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Community engagement and screening of 447 pesticides in the Gellibrand River catchment, Victoria, Australia

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The study presents a comprehensive water quality data collection along the Gellibrand River catchment, over three monitoring campaigns conducted in 2024, to investigate pesticides occurrence. Citizen scientists, guided by the Royal Melbourne Institute of Technology (RMIT) University and the European Commission Joint Research Centre (JRC), deployed and retrieved passive samplers (n = 10), collected grab composite samples (n = 10) and conducted Stir Bar Sorptive Extraction (SBSE) of the latter, while Solid Phase Extraction (SPE) of all the other samplers was performed by a PhD student. All samples were screened by Gas Chromatography Quadruple Time of Flight High Resolution Mass Spectrometry (GC-QToF-HRMS) for 447 pesticides. Passive samplers provided qualitative information on the presence/absence of pesticides, while grab composite samples provided quantitative information in form of pesticides concentrations. Concentrations were used to calculate Toxic Units (TU) to assess potential ecological risks for crustaceans *Daphnia magna*. A total of 18 different pesticides were detected in the first campaign, followed by 8 in the second, and 15 in the third campaign. The two different extraction methods deployed, SPE and SBSE, revealed different compounds. Across the campaigns, fungicides emerged as the most frequently detected class, although most screened chemicals were insecticides. Temporal and spatial analyses highlighted significant variability in pesticide presence, influenced by factors like weather conditions and surrounding land use, in particular, forestry. The study's ecological risk assessment identified potential toxicity risks to *Daphnia magna* from specific insecticides, such as carbaryl. The study successfully combined advanced sampling and extraction technologies with citizens participation, finding pesticides that are registered for and applied in surrounding forestry activities, providing valuable new insights regarding pesticides occurrence in Australia. Citizen scientists have been enthusiastically engaged in the collection, deployment, retrieval and extraction of samples. They contributed to communication and sharing of the results with the broader community, thus confirming the potential of citizen science to expand water quality monitoring capacity.

KEYWORDS

citizen science, forestry, passive samplers, pesticides, stir bar sorptive extraction, surface water

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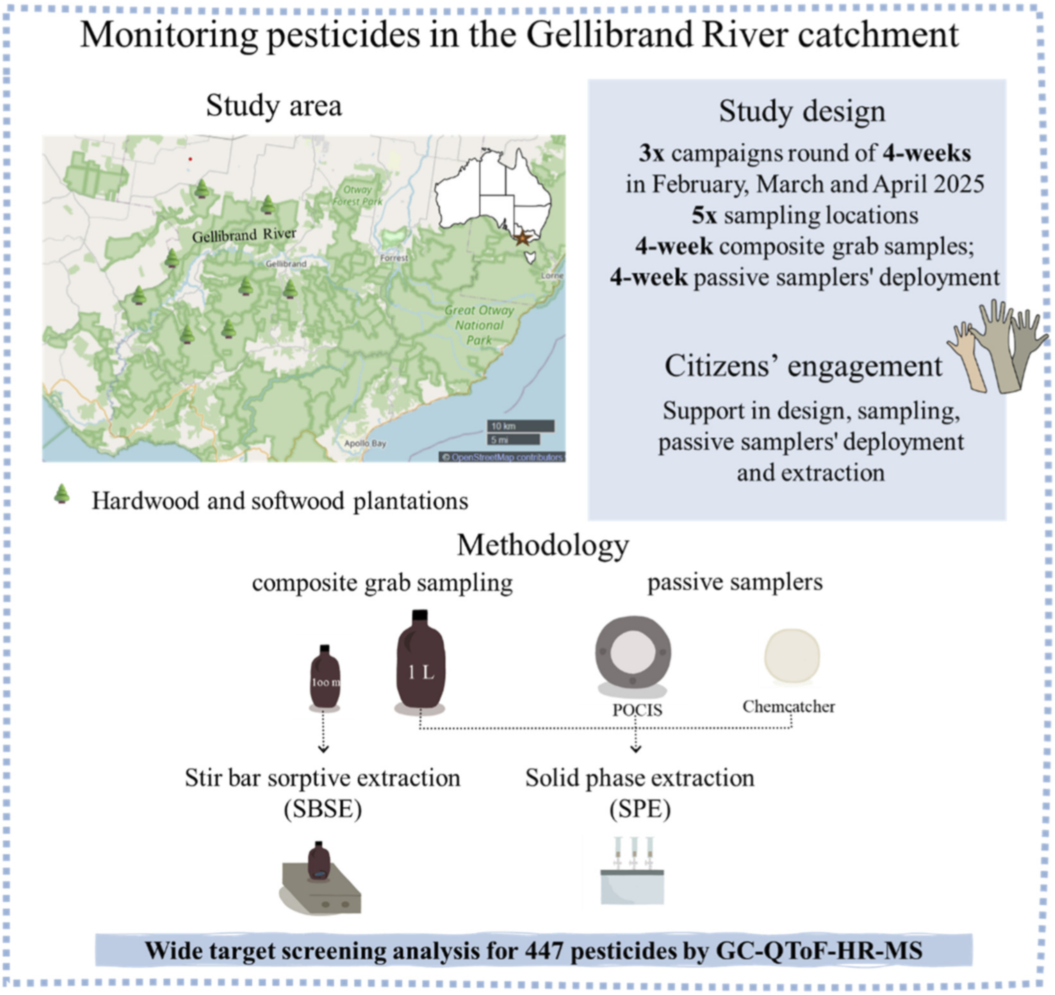


GRAPHICAL ABSTRACT

1 Introduction

Pesticides have become an essential ingredient in intensive agricultural practices to protect crops from pests and to provide consistent yields (Sharma et al., 2020). Australia's pesticide use lies at 59,634 tonnes of pesticides used per year (FAO, 2022). Currently over 8,000 pesticides and veterinary chemicals are registered in Australia for a variety of uses, including cropping, livestock, horticulture, forestry and domestic uses (Brodie and Landos, 2019). Those pesticides are often formulations of multiple active ingredients, some of which are already banned overseas (Laicher et al., 2022), due to their toxicity, persistency and bioaccumulation potential and the risk they represent for human and ecosystem health (Campanale et al., 2021; Moschet et al., 2017).

In Australia, the widespread and intensive use of pesticides is not counterbalanced by a comprehensive monitoring of waterways, with inconsistencies in regards to target pesticides and difficulties in selecting fit-for-purpose analytical methods (Serasinghe et al., 2022). The high number of pesticides to screen, the variability of their physical properties, application times and contamination sources contributes to complicating monitoring efforts (Campanale et al., 2021). Some long-term studies have reported pesticides occurrence in New South Wales, Queensland and South Australia (Laicher et al., 2022), while several research-led monitoring has investigated presence of pesticides in urban wetlands (Pettigrove et al., 2023), in coastal areas (Brodie and Landos, 2019; Laicher et al., 2022), in surface waters nearby agricultural areas (Allinson et al., 2016; Allinson et al., 2017; Wightwick et al., 2012) and wastewater inlets (Knight et al., 2023). These studies identified pesticides occurring at high frequencies in Australian waterways. Common contaminants include diuron, imidacloprid, pyrimethanil, MPCA, carbendazim, 2,4-D, hexazinone, simazine, atrazine, myclobutanil, trifloxystrobin, metalaxyl and iprodione. The majority of these pesticides are not routinely monitored, especially in the context of agricultural and domestic pesticides runoff in local waterways (Laicher et al., 2022).

Directing water quality monitoring towards more region-specific and cost-effective target screening should follow a prioritization step to identify which pesticides are more likely to occur (Serasinghe et al., 2022; Wightwick and Allinson, 2007). Serasinghe et al. (2022) has proposed a method of prioritization of pesticides based on data wide-screening, registered pesticides and land use. Community-led water quality monitoring can be an alternative way to carry out prioritization screening, helping to understand pollutant sources and characterize water quality conditions based on local concerns and knowledge (Tsatsaros et al., 2021). Indeed, citizen science in Australia has long been recognized to have potential to enhance data collection in environmental monitoring for more integrated policy making (Roger et al., 2019). To the best of our knowledge, two projects have focused on engaging citizens in water quality monitoring for pesticides in Australia. The Pesticide Detectives was established at RMIT University Aquatic Environmental Stress Research Group (AQUEST) and involved volunteers in the collection of sediments in local creeks all over Australia for screening of up to 110 pesticides in 2019–2020 (Chinathamby, 2021), while Pesticide Watch is an on-going citizen science activity run by Deakin University involving citizens in the monitoring of up to 400 pesticides in surface waters (Hamilton, 2025).

In this paper, we developed a unique approach which brings together target pesticide screening and citizens science. Using several sampling, passive samplers and grab composite samples, and extraction techniques, Solid Phase Extraction (SPE) and Stir Bar Sorptive Extraction (SBSE), this approach enables more comprehensive detection of pesticides than using a single sampling and extraction method. Citizens' participation provides a more context-specific, localized assessment of the pesticides present in the aquatic environment. The co-created monitoring activity was developed jointly by the local community (Land and Water Resources Otway Catchments - LAWROC) and scientists from RMIT University, in Victoria, Australia, and the European Commission Joint Research Centre (JRC), in Italy, which has been running such citizen science activities in other countries (Cacciatori et al., 2024; Cacciatori et al., 2025a; Cacciatori et al., 2025b). From the community's perspective, the monitoring should address concerns about whether pesticides used in local forestry plantations are impacting local aquatic ecosystems in the Gellibrand River catchment. For the scientists, study furthermore enabled evaluation of the workflow for citizen science collection of water quality data, where local participants conducted sampling and extraction, and the samples were sent to the JRC Water Laboratory for wide-screening analysis of 447 pesticides.

Therefore, more specifically, the aims of this project were to establish whether (1) the Gellibrand River catchment in the study is contaminated with pesticides and which land use activity are the likely sources; whether (2) combining different sampling and extraction methods increases the number of pesticides detected and if (3) the protocols and technologies are practical for citizen scientist to use in field sampling.

2 Materials and methods

2.1 Study area and design

Sampling was conducted in the upper catchment of the Gellibrand River (Figure 1), the whole catchment being 1,200 km^2. Grab composite samples were collected (n = 10), and passive samplers were deployed (n = 10), from five locations across four water bodies: the Gellibrand River (GV, R), Loves Creek (M), Charleys Creek (CC) and Boggy Creek (BC) (Figure 2). Gellibrand River was sampled at Valley Road (GV) near small family farms and livestock farming and further downstream (R) in the vicinity of livestock farms. Boggy Creek (BC) sampling point was in the vicinity of softwood (*Pinus radiata*) and hardwood (*Eucalyptus nitens*) plantations, native forests, small-scale agricultural, and livestock activities. Loves Creek (M) sampling site was located within a livestock farm, while Charleys Creek (CC) was sampled in a location surrounded by hardwood plantations. Coordinates of the sampling locations are provided in Supplementary Table S1 (Supplementary Material).

At the beginning of the first campaign, participants divided responsibilities among each other for the different sites, so that the same family, or group, collected samples at the same location throughout the seasons. Three monitoring campaigns were conducted within the Gellibrand River catchment in March (Campaign 1), April (Campaign 2) 2024 and November (Campaign 3) 2024. Campaign 1 was conducted during very dry weather (8 mm of rain recorded in March); Campaign 2 and 3 occurred during wet weather with 53.9 mm recorded in April and 60.4 mm recorded in November 2024. Rainfall information was obtained for the nearby measurement station of Irrewillipe (38.45° S, 143.66° E) (Australian Government Bureau of Meteorology, 2025).

During these campaigns, four different sampling methods were implemented (Figure 2). Chemcatcher with SDB-XC disk and Polar Organic Chemical Integrative Sampler (POCIS) passive samplers were deployed over a month in duplicates. 300 mL water samples were collected weekly over the deployment period of the passive samplers to make up a 4-week composite sample of 1 L, to be extracted by Solid Phase Extraction (SPE), and 100 mL, to be extracted by Stir Bar Sorptive Extraction. Stir Bar extracted samples were collected in duplicates for a total of 10 samples, while 1 L SPE samples were collected singularly, as control samples, for a total of 5 samples. It should be noted that, during the second campaign, due to misunderstanding in the collection of grab composite samples' volumes, 1 L samples to be extracted by SPE were not collected.

2.2 Citizen engagement

A total of ten families were engaged in the water quality monitoring initiative. Most participants were either part of LAWROC, or partners or family members of someone directly involved. Almost all participants were retirees, with few of them having a background in natural sciences (i.e., ecology, chemistry). Interest to participate in a water quality monitoring activity was initiated by LAWROC, due to concern raised by the vicinity of forestry plantations to the catchment of the Gellibrand River and a decline of endemic blackfish populations. After a couple of online meetings, an in-person meeting occurred to jointly define the sampling locations and initiate the first campaign of sampling. Citizen scientists provided land use maps of the area, which aided in the selection of the sampling locations, with the choice driven by presence of forestry, horticultural, livestock activities, and National Parks. Instructions on how to undertake sampling and extraction were provided in written and visual format to facilitate access and understanding (Cacciatori et al., 2023). During the first sampling campaign, a PhD student, together with the staff from AQUEST at RMIT University, trained participants in the deployment and retrieval of passive samplers, in the collection of composite samples, sample extraction and storage. The next two

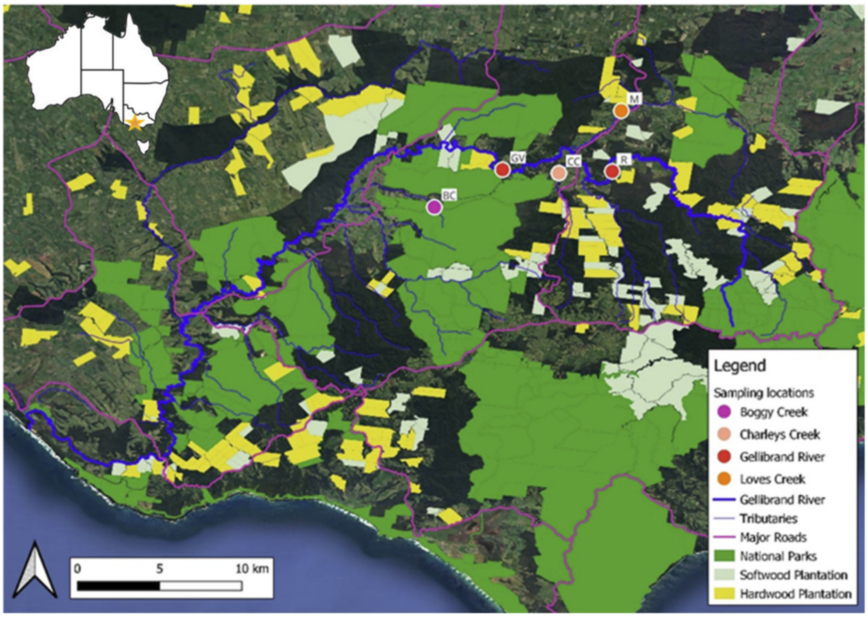
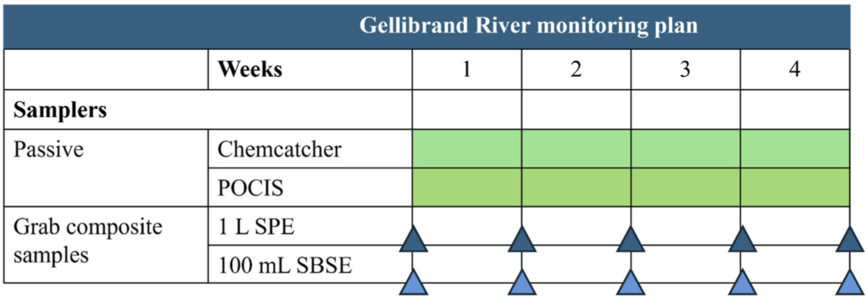


FIGURE 1
Map of sampling locations, with indicated surrounding landuse, along the Gellibrand River catchment, in Southeastern Victoria, Australia.



Gellibrand River monitoring plan

	Weeks	1	2	3	4
Samplers					
Passive	Chemcatcher	🟩	🟩	🟩	🟩
	POCIS	🟩	🟩	🟩	🟩
Grab composite samples	1 L SPE				
	100 mL SBSE				

FIGURE 2
Scheme of passive samplers (Chemcatcher and POCIS) deployment and grab composite samplers extracted by Solid Phase Extraction (SPE) and Stir Bar Sorptive Extraction (SBSE) over a period of 4 weeks. The same scheme has been applied for all three monitoring campaigns.

campaigns were conducted by LAWROC members (Figure 3) with sampling materials being delivered to them by courier.

To collect feedback on the engagement of the citizen scientists, a list of survey questions was shared with the group, while additional feedback was collected informally throughout the process. The feedback was instrumental to understand the suitability of protocols and instruction material, the ease in deploying the different sampling and extraction techniques, and whether any improvement could be implemented along the project rollout (i.e., communication strategy, logistics). The survey's questions are reported in Supplementary Material S2.

2.3 Sample collection, deployment and processing

The preparation of passive samplers was performed at RMIT AQUEST, where also bottles for grab sampling were pre-cleaned and labelled. Preparation of passive samplers followed protocols by Pettigrove et al. (2023), and further described in Cacciatori et al. (2026). In short, passive samplers' casings, polyether sulfone (PES) protective membranes and absorbent SDB-XC disks (Chemcatcher) were soaked in methanol and Milli-Q water for cleansing and chemical activation. For grab samples, pre-cleaning of new bottles was performed by acetone rinsing (3x), while used bottles were rinsed with acetone (3x), dish washed, autoclaved and rinsed again with acetone (3x).

LAWROC citizen scientists were involved in deployment and retrieval of passive samplers, as well as in the collection of grab samples and extraction using SBSE. The passive samplers were tied using rope to stable structures such as tree trunks or bridges and from a star picket. During the first campaign, passive samplers and deployment stainless steel casings were provided separately, yet to be assembled. This created some difficulties for the citizen scientists, as attaching the passive samplers onto the stainless-steel cases is a

FIGURE 3
Deployment of the passive samplers and collection of grab composite samples by the participants, during the monitoring campaigns.

rather meticulous step. Therefore, during the other two campaigns, these samplers were provided already attached on the cases, facilitating deployment greatly. LAWROC citizen scientists were provided with 500 mL, 5 L bottles. 500 mL bottles were used to collect grab samples each week for a total of 300 mL, indicated with a line. 5 L bottles were used for storage of the total sample amount (1.5L: 5 samples x 300 mL), which was eventually separated into 1 L and 100 mL for SPE and SBSE extraction.

All samples processing procedures were conducted at RMIT Laboratory, except for SBSE extraction, which was performed on 100 mL composite grab samples by LAWROC's members. Procedures of samples processing differed depending on samplers. Before elution, POCIS Hydrophilic Lipophilic Balance (HLB) absorbent material was transferred on an empty SPE cartridge, while Chemcatcher SDB disks were dried on a hot plate for 1–1.5 h at 35 °C. 1 L grab composite samples were filtered and extracted on a HLB cartridge according to the procedure described in (Cacciatori et al., 2025c). Extraction by SBSE consists of adding a stir bar to the water samples and letting it stir on a magnetic plate for 5 h at room temperature. Details of this extraction method are provided in Cacciatori et al. (2025c). After extraction, stir bars, dried SDB disks and HLB cartridges (POCIS and 1 L composite samples) were shipped via courier (DHL P/L) to the JRC Water Laboratory for analysis. All material used for sampling is thoroughly described in Cacciatori et al. (2026).

2.4 Quality assurance and quality control

Quality Assurance and Control was guaranteed deploying field and laboratory blanks for passive samplers' deployment and retrieval and preparation and processing in the laboratory, respectively. For composite grab samples, blank samples were processed alongside real samples. Furthermore, internal standard solution was added after elution of passive samplers and SPE extracted grab samples, and before SBSE extraction. The internal standard solution consisted of *trans*-Nonachlor ($^{13}C_{10}$, 98%, Cambridge Isotope Laboratories Inc., United States, quantifier ion: 418.8182; qualifier ion: 420.8154), diluted in acetone (1 mL), for a final concentration of 0.5 ng/L for the samples processed with SBSE and 5 ng/L for the water samples extracted with SPE and passive samplers.

2.5 Chemical analysis

Samples were target screened for 447 pesticides using a Gas Chromatograph 8,890 with Quadruple Time-Of-Flight 7,250 (Agilent Technology, United States). All chemicals were separated and analyzed on HP-5MS UI capillary column (length = 30 m, internal diameter = 0.25 mm, film thickness = 0.25 µm, Agilent Technology, Santa Clara, CA, United States). A thorough description of the analysis is reported in Cacciatori et al. (2026), while GC-QToF-HRMS method parameters are reported in Supplementary Table S3 (Supplementary Material).

Passive samplers provided qualitative information on the presence or absence of pesticides in the water, while grab composite sampling with SPE and SBSE allowed for the determination of their concentrations expressed in ng/L. Analysis and quantification of samples and calculation of the calibration curve was based on computation for 4 calibration levels (L1: 10 ng/L, L2: 25 ng/L, L3: 50 ng/L, L4: 100 ng/L). Calibration curve L1 was injected (SPE-extracted grab-samples) or desorbed (SBSE-extracted grab samples) 6 times to determine Limit of Detection (LOD) following Cacciatori et al. (2025c). Supplementary Table S4 of the Supplementary Material reports the list of all screened compounds, with their categories, and LODs for SPE and SBSE extracted samples. For compounds detected above 10 ng/L, Limit of Detection (LOD) were estimated using the signal-to-noise ratio method, with LOD defined as three times the baseline noise, respectively, following the approach described by Ravisankar et al., (2021).

A standard solution was not available for 12 compounds, namely 2,4,6-tribromoanisole, 2-chloronaphthalene, 2-phenylphenol, 4,4′-methylenebis(N,N-dimethylaniline), acetophenone, dichlorbenzamide, chlorocresol, epoxiconazole, isoxadifen, phthalimide, quinoline, resorcinol. They were excluded from quantitative analysis and are indicated with suspect target screening in Table S. Where possible, concentrations were compared to existing guideline values from the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC, 2020). The 99% species protection Default Guideline Value has been reported in the text when available, considering the vicinity of some of the sampling locations to National Parks. Acquisition data were analyzed with Quantitative Agilent Software (version 12.1).

2.6 Ecological risk assessment

An ecological risk assessment was conducted on a subset of the detected pesticides depending on the availability of data on the IUPAC Pesticide Properties Database (PPDB) (Agriculture & Environment Research Unit AERU, 2018). The toxic effects were calculated as acute risks to the freshwater crustacean *Daphnia magna* using Toxic Units (TU). TU are calculated by dividing the measured concentrations of each pesticide by the concentration that causes a 50% effect on a defined biological endpoint (Equation 1), indicated by the acute half-maximal effective concentration (EC50), or the median lethal concentration (LC50) (EFSA et al., 2019; SCHER and SCCS, 2011). Toxic units obtained for each compound were summed up for each sample, assuming concentration addition (EFSA et al., 2019) to provide information on the cumulative eco-toxicological risks at each sampling locations. EC(LC) 50 values used for the calculations are reported in Supplementary Table S6 of the Supplementary Material.

$$TU_{mixture} = \sum_{i}^{n} \frac{Ci}{EC\,(LC)50i} \tag{1}$$

This approach allows for the comparison of the effects of individual chemicals or their addition to an overall potential effect. Values exceeding 0.01 indicate potential toxicity to *D. magna* (EFSA Panel on Plant Protection Products and their Residues PPR, 2013). Only those pesticides with available toxicity data for *D. magna* were included in this assessment.

3 Results and discussion

A summary of the types, concentrations and frequencies of pesticides detected during the three monitoring campaigns across all sampling locations are reported in Supplementary Table S5 (Supplementary Material). While pesticide types and frequencies are reported for all samples as qualitative data, concentration levels refer specifically to composite grab samples extracted via SBSE and SPE. These extraction results are reported and analyzed together unless otherwise noted, as a detailed comparison of the two methods' recoveries and efficiencies is beyond the scope of this study (see Cacciatori et al., 2026). In case a compound was qualified by both methods, the highest value was reported.

3.1 Pesticides occurrence in the Gellibrand River catchment

During the first monitoring campaign, 18 pesticides or their metabolites of the 447 screened were detected (Table 1). The second and third monitoring campaigns yielded 8 and 15 pesticides respectively. Of all detected compounds, nine pesticides were found exclusively in Campaign 1, including six fungicides (azoxystrobin, boscalid, difenoconazole, fluquinconazole, imazalil, mefenoxam), two insecticides (deltamethrin, indoxacarb), and one metabolite (atrazine-desisopropyl). In contrast, only the insecticide carbaryl was detected uniquely in Campaign 2. During Campaign 3, 10 compounds were detected that had not previously been detected in previous campaigns. These pesticides were mainly fungicides and including fenhexamid, penconazole, propiconazole and tebuconazole.

The detected pesticides fall into several categories: fungicides, insecticides, herbicides, and other types such as intermediates, breakdown products and metabolites (Table 1). Throughout the three campaigns, most pesticides detected belonged to the class of fungicides, while most pesticides screened belonged to the class of insecticides (n = 176). Fungicides have been mentioned as an often overlooked pesticides class during surface water monitoring, although they are widely and intensively applied both in agricultural and urban environments (Zubrod et al., 2019).

3.1.1 Temporal observations over three monitoring campaigns: frequencies and concentrations

Those pesticides frequently detected during this study are presented in Table 2.

The herbicide simazine was the most frequently detected pesticide appearing in 95% of first-campaign samples, though detection dropped to 50% and <5% in subsequent campaigns. Despite simazine wide registered as an active ingredient (77 commercial products) to control weeds in various crops (APVMA, 2024),the concentrations detected in this study (up to 112.6 ng/L) are well below the Australian guidelines for protection of aquatic life (99% species protection 6,100 ng/L) (ANZECC, 2000).

The second most frequently detected (35%–70%) pesticide was bio-accumulative wood preservative 2,4,6-Trichlorophenol (2,4,6-TCP), (Table 2). Detected concentrations (3.3–22.1 ng/L) remained below The Australian default guideline value (3,000 ng/L) for 99% species protection. (ANZECC, 2000). Herbicide terbacil was detected in 65% of the samples during the third campaign (Table 2); however, no water quality guidelines currently exist for this herbicide terbacil is used in established forestry plantations like cottonwood and eucalypt forests and acts on annual grasses, broadleaved weeds and perennial weeds (Agriculture & Environment Research Unit AERU, 2018). There are ten approved pesticides using terbacil as active ingredient in Australia (APVMA, 2024).

Other notable pesticides included 2-Phenylphenol (50%, 1.1–2.7 ng/L), biphenyl (45% of samples) and herbicide hexazinone (35%–40%, 1.8–835.3 ng/L) and hexazinone (35%–40%, up to 2 ng/L). While hexazinone is below the 75,000 ng/l trigger value, no specific standards exist for 2-Phenylphenol or biphenyl. 2-Phenylphenol is used, in 5 registered products

TABLE 1 **Categories of pesticides screened and detected during the three monitoring campaigns, considering all sample types.**

Pesticide categories	Number of screened pesticides	Campaign 1	Campaign 2	Campaign 3
Insecticide	176	3	1	3
Fungicide	102	8	2	6
Herbicide	94	3	2	2
Intermediate	16	0	0	0
Breakdown product	12	0	0	1
Metabolite	12	1	0	0
Others	35	3	3	3
Total	447	18	8	15

TABLE 2 **Frequencies and concentration ranges of pesticides detected in >35% of samples across all campaigns. Y indicates that the compound has been detected by the passive samplers, but no concentration is calculated. Where available, Default guidelines values (DGV) for 99% species protection have been reported ANZECC (2000).**

Detected pesticides	Pesticide category	Campaign round	Frequency [%]	Concentration range [ng/L]	DGV [ng/L] (ANZECC, 2000)
2,4,6-TCP/2,4,6-Trichlorophenol	Wood preservative	1	55	5.9–18.5	3,000
		2	35	Y	
		3	70	3.3–22.1	
2-Phenylphenol	Microbiocide	1	50	1.1–2.9	NA
Biphenyl	Fungicide	1	45	7.7–11.2	NA
		2	45	1.8–835.3	
		3	45	3–7.6	
Hexazinone	Herbicide	1	35	1.6–1.8	NA
		3	40	3.9–4.1	
Simazine	Herbicide	1	95	8.7–112.6	6,100
		2	50	Y	
Terbacil	Herbicide	3	65	2.4–9.4	NA

(APVMA, 2024), as a coating agent protecting crops from storage diseases or for household purposes (Agriculture & Environment Research Unit AERU, 2018). Biphenyl is used as a fungicide preventing mould in citrus fruit and other crops, and as an intermediate product in organic synthesis (Agriculture & Environment Research Unit AERU, 2018). It also occurs naturally in coal tar, crude oil and natural gas. No specific environmental standards exist for it. Finally, hexazinone is commonly used in forestry and appears in 54 registered products as an active ingredient in Australia (APVMA, 2024). All other pesticides were detected in <35% samples.

Comparison of the three campaigns' concentrations is limited by varying sample sizes (n = 15–71) (Figure 4; Table 3), and by exclusive use of SBSE extraction during Campaign 2. Despite this, Campaign 2 showed concentrations an order of magnitude higher, with a median of 15.0 ng/L, than those recorded in Campaigns 1 and 3 (7.0–7.6 ng/L) (Figure 4; Table 3). The spike was driven by outliers like carbaryl (1,386.6 ng/L) and piperonyl butoxide (358 ng/L).

Peak concentrations varied by campaign: simazine (432.2 ng/L) was highest in Campaign 1, carbaryl (1,386.6 ng/L) and biphenyl (880.1 ng/L) in Campaign 2; and fenhexamid (415.4 ng/L) was highest in Campaign 3. All other compounds were detected in concentrations below 100 ng/L. These compounds have various use in local land use. Carbaryl, registered in 9 products in Australia, is a broad-spectrum insecticide, permitted for use on pines, eucalypt and nursery plants, on fruits, such as blueberries, raspberries, and strawberries, as well as for household purposes (APVMA, 2024). Piperonyl butoxide is indirectly used in forestry as a synergist to enhance effectiveness of insecticide formulations, like pyrethrins and pyrethroids. Fenhexamid, a protectant fungicide, is permitted in forestry for controlling *Botrytis cinerea* (grey mould) on ornamental conifers and non-bearing fruit trees. There are currently six products registered for use in Australia with fenhexamid as active ingredient, with three permits are currently active in Australia for fenhexamid for use on forest tree seedlings, on blueberries and cherries (APVMA, 2024). Other contributors included agricultural

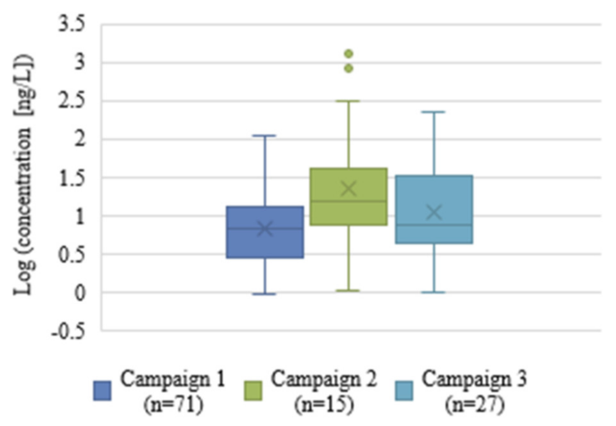


FIGURE 4
Logarithmic concentration distribution for the three monitoring campaigns. SBSE and SPE extracted concentrations have been considered together. In case a compound was qualified by both methods, the highest value was reported.

fungicides azoxystrobin (range 6.5–21.9 ng/L) and difenoconazole (range 6–29.2 ng/L), respectively registered in 110 products for use on blueberries, tomatoes, soybeans, and in 15 products for use on avocado, persimmons, plums and papaya (APVMA, 2024). There are currently no water quality guidelines values for these two fungicides.

3.1.2 Spatial variations at the five sampling locations

Fifteen pesticides were detected in average at the different sites during the first campaign, while 7 were detected during the second and the third campaigns. The first two campaigns showed little variability in terms of number of pesticides detected per sites, while during Campaign 3 pesticides detected at the different sites ranged from 3 (Charleys Creek - CC) to 11 (Loves Creek - M). Throughout the three monitoring events, the site with highest detection was Loves Creek (M - 33), followed by Boggy Creek (BC - 30), Gellibrand River at Valley Road (GV - 29), Charleys Creek (CC – 26) and Gellibrand River (R - 26). Figure 5 reports the total number of detected pesticides at the different sampling locations.

Charleys Creek (CC) reported by far the highest total concentration (1,378.7 ng/L) over all other sampling sites. This is linked to high concentrations detected during Campaign 2 for pesticides carbaryl, biphenyl and synergist piperonyl butoxide. In decreasing order, total concentrations were 255.2 ng/L at Boggy Creek (BC), 222.7 ng/L at Loves Creek (M), 215.4 ng/L at Gellibrand River at Valley Road (GV). Lowest total concentration was reported for Gellibrand River (R) with 73.4 ng/L. Figure 6 below reports total concentrations [ng/L] of detected pesticides at the different sampling locations.

Looking at contributions of single pesticides to the total concentrations, during Campaign 1, simazine contributed significantly to the samples from the Gellibrand River at Valley Road (GV - 112.6 ng/L), Loves Creek (M - 80.4 ng/L), Charley's Creek (CC- 66.4 ng/L), and in the Gellibrand River (R - 44.6 ng/L). At Boggy Creek (BC), the highest single pesticide concentration was recorded for 2,4,6 TCP (18.6 ng/L) (Table 2).

TABLE 3 SBSE and SPE extracted concentration distribution and simple statistics presented for the three campaigns, with indication of the number of detections considered. SBSE and SPE extracted concentrations have been considered together. In case a compound was qualified by both methods, the highest value was reported.

	Concentration [ng/L]		
	Campaign 1 (n = 71)	Campaign 2 (n = 15)	Campaign 3 (n = 27)
Average	12.7	175.0	27.3
Median	7.0	15.6	7.6
1st quartile	2.9	9.2	4.4
3rd quartile	12.7	34.7	28

During Campaign 2, as previously mentioned, at all sites, except at Gellibrand River (R), carbaryl was the compound found at the highest concentrations, ranging from 10.8 ng/L at Gellibrand River at Valley Road (GV) to 1,305.6 ng/L at Charleys Creek (CC). At Gellibrand River (R), the highest concentration was recorded for the fungicide biphenyl (19.8 ng/L). During campaign 3, fenhexamid contributed significantly to the total concentrations at Boggy Creek (222.4 ng/L), at Loves Creek (101 ng/L), and at the Gellibrand River at Valley Rd (92.0 ng/L), being the only compound detected at concentrations higher than 100 ng/L (Table 2). At Gellibrand River (R), the highest cumulative concentration was reached by diphenylamine (9.0 ng/L), while at Charleys Creek 2,4,6-Trichlorophenol had the highest recorded concentration (6.7 ng/L).

In terms of spatial distribution of pesticides, except for the spike at Charleys Creek (CC) from carbaryl occurrence, no pattern was identified; nonetheless Gellibrand River (R) appears to be the least contaminated site. Boggy Creek (BC), located nearby Natural Parks, did not show lower detections, nor concentrations in respect to the other sites. This might be due to atmospheric dispersal of some of the pesticides or due to aerial spray applications. Despite these differences, all recorded concentrations remained below the National Guideline values where applicable, except for endrin aldehyde (Campaign 3). Low concentrations are in accordance to Pesticide Detectives (Chinathamby, 2021), which found at the Gellibrand samples that all pesticides tested/screened for where below the detection limit of <0.01 mg/kg in the sediment. Nonetheless, calculated concentrations remain a limited instantaneous snapshot in the level of contamination of the samples. Indeed, while composite sample can increase the chance of detecting a pesticide, it can also lead to under-estimation of or miss out peak concentrations in waterways due to shorter run off events anddilution in weeks with lower use or lower runoff (Laicher et al., 2022).

3.1.3 Eco-toxicological risk assessment

Calculated toxic units for samples extracted by SBSE and SPE are reported in Supplementary Table S6 (Supplementary Material).

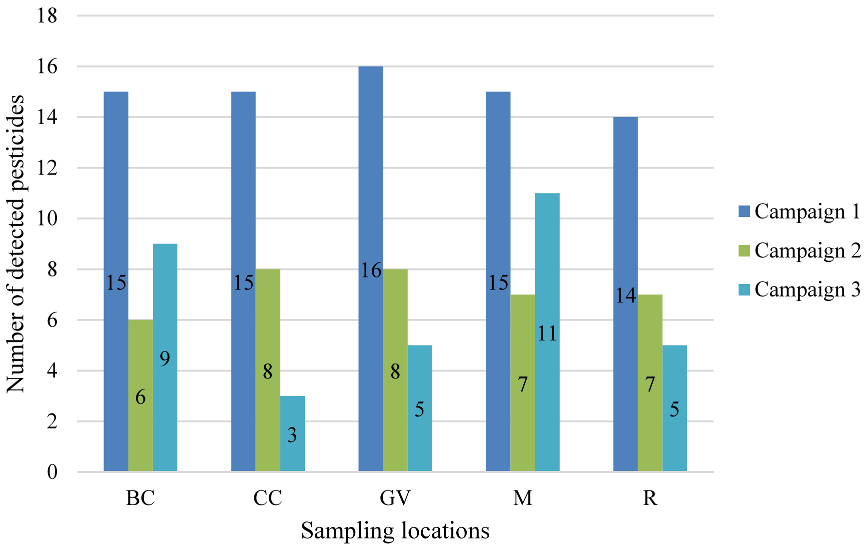


FIGURE 5
Number of detected pesticides at the different sampling locations Boggy Creek (BC), Charleys Creek (CC), Gellibrand River at Valley Road (GV), Loves Creek (M), Gellibrand River (R) for the three sampling campaigns.

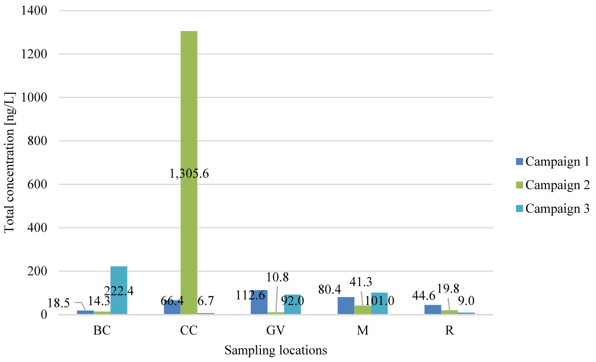


FIGURE 6
Total concentrations [ng/L] of detected pesticides at the different sampling locations Boggy Creek (BC), Charleys Creek (CC), Gellibrand River at Valley Road (GV), Loves Creek (M), Gellibrand River (R) for the three sampling campaigns.

During the Campaign 1, the total acute toxic unit threshold for crustaceans (TU = 0.01) was reached at the Gellibrand River at Valley Road (GV), Boggy Creek (BC) and Charleys Creek (CC) (Figure 7). In all these cases, the potential risk of toxicity to the crustacean *Daphnia magna* was attributed to the insecticide deltamethrin. Deltamethrin is commonly used to control sucking and chewing pests and is also applied to timbers to protect against borers. It is approved for use in Australia in 80 products, including those for household applications. Deltamethrin is linked to high chronic and acute toxicity to fish (Agriculture & Environment Research Unit AERU, 2018).

During the Campaign 2, the total acute toxic unit threshold for crustaceans was exceeded in Loves Creek (M) and Charleys Creek (CC) (Figure 8). In both cases, the exceedance was due to the presence of the insecticide carbaryl. Carbaryl is linked to moderate toxicity to fish (Agriculture & Environment Research Unit AERU, 2018).

In the Campaign 3, the cumulative acute toxicity threshold for crustaceans was exceeded at Loves Creek (M) and Boggy Creek (BC), mainly due to endrin aldehyde and 2,4′-Methoxychlor. Both 2,4′-Methoxychlor and endrin aldehyde are no longer registered or permitted for use in Australia, which hints to legacy contamination. 2,4′-Methoxychlor is an isomer of insecticide methoxychlor, with which it shares insecticidal and endocrine-disrupting properties. 2,4′-Methoxychlor was found in a sample at 4.4 ng/L, below the low reliability trigger value of 5 ng/L (ANZECC, 2000). Endrin aldehyde is a breakdown product of insecticide endrin. Freshwater moderate reliability guideline is 10 ng/L for 99% species protection. Endrin was found at only one sample location with concentration equal to 21.3 ng/L, thus above both reliability guideline values (ANZECC, 2000). Both 2,4′-Methoxychlor and endrin aldehyde are linked to acute toxicity to fish (Agriculture & Environment Research Unit AERU, 2018).

Ecotoxicological risk assessments from all campaigns indicated potential risks to the crustacean *D. magna*, attributed to specific toxic insecticides, namely deltamethrin and carbaryl, 2,4′-Methoxychlor and endrin aldehyde. Nevertheless, it is hard to draw conclusions on the toxicity risk for the endemic blackfish. As May and Hahn (2014) observed, based on a comparison on 240 substances, a generalization on whether fish or *D. magna* is more sensitive cannot be drawn, as chronic fish test would be requires for about 13% of the studied substances.

3.2 Likely sources of pesticides pollution

There was not necessarily concordance in terms of individual pesticides detected in the three different monitoring campaigns. Different uses, point sources application and rainfall patterns throughout the year might explain these variations. Trace

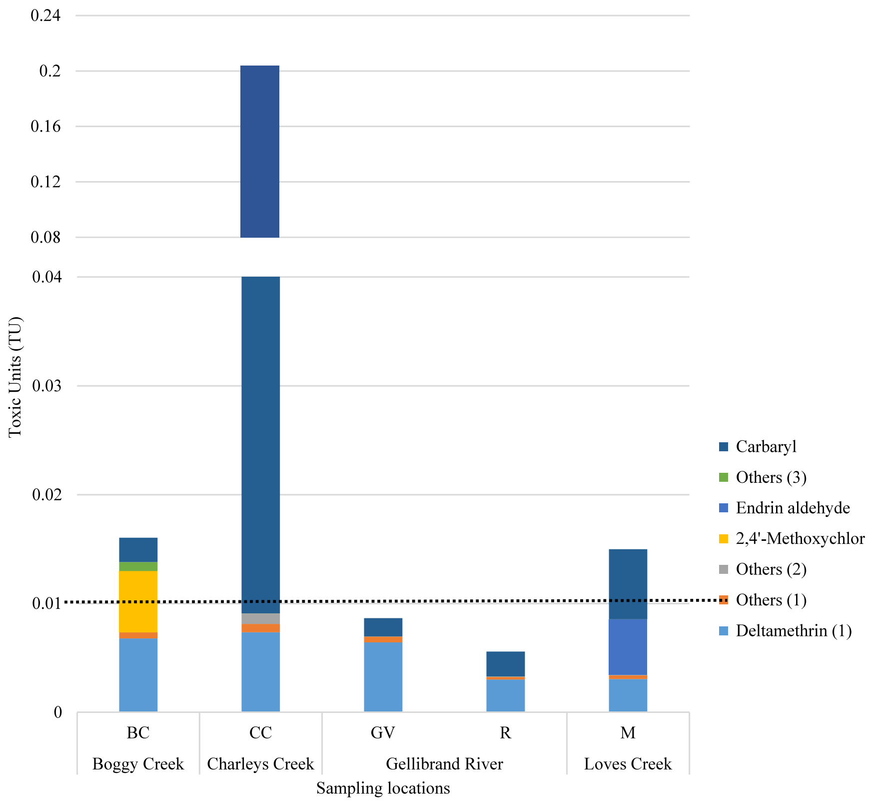


FIGURE 7
Toxic Units [TU] reported for the different sampling locations. The dotted line indicates the risk threshold for crustacean *D. magna.* (TU = 0.01). In case of two different concentrations and consequently toxic units detected by the different extraction techniques, the highest calculated TU has been considered.

concentrations of some legacy pesticides might be linked to groundwater recharge because of low rainfall during March 2024. During Campaign 2 spikes in concentrations of carbaryl, piperonyl butoxide and biphenyl, which were not detected during the other two exercises, might hint to recent application in nearby orchards and farms. Combined use of carbaryl with synergist piperonyl butoxide has been mentioned to enhance insecticide's efficacy (US EPA, 2021). Indeed, the area is characterized by orchards and horticulture, including blueberries, for which carbaryl is registered for use in Australia (APVMA, 2024). The second campaign occurred in April, beginning of the autumn season in Australia. According to literature, pesticides use on blueberries is greatest during the wet season (summer) when summer crops are in fruit, although some blueberry farms produce winter crops which may be sprayed to protect the fruit (Laicher et al., 2022).

Other pesticides have been detected consistently throughout the three campaigns, although at concentration <100 ng/L, except for biphenyl spike in Campaign 2. Those pesticides are wood preservative 2,4,6-TCP, fungicide biphenyl, herbicide hexazinone, simazine and terbacil. Except biphenyl, which frequent detection can be explained with its wide natural occurrence, all other compounds can be linked to forestry applications. According to survey results published by the Australian Government (Jenkin and Tomkins, 2006), at the time of publishing, hexazinone covered 19.8%, simazine 13.1% and terbacil 1.9% of active ingredients' use in forestry in the country. A traditional cycle of pesticides spraying in forestry includes using herbicides for site clean-up from previous land use, pre-plant cleanup, application during the first 2 years for post-planting weed control (Jenkin and Tomkins, 2006). In a similarly old research looking at pesticides contamination in waterways near forestry in Victoria, Wightwick and Allinson (2007) detected 2,4,5-TCP and hexazinone nearby areas of aerial spraying, while a study in Tasmania detected 2,4-D, MCPA, hexazinone and simazine (WIST, 2014).

3.3 Comparison of samplers

Grab samples coupled with SPE detected a total of 19 different pesticides, while SBSE extracted 15. Both sampling and extraction techniques yielded 11 unique pesticides, not recovered by the other methods (Figure 8). Therefore, the two techniques appear complementary. Passive samplers, POCIS and Chemcatcher, detected respective 9 and 6 different compounds, with POCIS detecting only 1 unique compound, while Chemcatcher none. The similarity in absorbent material (HLB, SDB) and target polarity range of POCIS, Chemcatcher and SPE sorbent might explain the overlap in detected compounds. During the second campaign when no SPE was performed on grab samples, therefore, POCIS guaranteed detection of a wider range of compounds, than if SBSE would have been deployed alone. Certainly the use of multiple

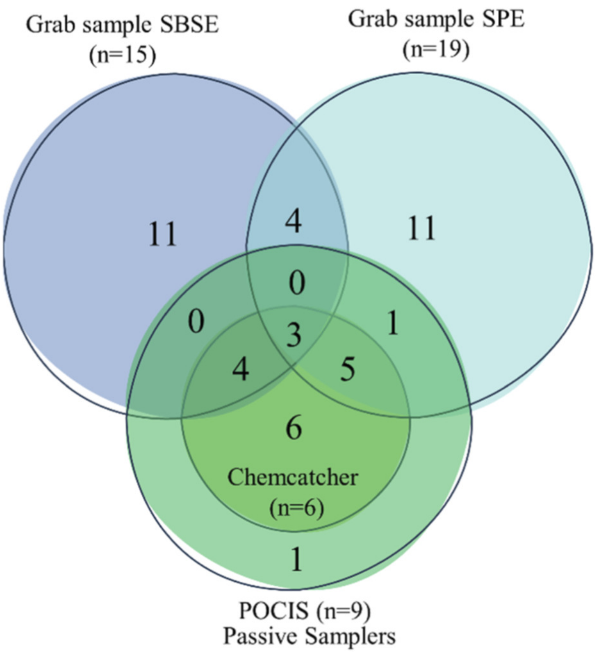


FIGURE 8
Number of pesticides detected by the different sampling and extraction techniques, grab samples coupled with SBSE and SPE, and passive samplers, Chemcatcher and POCIS with indication of total number of pesticides detected in parentheses, and commonly detected compounds in the intersections.

techniques during the campaigns expanded the type and number of compounds detected (Cacciatori et al., 2026).

3.4 Citizen science feedback

The survey on the participation in the initiative received one collective response by the group. Although the overall level of satisfaction was rated with the highest score, some issues and improvements have been identified. In particular, the respondents hinted at how the online details were not necessarily consistent with the apparatus set up in the field. The participants particularly valued the in-person demonstration which facilitated the understanding of the protocols and clarified potential disagreement between written material and actual set up. The participants highlighted, through informal feedback, the difficulties in assembling the passive samplers in the first campaign, a preparation step which was modified for the two following campaigns to provide already set up samplers. As general feedback on the different sampling and extraction techniques, participants found passive samplers initially as the hardest, because of the handiness required during deployment and retrieval, and later “less engaging”, due to the limited participation envisioned. Collection of composite samples was not deemed particularly challenging, although it created misunderstanding in the second campaign regarding the volume to be sampled. SBSE was considered exciting because of the extraction procedure, which allowed for a more analytical experience. As lessons learnt, participants voiced the importance of understanding and slowly following each part of the process, for which continuous training, even when deemed unnecessary, remains fundamental. Finally, participants said they were satisfied with the project, although it did not necessarily answer the questions they had on the impact of forestry on declining populations of blackfish. Relevant was the possibility to share this experience in a team with common goal and enthusiasm and, to socialize with members of the same community.

Local citizen engagement was instrumental in the collection of pesticides monitoring data in the Gellibrand catchment and in the assessment of applicability of different sampling and extraction techniques. Although results did not necessarily respond to the expectations of participants on the link between pesticides use for forestry and a decreasing endemic blackfish population, they contributed to increasing the knowledge on the water quality status of local water bodies, sharing information on the types of pesticides used and found in the area, while facilitating the communication with researchers and scientists, which might be seen with reservations and doubts. The implemented techniques resulted not only to be complementary in terms of pesticides detected, but also in terms of applicability in citizen science initiatives. The choice should therefore be based on the time volunteers can invest (i.e., once a month, every week, . . .), the information to be obtained (quantitative or qualitative), the financial and human resources available.

4 Conclusion

The study demonstrates the successful integration of citizen science with advanced monitoring techniques to assess pesticide contamination in the Gellibrand River catchment. By coupling various sampling and extraction methods, the project expanded its ability to detect a wide range of pesticides. This comprehensive approach not only provided valuable insights into the types and concentrations of pesticides present in an area of wood plantations but also allowed for an ecological risk assessment, highlighting potential risks to aquatic life from specific toxic insecticides like deltamethrin and carbaryl. The involvement of local citizens was crucial in collecting data and enhanced community awareness and engagement with water quality issues. Although the results did not fully address participants’ concerns about the impact of forestry activities on local blackfish populations, they contributed significantly to understanding local water quality conditions and pollutant sources. This initiative exemplifies the potential for community-led environmental monitoring to inform policy and management strategies, and it underscores the importance of continued efforts to identify and manage pesticide contamination, particularly in sensitive ecological areas. Future work should consider expanding the scope to include additional compounds, such as polar contaminants and neonicotinoids, and further investigate the impacts of land use on the ecosystem of the Gellibrand River. A final recommendation would be for the forestry companies to share transparently with this community what and when pesticides are being applied on the surrounding plantations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

CC: Writing – review and editing, Conceptualization, Formal Analysis, Methodology, Writing – original draft, Data curation, Software, Visualization, Investigation. JM: Supervision, Writing – review and editing, Methodology, Investigation, Resources, Conceptualization. GM: Software, Conceptualization, Writing – review and editing, Methodology, Formal Analysis, Supervision. BG: Writing – review and editing, Project administration, Resources, Conceptualization, Supervision. VP: Methodology, Supervision, Conceptualization, Validation, Resources, Writing – review and editing, Project administration.

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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

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Supplementary material

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