainability is increasingly challenged by climate change, particularly the intensification of extreme weather events (Abbass et al., 2022). In China, for instance, the frequency and severity of heat waves and heavy rainfall have markedly increased in recent years, substantially elevating the national climate risk index (China Climate Change Blue Book, 2023). Such climatic extremes disrupt aquatic environments, often exceeding the tolerance limits of cultured species and triggering cascading ecological and economic impacts (Yadav et al., 2024). Elevated water temperatures alter key water quality parameters (e.g., dissolved oxygen, pH, and ammonia levels) and compromise the physiological resilience of farmed animals, leading to reduced feed efficiency, weakened immune defenses, and increased susceptibility to disease outbreaks (Yadav et al., 2024). Consequently, mass mortality events and pond collapses have become more frequent, posing serious threats to global aquaculture productivity and profitability.

Shrimp aquaculture, a cornerstone of tropical and subtropical aquaculture in China, exemplifies the vulnerability of crustaceans to thermal stress (F.A.O., 2024). Temperature strongly influences shrimp physiology, regulating metabolism, molting, growth, and feeding behavior (Wang et al., 2020). Sustained heat stress impairs antioxidant defenses, immune responses, and reproductive function (Liu et al., 2022b; Liu et al., 2022a; Barajas-Sandoval et al., 2023). Prolonged exposure to high temperatures or abrupt fluctuations can exceed tolerance thresholds, causing mortality. Moreover, persistently high pond temperatures favor pathogen proliferation, increasing the incidence of infectious diseases and jeopardizing the stability of intensive shrimp culture systems (Han et al., 2020). To address these challenges, researchers increasingly employ model organisms to investigate stress physiology and screen functional additives that may enhance thermal resilience in cultured crustaceans.

Artemia franciscana, a branchiopod crustacean widely used both as a live feed in shrimp hatcheries and as an experimental model, provides a practical bridge between fundamental stress biology and aquaculture applications. *Artemia* is easy to culture, has a short life cycle, and exhibits well-characterized responses to environmental stressors (Morgan, 2018; Tiong et al., 2025). Many aspects of its stress physiology, immune signaling, and energy metabolism are conserved with penaeid shrimp, making it a valuable model for mechanistic studies (Tiong et al., 2025). However, differences in osmoregulation and lipid metabolism limit the direct extrapolation of findings to marine shrimp. Despite these differences, insights from *Artemia* remain valuable for hypothesis-driven studies and preliminary screening of functional additives. Furthermore, elevated temperature markedly reduces *Artemia* viability and nutritional quality, affecting hatchery performance and live-feed reliability (Junprung et al., 2024; Xue et al., 2024). Therefore, improving its thermal tolerance would benefit both basic crustacean research and aquaculture production.

Plant-derived essential oils have gained increasing attention in aquaculture due to their antioxidant, antimicrobial, and immunomodulatory properties (Hossain et al., 2022; Jeyavani

et al., 2022; Muñoz-Acevedo et al., 2022). The essential oil of *Litsea citrata* (LC-EO), rich in citral—a mixture of the isomers geranial and neral, typically comprising 70–85% of the total oil—has shown strong bioactivity in aquatic models. Previous studies demonstrated that LC-EO enhances survival and modulates immune-related gene expression in gnotobiotic *A. franciscana* (Zheng et al., 2021; Zheng et al., 2024), suggesting its potential to improve resilience under environmental stress. However, the molecular mechanisms underlying its protective effects against thermal stress remain unclear. Specifically, it is not yet known whether LC-EO acts through Nrf2-mediated antioxidant signaling, induction of heat shock proteins (HSPs), or modulation of apoptotic pathways in crustaceans.

Although essential oils, including LC-EO and its major components, have been reported to enhance survival or immune competence in *Artemia* under pathogenic challenges (Zheng et al., 2021; Zheng et al., 2024), their role in modulating molecular responses to abiotic stress—particularly thermal stress—remains largely unexplored. To our knowledge, no previous study has combined phenotypic survival assays with transcriptome-wide analysis to elucidate the molecular pathways potentially underlying LC-EO-mediated thermal resilience in *Artemia*. This integrative approach represents the primary novelty of the present work.

In this study, we address this gap by employing comparative transcriptomic analysis to systematically characterize the molecular responses of *A. franciscana* to acute heat stress and to elucidate how supplementation with LC-EO reshapes these responses. By integrating differential gene expression and pathway enrichment analyses, we specifically examine whether LC-EO-mediated thermal protection involves coordinated regulation of antioxidant signaling, protein homeostasis, and apoptosis-related pathways. This work provides mechanistic insight into essential-oil-assisted thermal resilience at the systems level and establishes *Artemia* as an effective model for screening functional additives aimed at improving aquaculture robustness under climate-driven temperature extremes.

2 Materials and methods

2.1 *Artemia* hatching and rearing

The *San Francisco Bay* strain of *A. franciscana* (AR-ARC code: USA-SL-40) was obtained from the Asia Regional *Artemia* Reference Center, College of Marine and Environmental Sciences, Tianjin University of Science and Technology, Tianjin, China. Cysts were hatched under controlled conditions at 28°C, salinity of 30 g/L, light intensity of 2000 lx, and continuous aeration. After 24 h of incubation, newly hatched nauplii were collected and transferred to glass aquaria (50 × 30 × 40 cm; water depth 20 cm) for rearing. Conditions were maintained at 28°C and 30 g/L salinity, with a stocking density of 1 individual/mL and a 14 h:10 h light–dark cycle. Water was exchanged every two days (50% volume replacement), and nauplii were fed once daily with *Spirulina* powder (Kejing brand; Tianjin Fengnian Aquaculture Co., Ltd., Tianjin, China) at 8 mg/L.

2.2 Experimental design

Two-day-old nauplii were used to assess the protective effects of *Litsea citrata* essential oil (LC-EO; Pranarôm International S.A., Ghislenghien, Belgium) under heat stress. LC-EO was first dissolved in 1% ethanol as a vehicle solution. To evaluate concentration-dependent effects, nauplii ($n = 20$ per replicate, triplicated) were placed in 50 mL centrifuge tubes containing 20 mL seawater supplemented with *Spirulina* powder and exposed to five LC-EO concentrations: 0% (control, C), 0.005% (T1), 0.01% (T2), 0.02% (T3), and 0.04% (T4). The concentration range of LC-EO and the solvent level (1% ethanol) were determined based on preliminary range-finding trials conducted in our laboratory (unpublished), which identified doses that were biologically effective yet non-lethal. The ethanol content was maintained at 1% across all treatments, including controls, to ensure a consistent solvent background.

For the acute heat-stress challenge, two temperature regimes were applied. Under non-stress conditions, water temperature was maintained at 28°C; under heat stress, temperature was gradually increased at 2°C h⁻¹ until reaching 34°C and maintained for 24 h. Survival was recorded at the end of exposure by counting live nauplii. The 0.005% LC-EO concentration was selected for transcriptomic analysis as the lowest dose that consistently improved survival under heat stress while maintaining survival comparable to controls under non-stress conditions.

2.3 Sample collection

The survival assay and transcriptomic experiment were conducted as independent experiments following identical exposure and temperature ramping protocols; RNA samples were collected from separate populations and do not represent the same individuals used for survival assessment. For RNA-seq, nauplii were cultured in 1 L glass bottles rather than 50 mL tubes to obtain sufficient biomass for RNA extraction and to avoid potential density-induced transcriptional bias.

Four experimental treatments were established for transcriptomic analysis: C = control (28°C, no LC-EO), H = heat stress (34°C, no LC-EO), EC = LC-EO exposure (28°C + 0.005% LC-EO), and EH = combined treatment (34°C + 0.005% LC-EO). Two-day-old nauplii were transferred into 1 L glass bottles containing 500 mL of filtered seawater enriched with *Spirulina* powder. The heat-stress treatment followed the same gradual temperature-increase protocol.

Samples were collected at 24 h after the onset of heat stress to evaluate temporal physiological and molecular responses. At 24 h, approximately 25 mg of nauplii were harvested, rinsed with distilled water to remove residual seawater and feed, immediately frozen in liquid nitrogen, and stored at -80°C until transcriptomic analyses. All treatments were conducted in triplicate to ensure reproducibility and statistical rigor. All nauplii originated from the same cyst batch to ensure genetic consistency. For each treatment, biological replicates were maintained in independent culture containers, which served as the experimental units for both survival and transcriptomic analyses. The use of larger culture volumes for

RNA-seq is a common and necessary practice in *Artemia* transcriptomic studies to obtain sufficient biomass and minimize density-related transcriptional artifacts (Junprung et al., 2019; Zheng et al., 2021).

2.4 RNA extraction, library construction, and transcriptome sequencing

Total RNA was extracted using TRIzol reagent (Invitrogen, CA, USA) following the manufacturer's protocol. RNA quantity and purity were measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA), and integrity was assessed with a Bioanalyzer 2100 (Agilent, CA, USA) and agarose gel electrophoresis. Samples meeting quality thresholds (concentration > 50 ng/μL, RIN > 7.0, OD260/280 > 1.8, total RNA > 1 μg) were used for sequencing.

Polyadenylated mRNA was specifically captured using oligo (dT) magnetic beads (Dynabeads Oligo(dT), Thermo Fisher, USA) through two rounds of purification. The isolated mRNA was fragmented at 94°C for 5–7 min using the NEBNext[®] Magnesium RNA Fragmentation Module (NEB, USA). First-strand cDNA synthesis was performed using SuperScript[™] II Reverse Transcriptase (Invitrogen, CA, USA), followed by second-strand synthesis with *E. coli* DNA polymerase I (NEB, USA) and RNase H (NEB, USA), incorporating dUTP into the second strand. The resulting double-stranded DNA fragments were end-repaired, adenylated at the 3' ends, and ligated with sequencing adapters containing T-overhangs. Size selection and purification were performed using magnetic beads. The second strand was digested with UDG enzyme (NEB, USA), and the libraries were amplified by PCR (pre-denaturation at 95°C for 3 min, followed by 8 cycles of 98°C for 15 s, 60°C for 15 s, and 72°C for 30 s, with a final extension at 72°C for 5 min) to obtain fragment sizes of 300 ± 50 bp. Paired-end sequencing (PE150) was performed on an Illumina NovaSeq[™] 6000 platform (LC Bio Technology Co., Ltd., Hangzhou, China) according to the standard protocol.

2.5 Bioinformatic analysis

Raw sequencing data in FASTQ format were quality-controlled using fastp (<https://github.com/OpenGene/fastp>) to remove adapters, low-quality reads, and duplicates (default parameters). Clean reads were then aligned to the publicly available *Artemia franciscana* reference genome (NCBI_ASM3288406v1) using HISAT2 (<https://ccb.jhu.edu/software/hisat2>), generating BAM files. Transcript assembly and quantification were performed using StringTie (<https://ccb.jhu.edu/software/stringtie>), and raw read counts were obtained for each gene.

Differentially expressed genes (DEGs) between samples were identified using the R package DESeq2 (<https://bioconductor.org/packages/release/bioc/html/DESeq2.html>) with thresholds of $|\log_2FC| \geq 1$ and FDR-adjusted p -value (q -value < 0.05). Raw counts were used as input for DESeq2 to ensure accurate statistical inference. Functional annotation and enrichment analyses of DEGs were conducted using DAVID (<https://davidbioinformatics.nih.gov/>) for Gene Ontology (GO) terms and KEGG pathways.

2.6 Quantitative real-time-PCR analysis

Eight DEGs were selected further validation using qRT-PCR to validate our Illumina sequencing data. RNA samples were generated from four groups (with three replicate samples each group) in the preceding experiment. Primers were designed based on the assembled transcriptome sequence using the Primer5 software (Premier Biosoft International) and listed in Table 1. The PrimerScriptTM RT Reagent Kit with gDNA Eraser (TaKaRa, Dalian, China) was used to synthesize first-strand cDNA. The expression of each sample was measured in a 20- μ L reaction volume in triplicate using SYBR[®] Premix Ex Taq (TaKaRa, Dalian, China) and a real-time PCR instrument (Shanghai Hongshi MedicalTechnology, China).

Elongation factor (EF-1) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was thus used as an internal reference (Zheng et al., 2021). The cycling parameters were as follows: 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s and 60 °C for 20 s. A dissociation curve analysis was performed to check amplification specificity. Relative fold changes were calculated using the Relative Expression Software Tool version 2009 based on the threshold values generated by qRT-PCR.

2.7 Statistical analyses

Data were expressed as mean \pm SD. The *Artemia* survival data were arcsin transformed to satisfy normal distribution and homoscedasticity requirements as necessary. In the interaction studies, statistical analysis was performed using two-way ANOVA followed by Bonferroni *post hoc* test. Unless stated otherwise, all other data were compared with one-way ANOVA, followed by Tukey's *post hoc* test. Statistical analysis was performed using the IBM statistical software SPSS (version 22.0, IBM Corp., Armonk,

New York, USA), and the results were shown using GraphPad Prism software (version 7, GraphPad Software, Inc., San Diego, CA, USA) and included all biological repeats. The significance level was set at $P < 0.05$.

3 Result

3.1 survival rate

Survival was significantly influenced by essential oil concentration (EC), heat stress (HS), and their interaction (EC \times HS, $p < 0.001$; Figure 1). Under non-stress conditions (28°C), survival remained close to 100% in the control, 0.005%, and 0.01% LC-EO groups, with no significant differences among them. In contrast, survival decreased moderately at 0.02% and markedly at 0.04%, suggesting potential concentration-dependent toxicity at higher doses.

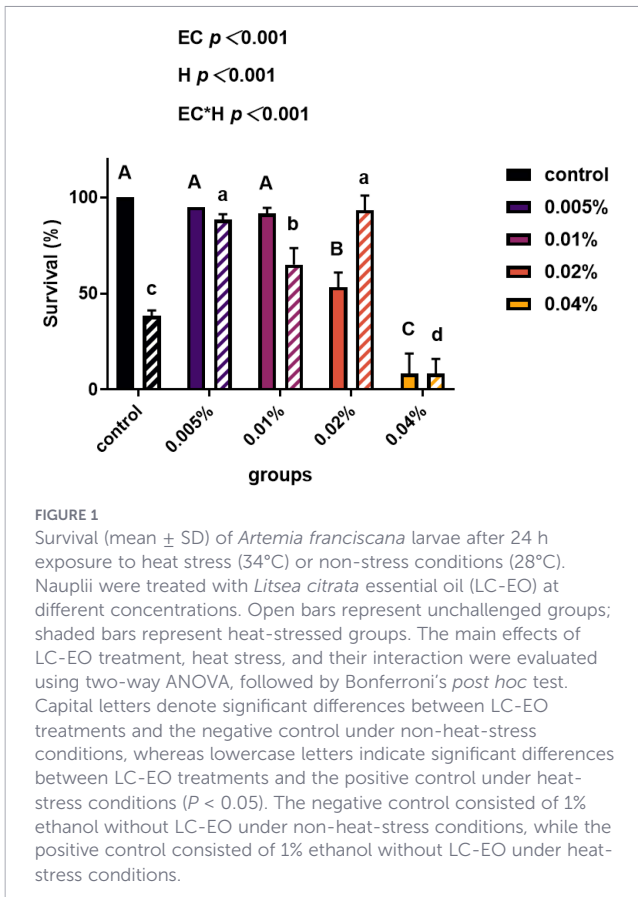
Under heat stress (34°C), all groups exhibited reduced survival. The positive control (without LC-EO) showed a pronounced decline, while the 0.005% and 0.01% LC-EO groups maintained significantly higher survival, indicating partial protective effects of LC-EO at low concentrations. The 0.02% group displayed intermediate survival, and the 0.04% group showed the lowest values, consistent with a narrow beneficial concentration range for thermotolerance enhancement.

3.2 Raw sequencing data and quality statistics

Twelve cDNA libraries were constructed and sequenced using the Illumina NovaSeqTM 6000 platform, yielding a total of 528 million paired-end reads. After quality filtering, 510 million high-

TABLE 1 Sequences and efficiencies of primers used for qPCR in *Artemia franciscana*.

Gene ID or name	Primer sequence (5'-3')	Annealing temperature (°C)	Product length (bp)
EF-1	TCGACAAGAGAACCATTGAAAA ACGCTCAGCTTTAAGTTTGTC	60	101
GAPDH	GTTGATGGCAAACCTCGTCATA CCACCTCCAAGTGAGCATT	61	158
136028742	TGCAGTACAAAGGTCAATCAT CGTTATCACCTCGTTTTATCA	55	139
136041384	GGAATGTCACCTCTCGCCTTGTTT TACCGTTATCAGCCCACTCATCT	65	144
136028323	GATGGCGGCAATGAAGAAG CAGCAGGGTTTGTGGAATAG	55	160
136024719	AGGCGTCTTGATGCTCTTCTTT ATTTGCAGCGTCGACAGTAACAC	65	181
136028324	ATGGCGGCAATGAAGAAGG GCAGGGTTTGTGGAATAGGTG	60	157
136032317	GAGACGTCCCCTGGCAAGTTT ATCAGACATCGGAGCAGGAAGGT	60	332
136037306	GGTGGTACGTCGCCTGTTGTTT CGTTCCGGTTAGAGCGTTGAGTAT	65	249
136028626	CGTTGTTTCGGGTCTTATTC AGCTGCAACTCTGTCATCTGTG	65	199



quality reads (96.26–96.96%) were retained, representing 5.69–7.19 Gb per sample (mean = 6.38 Gb). Q20 and Q30 values exceeded 99%, and GC content was 39.5–40.0%, confirming excellent sequencing quality suitable for transcriptomic analysis (Table 2).

Read mapping rates ranged from 92.9% to 94.4%, with the majority of reads mapping to exonic regions (82–86%), providing a reliable dataset for downstream DEG and pathway analyses.

3.3 Differentially expressed genes and enrichment analysis

A total of 1,082 genes were differentially expressed ($|\log_2FC| \geq 1$, $q < 0.05$) following LC-EO treatment under different temperature conditions (Figure 2). Of these, 318 DEGs were identified under non-stress conditions (EC vs C), and 764 DEGs under heat stress (EH vs H), indicating a more extensive transcriptional response when LC-EO was combined with thermal challenge. This pronounced increase in DEG number under heat stress suggests that LC-EO exerts a stress-contingent modulatory effect, eliciting broader transcriptional reprogramming only when physiological homeostasis is challenged. In contrast, under non-stress conditions, LC-EO exposure is associated with relatively limited gene expression changes, indicating that its regulatory influence is context dependent rather than constitutively activated.

The majority of DEGs under heat stress were upregulated and associated with metabolic regulation, antioxidant defense, and

protein stability. Although several DEGs were annotated as “hypothetical proteins,” many exhibited expression patterns consistent with stress adaptation, such as co-expression with genes involved in oxidation–reduction processes and proteostasis. Nevertheless, their exact molecular roles remain to be characterized and were not inferred here.

Among annotated DEGs, *heme-binding protein 2-like* showed consistent upregulation under both temperature conditions. Given the known roles of heme-binding and cytochrome-associated proteins in oxidative stress mitigation and cellular respiration, its induction suggests potential involvement in redox homeostasis under LC-EO treatment. However, the direct link between this transcript and improved thermotolerance requires further validation.

Gene Ontology (GO) enrichment revealed distinct transcriptional patterns between temperature conditions. Under 28°C, enriched terms were mainly related to metabolism, membrane integrity, and catalytic activity, with several metabolic genes being downregulated—suggesting mild metabolic reprogramming in response to LC-EO exposure. Under 34°C, however, LC-EO treatment significantly enriched GO terms associated with proteolysis, oxidative stress response, and organelle organization, indicating a stronger activation of cellular protection and repair mechanisms (Figure 3).

KEGG pathway enrichment analysis revealed that LC-EO-responsive genes could be broadly categorized into several major functional modules. These included pathways related to antioxidant defense and redox homeostasis, such as glutathione metabolism (map00480); detoxification and xenobiotic metabolism, represented by cytochrome P450-associated pathways (map00982); and cellular degradation and turnover, including lysosome-related pathways (map04142) (Figure 4).

Under non-stress conditions (28°C), enrichment was largely confined to detoxification- and antioxidant-related modules, suggesting a mild priming effect. In contrast, under heat stress (34°C), additional enrichment of pathways associated with transcriptional regulation and nucleotide metabolism (e.g., RNA polymerase, purine and pyrimidine metabolism) was observed, indicating a broader activation of cellular maintenance and recovery-related processes.

Overall, the transcriptomic data suggest that LC-EO supplementation primes *A. franciscana* for enhanced cellular stress management, particularly by regulating redox processes, macromolecule turnover, and metabolic homeostasis under heat stress.

3.4 Validation by qRT-PCR

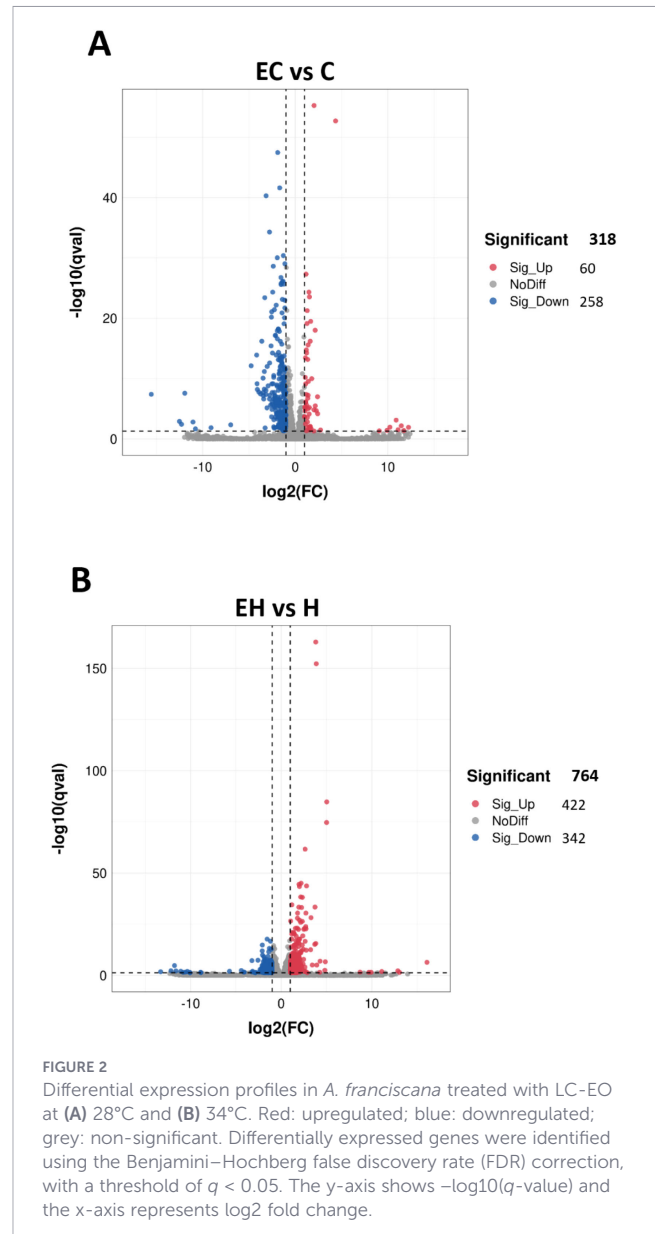
Eight DEGs representing both up- and downregulated genes were selected for qRT-PCR validation. Expression trends from qRT-PCR were highly correlated with RNA-Seq data ($r = 0.83$ – 0.94 , $P < 0.001$), confirming the reliability of transcriptomic quantification (Figure 5).

These consistent expression patterns strengthen confidence in the identified transcriptional changes and provide a foundation for future functional characterization of LC-EO-responsive genes in stress adaptation.

TABLE 2 Summary of sequencing and mapping statistics for 12 libraries.

Sample name	Raw reads	Clean reads	Clean bases	Q20 (%)	Q30 (%)	GC content (%)	Total mapped (%)	Uniquely mapped (%)	Multiple mapped (10%)	Exon	Intron	Intergenic
C1	49517498	47909404	7.19G	100.00	99.36	40	93.92	73.06	20.86	86.23	11.66	2.11
C2	44015516	42594064	6.39G	100.00	99.36	40	94.12	73.92	20.21	86.25	11.74	2.01
C3	47502542	45954066	6.89G	100.00	99.38	39.50	94.34	72.10	22.24	85.22	12.66	2.12
H1	39946582	38528084	5.78G	100.00	99.34	39.50	93.78	73.09	20.69	84.97	12.64	2.39
H2	39329164	37943214	5.69G	100.00	99.36	39.50	93.70	72.62	21.08	84.46	13.07	2.47
H3	41121890	39636662	5.95G	100.00	99.35	39.50	93.81	72.10	21.71	84.35	13.28	2.37
EC1	49242890	47722710	7.16G	100.00	99.40	39.50	94.21	70.89	23.32	83.91	13.83	2.27
EC2	45422466	43894598	6.58G	100.00	99.38	39.50	94.39	69.51	24.88	82.86	14.80	2.34
EC3	43840756	42322598	6.35G	100.00	99.34	39.50	93.08	67.59	25.49	82.44	15.22	2.34
EHI	42344428	40878350	6.13G	100.00	99.33	39.50	93.73	74.43	19.30	86.37	11.41	2.22
EH2	41978662	40452416	6.07G	100.00	99.31	40	92.89	69.83	23.06	84.29	13.39	2.32
EH3	44139868	42659082	6.40G	100.00	99.35	39.50	93.93	70.31	23.63	84.11	13.67	2.22

C: *Artemia* without LC-EO treatment under 28°C; H: *Artemia* without LC-EO treatment under 34°C; EC: *Artemia* with LC-EO treatment under 28°C and EH: *Artemia* with LC-EO treatment under 34°C.



4 Discussion

Artemia franciscana has long served as a tractable model for investigating environmental stress physiology due to its short life cycle, transparent body, and well-characterized development (Criel, 2018; Morgan, 2018). Its sensitivity to temperature fluctuations and chemical exposure further make it suitable for exploratory screening of bioactive compounds and for hypothesis generation regarding stress-response pathways (Ma et al., 2024; Xue et al., 2024; Ravantab et al., 2025). *Litsea citrata* essential oil (LC-EO), characterized by a high content of citral and other bioactive terpenoids, has been widely reported to exhibit antioxidant- and immune-related bioactivities in terrestrial animal systems (Goh et al., 2024; Xia et al., 2024). In terrestrial animals, particularly livestock and poultry, LC-EO has been extensively investigated as a natural feed additive that reduces oxidative stress, enhances immune function, and improves growth performance (Thielmann and Muranyi, 2019). For example, dietary supplementation of 250

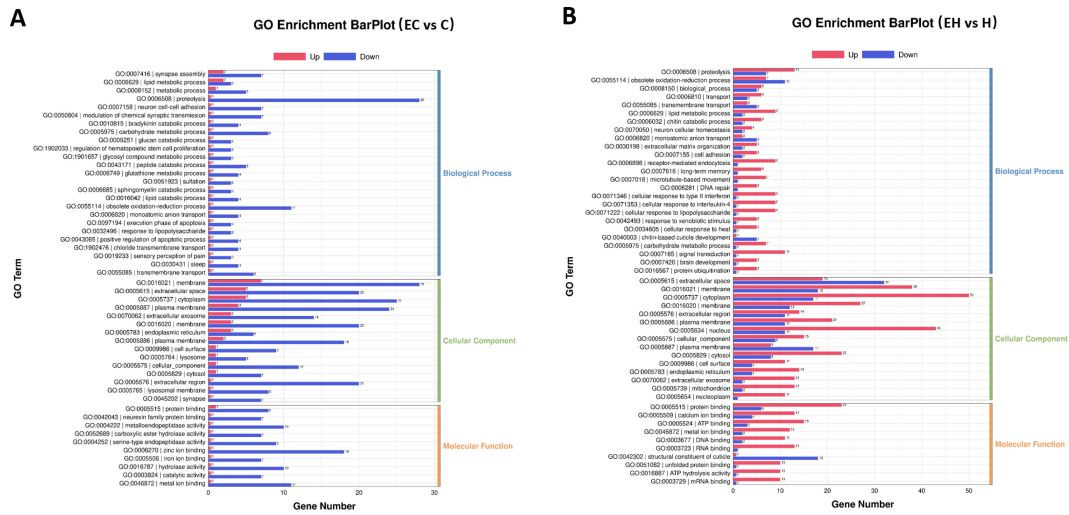


FIGURE 3 The most enriched Gene Ontology (GO) term analysis and function classifications of *Artemia franciscana* with LC-EO treatment under (A) 28°C (EC vs C) and (B) 34°C (EH vs H).

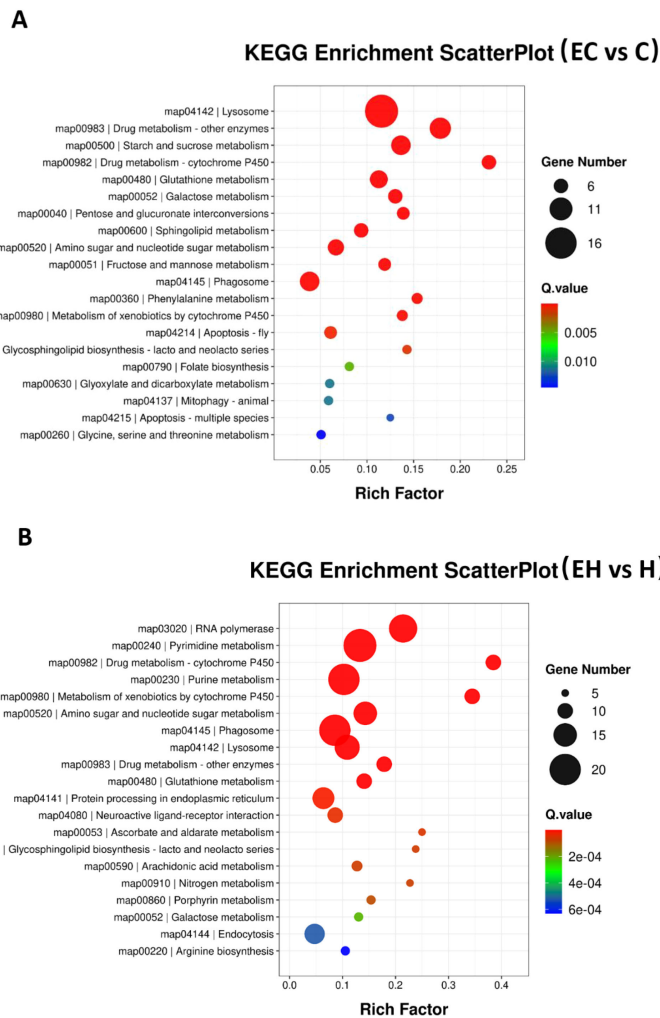
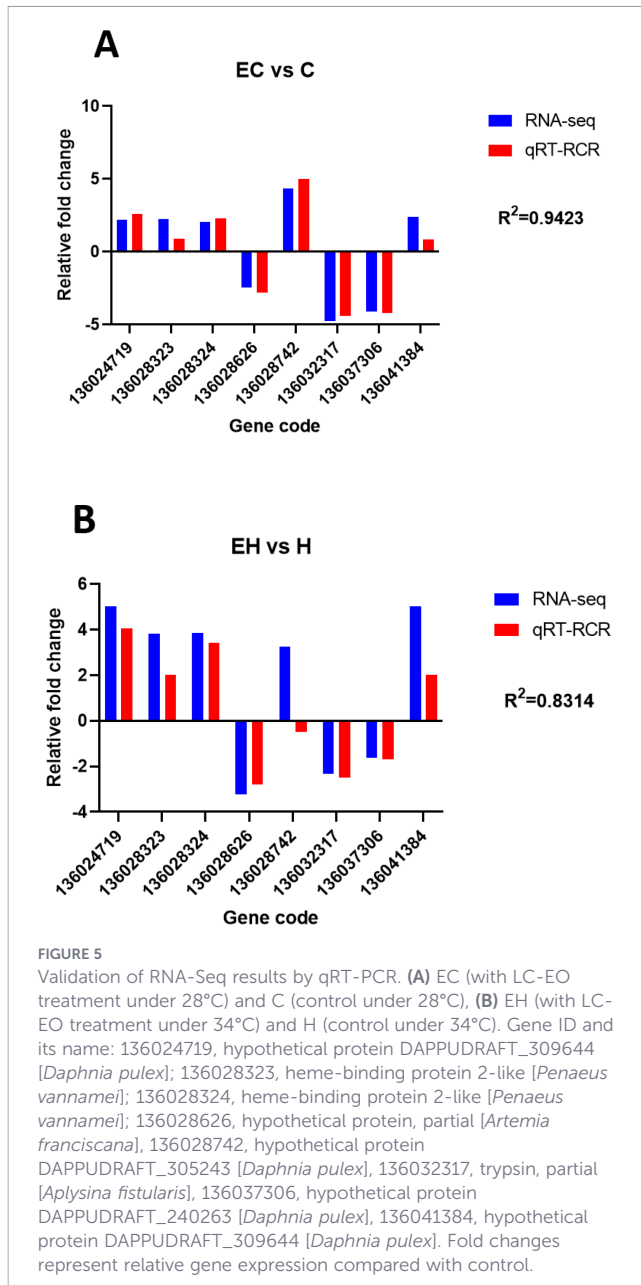


FIGURE 4 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of transcriptomic responses in *Artemia franciscana* with LC-EO treatment under (A) 28°C (EC vs C) and (B) 34°C (EH vs H).



mg/kg LC-EO in pigs significantly improved growth performance, antioxidant capacity, and nutrient utilization while reducing dependence on antibiotics (Chen et al., 2023). Similarly, supplementation with *L. citrata* extract (1.25–2.50 g/kg) improved amino acid and unsaturated fatty acid profiles in broiler meat, enhancing both nutritional value and flavor (Luo et al., 2024). However, evidence for its biological effects in aquatic organisms, particularly at the molecular level, remains limited.

Against this background, the present study aimed to characterize the phenotypic survival response and associated transcriptomic signatures of *A. franciscana* exposed to LC-EO under thermal stress. Importantly, the study was designed as an exploratory investigation to identify transcriptional patterns associated with LC-EO exposure.

4.1 Phenotypic response and dose effects

LC-EO supplementation exhibited a clear concentration-dependent association with survival performance. Under normal temperature (28°C), concentrations up to 0.01% did not significantly affect viability, whereas higher concentrations (0.02% and 0.04%) were associated with reduced survival, indicating potential toxicity at elevated doses. Under acute heat stress (34°C), nauplii exposed to 0.005–0.01% LC-EO showed higher survival than negative controls, suggesting that low-dose LC-EO exposure is associated with improved tolerance to elevated temperature.

The reduced survival observed at higher LC-EO concentrations ($\geq 0.02\%$) under both temperature conditions suggests that LC-EO exhibits a narrow beneficial concentration window. At elevated doses, essential oil components may exert cytotoxic or pro-oxidant effects, as reported for other plant-derived essential oils in aquatic invertebrates (Zheng et al., 2021). These findings highlight the importance of dose optimization and caution against extrapolating beneficial effects observed at low concentrations to higher exposure levels.

Previous gnotobiotic studies have reported a lower safety threshold ($\leq 0.002\%$) for earlier developmental stages (Zheng et al., 2021). The higher tolerance observed in the present study may reflect differences in developmental stage, culture conditions, or microbial context, as the nauplii used here were older and reared in a non-germ-free system. In the present study, the nauplii were approximately two days older than instar II larvae (Gollas-Galvan et al., 2017), and were cultured in a non-germ-free system, which could confer greater physiological resilience. Interactions between *Artemia* and associated microbiota have been proposed to influence stress resilience in aquatic invertebrates, analogous to observations in germ-free versus conventionally reared animals and plants (Lamanna and Ward, 1970; Sahashi et al., 1989). However, as these factors were not experimentally manipulated in this study, their contribution remains speculative and warrants targeted investigation.

4.2 Transcriptomic modulation under LC-EO treatment

Transcriptomic profiling revealed extensive gene expression changes associated with LC-EO exposure, particularly under heat stress, where the number of differentially expressed genes (DEGs) more than doubled compared to the non-stress condition. This finding indicates that LC-EO exposure is associated with broad transcriptional reprogramming during thermal challenge (Li et al., 2020; Shang et al., 2024). However, it is important to emphasize that such changes represent correlational associations rather than direct evidence of functional enhancement. Accordingly, the transcriptomic profiles should be interpreted as population-level molecular responses associated with LC-EO exposure under heat stress, rather than as direct molecular correlates of individual survival outcomes.

Notably, the top-ranked upregulated genes identified in Table 3 are predominantly annotated as hypothetical proteins with

unknown or poorly characterized functions. At present, these transcripts cannot be confidently assigned to specific physiological processes, and their roles in thermal stress tolerance or LC-EO responsiveness remain unresolved. Consequently, gene-level rankings alone do not permit mechanistic inference regarding the primary molecular drivers underlying the observed transcriptional differences. This predominance of uncharacterized genes highlights current limitations in *Artemia* genome annotation and underscores the exploratory nature of the present transcriptomic analysis.

Under normal temperature (28°C), LC-EO exposure was associated with downregulation of genes related to metabolic and catalytic processes, suggesting a modest metabolic adjustment rather than activation of overt stress-response pathways. In contrast, under heat stress (34°C), enrichment of genes associated with proteolysis, oxidative stress-related processes, and organelle organization suggests activation of cellular maintenance and defense pathways. While these transcriptomic patterns are consistent with known stress-response frameworks in *Artemia*, they cannot be interpreted as direct evidence of enhanced antioxidant capacity, immune activation, or cellular repair in the absence of supporting physiological data.

Among the annotated DEGs, a heme-binding protein 2-like gene was consistently upregulated under both temperature conditions. Given the known associations of heme-binding and cytochrome-associated proteins are often implicated in redox regulation and detoxification processes (Souza et al., 2019), suggesting a potential link between LC-EO exposure and redox-related gene networks. Nevertheless, without biochemical measurements—such as reactive oxygen species levels, antioxidant enzyme activities, or cytochrome P450 function—this association remains hypothetical. The predominance of hypothetical proteins among other LC-EO-responsive DEGs further underscores the exploratory nature of the transcriptomic findings.

A limitation of the present study concerns the use of ethanol as a solvent for LC-EO administration. Because all treatments, including controls, contained 1% ethanol, the present study does not disentangle LC-EO-specific effects from potential solvent-related baseline effects. Although this solvent concentration is commonly used for essential oil delivery in crustacean studies, its

biological neutrality in *Artemia* was not independently validated here. Importantly, all phenotypic and transcriptomic comparisons were conducted within a uniform solvent background, ensuring that observed differences are attributable to LC-EO exposure relative to matched controls.

Another notable limitation arises from the incomplete functional annotation of the *A. franciscana* genome. As a non-model organism, a substantial proportion of LC-EO-responsive differentially expressed genes were annotated as hypothetical or uncharacterized proteins, which restricts gene-level functional interpretation. Consequently, mechanistic inferences in this study rely primarily on pathway-level enrichment analyses rather than on the biological roles of individual transcripts. While such enrichment-based approaches provide robust systems-level insights into the molecular responses associated with thermal stress adaptation, targeted functional validation will be required in future studies to clarify the roles of these uncharacterized genes and to refine mechanistic understanding.

4.3 Pathway-level implications

Importantly, the interpretation of antioxidant-, detoxification-, and stress-response processes in this study is derived primarily from pathway-level enrichment analyses across broader DEG sets, rather than from the functional annotation of the top-ranked individual DEGs. This distinction is critical, as enrichment analyses capture coordinated transcriptional shifts across multiple genes, even when individual high-ranking transcripts remain functionally uncharacterized.

KEGG pathway analysis revealed recurrent enrichment associations with lysosome, glutathione metabolism, and cytochrome P450-related pathways under both temperature conditions (Magouz et al., 2022). These pathways are central to cellular detoxification and redox homeostasis, aligning with the known bioactivities of plant-derived compounds (Magouz et al., 2021). Comparable pathway signatures have been repeatedly reported in *Artemia* spp. subjected to thermal stress, oxidative challenge, or chemical exposure, where enhanced antioxidant capacity and detoxification efficiency are regarded as central elements of stress adaptation (Clegg, 2001).

TABLE 3 Differentially expressed genes in nauplii supplemented with LC-EO for 24h under two temperature conditions (28°C and 34°C).

Gene ID	Gene name	log ₂ (fold change)	
		EC vs C	EH vs H
Up-regulated			
136028742	hypothetical protein DAPPUDRAFT_305243 [<i>Daphnia pulex</i>]	4.36	3.25
136041384	hypothetical protein DAPPUDRAFT_309644 [<i>Daphnia pulex</i>]	2.41	5.03
136028323	heme-binding protein 2-like [<i>Panaeus vannamei</i>]	2.27	3.82
136024719	hypothetical protein DAPPUDRAFT_309644 [<i>Daphnia pulex</i>]	2.17	5.01
136028324	heme-binding protein 2-like [<i>Panaeus vannamei</i>]	2.03	3.87
Down-regulated			
136032317	trypsin, partial [<i>Aplysina fistularis</i>]	-4.76	-2.32
136037306	hypothetical protein DAPPUDRAFT_240263 [<i>Daphnia pulex</i>]	-4.10	-1.61
136028626	hypothetical protein, partial [<i>Artemia franciscana</i>]	-2.46	-3.23

In *Artemia*, molecular responses to heat stress are classically characterized by the activation of antioxidant defense systems—particularly glutathione-based redox regulation—together with lysosome-mediated degradation and metabolic reprogramming aimed at maintaining cellular homeostasis. The enrichment of lysosomal pathways observed in the present study is in agreement with earlier transcriptomic and physiological analyses demonstrating that lysosome-associated autophagy plays a critical role in removing damaged proteins and organelles during thermal stress in *A. franciscana* and other crustaceans (Kawsar et al., 2025). Similarly, the involvement of cytochrome P450-related pathways aligns with previous findings that P450-mediated biotransformation constitutes a major detoxification mechanism in *Artemia*, facilitating the clearance of xenobiotics and oxidative by-products generated under stressful conditions (Liu et al., 2015).

Under heat stress, LC-EO treatment was additionally associated with enrichment of RNA polymerase and nucleotide metabolism pathways. Similar transcriptional activation patterns have been documented in *A. franciscana* during post-stress recovery phases and are thought to reflect increased demand for RNA synthesis, DNA repair, and cellular maintenance following thermal insult (Junprung et al., 2024). Such responses are widely interpreted as hallmarks of stress recovery rather than acute stress signaling, suggesting that LC-EO may contribute to a more rapid or efficient re-establishment of transcriptional and metabolic equilibrium after heat exposure.

Overall, the pathway-level responses identified in this study substantially overlap with well-established molecular stress-response frameworks in *Artemia*, including antioxidant defense, lysosome-mediated quality control, detoxification via cytochrome P450, and recovery-associated transcriptional activation. However, the predominance of hypothetical proteins among the most strongly regulated transcripts highlights a key limitation of the current dataset. While pathway enrichment provides biologically meaningful context, functional roles of individual uncharacterized genes cannot be inferred from transcriptomic data alone. Targeted functional validation will be required to determine whether and how these transcripts contribute to LC-EO-associated thermal resilience.

5 Conclusions

This study demonstrates that exposure to *L. citrata* essential oil (LC-EO) at 0.005% is associated with improved survival of *A. franciscana* under acute heat stress, without adverse effects under normal temperature conditions. Transcriptomic analysis revealed that LC-EO exposure is associated with modulation of gene expression patterns linked to antioxidant defense, detoxification, immune regulation, and proteolysis and nucleotide metabolism. The strong concordance between RNA-Seq and qRT-PCR results supports the robustness of the transcriptional dataset.

However, these findings should be interpreted with appropriate caution. The observed transcriptional changes represent associative evidence and do not establish causal mechanisms by which LC-EO

enhances thermal tolerance. Moreover, interpretation is constrained by the short-term exposure design and the absence of an ethanol-free control, meaning that LC-EO-associated effects were assessed under a uniform solvent background. Future studies integrating functional and biochemical validation, longer-term and multi-stage exposure designs, and refined solvent controls will be required to determine whether the observed transcriptomic modulation translates into sustained physiological protection and to strengthen mechanistic inference.

Data availability statement

The RNA-seq datasets generated during this study have been deposited in the Genome Sequence Archive (GSA) Accession number: CRA037791.

Author contributions

XZ: Writing – review & editing, Conceptualization, Funding acquisition, Investigation, Writing – original draft. DW: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. LZ: Methodology, Writing – review & editing. AD: Methodology, Resources, Visualization, Writing – review & editing. AL: Methodology, Writing – review & editing. HD: Funding acquisition, Supervision, Writing – review & editing. JZ: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This research was supported by the International Science and Technology Cooperation Programs of Hainan Province in 2025 (GHYF2025011), the Guangdong Basic and Applied Basic Research Foundation (2023A1515110447), the Natural Science Foundation of Hainan Province (324QN368), the Science and Technology Projects in Guangzhou (2025A04J3979), the Central Public-interest Scientific Institution Basal Research Fund, South China Sea Fisheries Research Institute, CAFS (2023RC02, 2023TD97 and 2024XT01), Key Research and Development Task Special Project of the Xinjiang Uygur Autonomous Region (2024B02014) and Ningbo Key Research and Development Programs and "Listed and Commanded" Project (2023Z113).

Acknowledgments

We sincerely thank prof. Liying Sui from the Asian Regional *Artemia* Reference Center (AR-ARC) of Tianjin University of

Science and Technology, China, for generously providing the *Artemia franciscana* cysts for this study.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

References

- Abbass, K., Qasim, M. Z., Song, H., Murshed, M., Mahmood, H., and Younis, I. (2022). A review of the global climate change impacts, adaptation, and sustainable mitigation measures. *Environ. Sci. Pollut. Res.* 29, 42539–42559. doi: 10.1007/s11356-022-19718-6
- Barajas-Sandoval, D., Escobedo-Fregoso, C., Peña-Rodríguez, A., Barajas-Sandoval, D., Escobedo-Fregoso, C., and Peña-Rodríguez, A. (2023). Temperature and fasting effect on compensatory growth and transcriptomic response in the Pacific white shrimp *Penaeus vannamei*. *Aquaculture* 574, 739711. doi: 10.1016/j.aquaculture.2023.739711
- Chen, F., Wang, Y., Wang, K., Chen, J., Jin, K., Peng, K., et al. (2023). Effects of *Litsea cubeba* essential oil on growth performance, blood antioxidation, immune function, apparent digestibility of nutrients, and fecal microflora of pigs. *Front. Pharmacol.* 14, 1166022. doi: 10.3389/fphar.2023.1166022
- Clegg, J. S. (2001). Cryptobiosis—a peculiar state of biological organization. *Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol.* 128, 613–624. doi: 10.1016/S1096-4959(01)00300-1
- Criel, G. R. J. (2018). *Morphology of artemia, artemia biology* (CRC Press, Ghent, Belgium), 119–154.
- F.A.O (2024). *The state of world fisheries and aquaculture 2024 blue transformation in action*. Rome: Food and Agriculture Organization of the United Nations. 98.
- Goh, M. P. Y., Samsul, R. N. E., Mohaimin, A. W., Goh, H. P., Zaini, N. H., Kifli, N., et al. (2024). The analgesic potential of *Litsea* species: a systematic review. *Molecules* 29, 2079. doi: 10.3390/molecules29092079
- Gollas-Galvan, T., Cabanillas-Gámez, M., Hernández-López, J., Coronado-Molina, D., and Martínez-Porchas, M. (2017). Transcriptional expression of immune system genes in *Litopenaeus vannamei* during ontogenetic development. *Aquaculture Res.* 48, 1110–1118. doi: 10.1111/are.12953
- Han, J., Lee, J.-S., Park, J. C., Hagiwara, A., Lee, K.-W., and Lee, J.-S. (2020). Effects of temperature changes on life parameters, oxidative stress, and antioxidant defense system in the monogonont marine rotifer *Brachionus plicatilis*. *Mar. Pollut. Bull.* 155, 111062. doi: 10.1016/j.marpolbul.2020.111062
- Hossain, A., Habibullah-Al-Mamun, M., Nagano, I., Masunaga, S., Kitazawa, D., and Matsuda, H. (2022). Antibiotics, antibiotic-resistant bacteria, and resistance genes in aquaculture: risks, current concern, and future thinking. *Environ. Sci. Pollut. Res.* 29, 1–22. doi: 10.1007/s11356-021-17825-4
- Jeyavani, J., Sibiy, A., Sivakamavalli, J., Divya, M., Preetham, E., Vaseeharan, B., et al. (2022). Phytotherapy and combined nanoformulations as a promising disease management in aquaculture: a review. *Aquaculture Int.* 30, 1–16. doi: 10.1007/s10499-022-00848-0
- Junprung, W., Nanakorn, Z., Norouzitallab, P., Supungul, P., Vanrompoy, D., Bossier, P., et al. (2024). Thermal adaptation affects expression and regulation of metabolism-, stress-, and immune-related genes in *Artemia franciscana* populations. *Aquaculture Rep.* 39, 102511. doi: 10.1016/j.aqrep.2024.102511
- Junprung, W., Norouzitallab, P., De Vos, S., Tassanakajon, A., Nguyen Viet, D., Van Stappen, G., et al. (2019). Sequence and expression analysis of HSP70 family genes in *Artemia franciscana*. *Sci. Rep.* 9, 8391. doi: 10.1038/s41598-019-44884-y
- Kawsar, M. A., Adikari, D., and Zhang, Y. (2025). Autophagy in aquatic animals: mechanisms, implications, and future directions. *Front. Immunol.* 16, 1612178. doi: 10.3389/fimmu.2025.1612178
- Lamanna, C., and Ward, T. G. (1970). Enhanced toxicity of nervous system drugs for germ-free mice. *Infection Immun.* 1, 139–141. doi: 10.1128/iai.1.2.139-141.1970
- Li, L., Zhang, Z., and Huang, Y. (2020). Integrative transcriptome analysis and discovery of signaling pathways involved in the protective effects of curcumin against oxidative stress in tilapia hepatocytes. *Aquat. Toxicol.* 224, 105516. doi: 10.1016/j.aquatox.2020.105516
- Liu, J., Chen, S., Ren, Z., Ye, Y., Wang, C., Mu, C., et al. (2022a). Effects of diurnal temperature fluctuations on growth performance, energy metabolism, stress response, and gut microbes of juvenile mud crab *Scylla paramamosain*. *Front. Mar. Sci.* 9, 1076929. doi: 10.3389/fmars.2022.1076929
- Liu, J., Shi, C., Ye, Y., Ma, Z., Mu, C., Ren, Z., et al. (2022b). Effects of temperature on growth, molting, feed intake, and energy metabolism of individually cultured juvenile mud crab *Scylla paramamosain* in the recirculating aquaculture system. *Water* 14, 2988. doi: 10.3390/w14192988
- Liu, N., Li, M., Gong, Y., Liu, F., and Li, T. (2015). Cytochrome P450s—Their expression, regulation, and role in insecticide resistance. *Pesticide Biochem. Physiol.* 120, 77–81. doi: 10.1016/j.pestbp.2015.01.006
- Luo, Y., Bi, Y., Xu, Z., Shan, L., He, J., Wang, K., et al. (2024). Exploring possible benefits of *Litsea cubeba* Pers. extract on growth, meat quality, and gut flora in white-feather broilers. *Front. Veterinary Sci.* 10, 1335208.
- Ma, D., Zhou, Q., Sui, L., Guo, Q., Liu, H., Liang, H., et al. (2024). Phlorizin alleviates deltamethrin-induced oxidative stress in brine shrimp *Artemia*. *J. Oceanology Limnology* 42, 163–173. doi: 10.1007/s00343-023-2387-z
- Magouz, F. I., Amer, A. A., Faisal, A., Sewilam, H., Aboelenin, S. M., and Dawood, M. (2022). The effects of dietary oregano essential oil on the growth performance, intestinal health, immune, and antioxidative responses of Nile tilapia under acute heat stress. *Aquaculture* 548, 737632. doi: 10.1016/j.aquaculture.2021.737632
- Magouz, F. I., Mahmoud, S. A., El-Morsy, R., Paray, B. A., Soliman, A. A., Zaineldin, A. I., et al. (2021). Dietary menthol essential oil enhanced the growth performance, digestive enzyme activity, immune-related genes, and resistance against acute ammonia exposure in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 530, 735944. doi: 10.1016/j.aquaculture.2020.735944
- Morgan, M. A. (2018). *Artemia franciscana as a model for stress in saltwater lakes: an environmental metabolomics approach* (Riverside: University of California).
- Muñoz-Acevedo, A., González, M. C., Castillo, O. J., Guzmán, C. P., Rodríguez-Acosta, S., Cervantes-Díaz, M., et al. (2022). *Promising antimicrobial agents from some Latin American medicinal plants against disease-causing pathogens in humans and animals, Promising Antimicrobials from Natural Products* (Springer, Netherlands), 241–279.
- Nabi, N., Ahmed, I., Qadir, M., and Reshi, Q. M. (2025). *Global aquaculture: scenarios and nutritional implications, Aquaculture: Enhancing Food Security and Nutrition* (Springer, London, UK), 121–137.
- Ravansh, N., Ghasemi, Z., Johari, S. A., and Handy, R. D. (2025). Toxicity of mercuric chloride when combined with ionic and nanoparticulate silver on *Artemia salina*: growth, fatty acid composition, oxidative stress, and lipid peroxidation. *Environ. Science: Nano* 12, 1626–1637.
- Sahashi, N., Tsuji, H., and Shishiyama, J. (1989). Barley plants grown under germ-free conditions have increased susceptibility to two powdery mildew fungi. *Physiol. Mol. Plant Pathol.* 34, 163–170. doi: 10.1016/0885-5765(89)90023-4
- Shang, X., Geng, L., Wei, H. J., Liu, T., Che, X., Li, W., et al. (2024). Analysis revealed the molecular mechanism of oxidative stress-autophagy-induced liver injury caused by

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- high alkalinity: integrated whole hepatic transcriptome and metabolome. *Front. Immunol.* 15, 1431224. doi: 10.3389/fimmu.2024.1431224
- Souza, C. D. F., Baldissera, M. D., Baldissarroto, B., Heinzmann, B. M., Martos-Sitcha, J. A., and Mancera, J. M. (2019). Essential oils as stress-reducing agents for fish aquaculture: a review. *Front. Physiol.* 10, 785. doi: 10.3389/fphys.2019.00785
- Thielmann, J., and Muranyi, P. (2019). Review on the chemical composition of *Litsea cubeba* essential oils and the bioactivity of its major constituents citral and limonene. *J. Essential Oil Res.* 31, 361–378. doi: 10.1080/10412905.2019.1611671
- Tiong, I. K. R., Lau, C. C., Taib, M. I. M., Waiho, K., Sorgeloos, P., and Sung, Y. Y. (2025). Artemia as a model organism in stress response studies: Current progress and future prospects. *Mar. Biol.* 172, 16. doi: 10.1007/s00227-024-04569-1
- Wang, Z., Zhou, J., Li, J., Lv, W., Zou, J., and Fan, L. (2020). A new insight into the intestine of Pacific white shrimp: regulation of intestinal homeostasis and regeneration in *Litopenaeus vannamei* during temperature fluctuation. *Comp. Biochem. Physiol. Part D: Genomics Proteomics* 35, 100687. doi: 10.1016/j.cbcd.2020.100687
- Xia, L., Li, R., Tao, T., Zhong, R., Du, H., Liao, Z., et al. (2024). Therapeutic potential of *Litsea cubeba* essential oil in modulating inflammation and the gut. *Interact. between Bioactive Food Ingredients Intestinal Microbiota* 2, 63.
- Xue, Y., Jiang, G., Shu, H., Wang, W., and Huang, X. (2024). Effects of temperature and salinity on the growth, reproduction, and carotenoid accumulation in *artemia sinica* and transcriptome analysis. *Fishes* 9, 437. doi: 10.3390/fishes9110437
- Yadav, N. K., Patel, A. B., Singh, S. K., Mehta, N. K., Anand, V., Lal, J., et al. (2024). Climate change effects on aquaculture production and its sustainable management through climate-resilient adaptation strategies: a review. *Environ. Sci. Pollut. Res.* 31, 31731–31751. doi: 10.1007/s11356-024-33397-5
- Zheng, X., Declercq, A. M., Vanrompay, D., Feyaerts, A. F., Van Dijck, P., and Bossier, P. (2024). *In vitro* and *in vivo* characterization of citral and limonene combinations against *Vibrio campbellii* in brine shrimp. *Aquaculture* 593, 741343. doi: 10.1016/j.aquaculture.2024.741343
- Zheng, X., Han, B., Kumar, V., Feyaerts, A. F., Van Dijck, P., and Bossier, P. (2021). Essential oils improve the survival of gnotobiotic brine shrimp (*Artemia franciscana*) challenged with *Vibrio campbellii*. *Front. Immunol.* 12, 693932. doi: 10.3389/fimmu.2021.693932