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Review

Toxicity Effects and Biomarker Responses in Two South American Freshwater Prawns Exposed to Pesticides

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Abstract

The freshwater crustaceans *Macrobrachium borellii* and *Palaemon argentinus* are key components of benthic communities and are widely distributed throughout South American freshwater ecosystems. As native species, they inhabit environments frequently impacted by pollutants from agricultural activity. Due to their marked sensitivity to these chemical stressors, both species have been proposed as valuable bioindicators for monitoring freshwater pollution. The aim of this study was to synthesize available information regarding the effects of several pesticide classes on standard ecotoxicological endpoints. Specifically, the review examines lethal concentration values (e.g., LC_{50}) and sublethal effects including metabolic disorders, histopathological alterations, and behavioral changes used as biomarkers. These biological responses were evaluated across different life stages, including embryos, larvae, and adults. Reports to date show that both species are highly sensitive to pesticides with different mechanisms of toxicity. Such biological responses are significantly influenced by concentration, exposure time, and developmental stage. The information collected on the biological and ecological characteristics of *M. borellii* and *P. argentinus* supports their suitability for ecotoxicological research and underscores their role as reliable indicator organisms for assessing the health of South American aquatic ecosystems.

Keywords: aquatic toxicology; bioindicator; biomarker; crustaceans

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Academic Editor: Juan Antonio Baeza

Received: 19 March 2026
Revised: 19 June 2026
Accepted: 26 June 2026
Published: 6 July 2026

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1. Introduction

Due to their high sensitivity to diverse environmental stressors, crustaceans have been extensively studied in standardized toxicity tests and considered for the ecotoxicological risk assessment [1]. A primary rationale for this is their notable success, evidenced by their distribution across diverse habitats, including marine, terrestrial, and freshwater environments. This distribution renders them suitable candidates for comparative research [2]. Branchiopods, copepods, and amphipods are among the taxa that have been

most extensively studied in this field [1]. Nevertheless, decapod crustaceans are regarded as a pertinent model for environmental monitoring studies as they possess both advantageous biological characteristics and commercial importance in food production [3,4]. In this context, the genera *Macrobrachium* and *Palaemon* have been identified as the most studied in South America, using a wide range of biochemical, physiological, and morphological parameters to monitor ecosystem health [5].

The freshwater prawns *Macrobrachium borellii* (Nobili, 1896) and *Palaemon argentinus* (Nobili, 1901) are sympatric species with a wide distribution in northern and central Argentina, Paraguay, Uruguay, and Brazil [6,7] (Figure 1). As key representatives of communities in lotic and lentic environments in the Pampas region [8], both species perform fundamental and similar ecological functions within the local food web [9]. The rationale behind the selection of these decapods as suitable biological models for environmental and ecotoxicological studies is threefold. Firstly, they possess the ability to adapt to stressors. Secondly, they are readily identified taxonomically. Thirdly, their life cycles are brief, and they can be maintained under controlled laboratory conditions [10,11]. It has been demonstrated in several reports that both species exhibit a high degree of sensitivity to exposure to organic and inorganic pollutants. This sensitivity is manifested through lethal and sublethal responses, which has led to their proposal as indicator organisms for environmental quality [4,12–16].

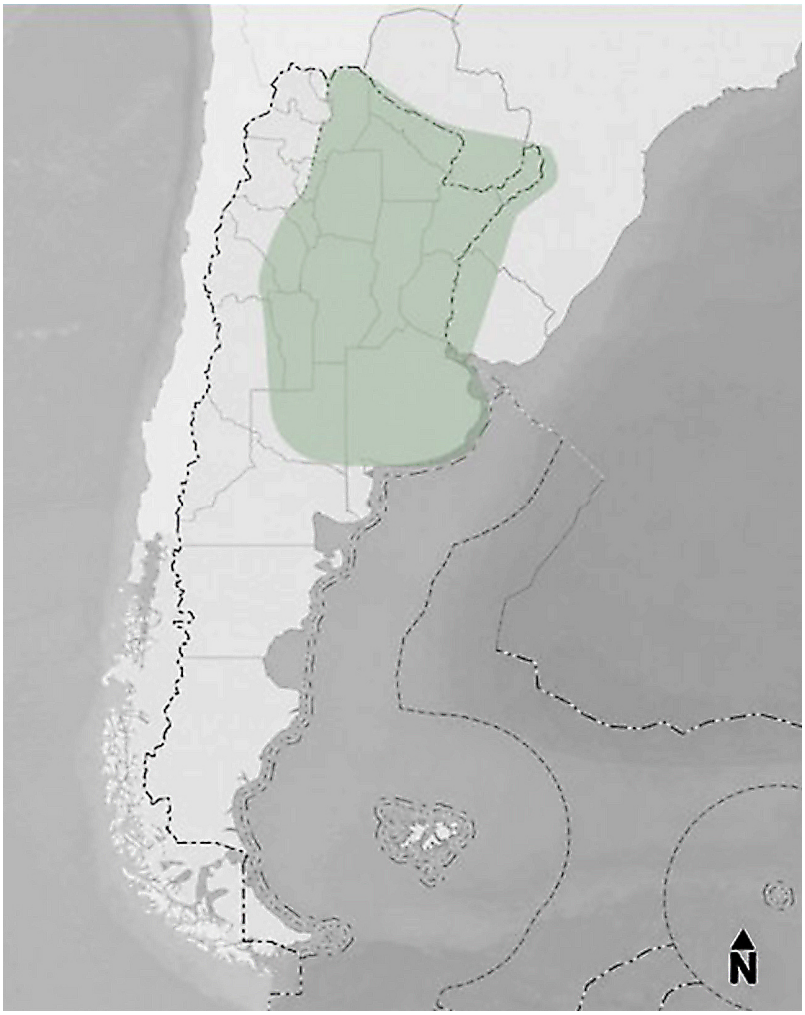


Figure 1. Joint geographic distribution of *Palaemon argentinus* and *Macrobrachium borellii* prawns [7].

These organisms inhabit freshwater environments that are perpetually exposed to pesticide contamination, which is a primary factor contributing to the degradation of water quality. Over the past several decades, there has been a shift in the global utilization of pesticides, with a transition from highly persistent compounds, such as organochlorines (e.g., DDT and related chemicals), to products that exhibit faster environmental degradation. This transition was driven by mounting evidence of the long-term ecological and health impacts associated with persistent organic pollutants, including bioaccumulation and long-range transport. Consequently, regulatory restrictions and international agreements have encouraged the replacement of legacy pesticides with current-use pesticides (e.g., organophosphates, carbamates, pyrethroids, and newer herbicides and insecticides), which generally have shorter environmental half-lives. Nevertheless, the continuous application of these compounds, their mobility, and their potential for sublethal and mixture effects mean they remain a significant concern for aquatic ecosystems and non-target organisms worldwide [17]. The presence of pesticides has been extensively documented on a global scale, including in South America, where countries such as Argentina are characterized by large-scale agricultural production. Furthermore, some compounds, such as glyphosate, have been reported as *pseudopersistent* pollutants due to their extensive use in horticulture and crop production [18]. The integrity of water resources is compromised by chemicals through multiple routes, including surface runoff, spills, or direct spraying [19]. Moreover, earlier research has already drawn parallels between environmental concentrations and the documented effects on exposed organisms, thereby highlighting significant ecological risk to aquatic ecosystems [20–22].

Conventional physicochemical analyses of water and sediments proved unsuccessful in revealing the biological implications of contaminants on biota, underscoring the necessity for strategies based on bioindicators to evaluate the stress levels in these environments [20]. In ecotoxicology, endpoints are defined as the responses of organisms to contaminant exposure, with these responses correlating with contaminant concentration and exposure duration. Mortality, a primary endpoint in short-term bioassays, is commonly expressed as the median lethal concentration (LC_{50}), contributing significantly to traditional ecotoxicological data [21]. Conversely, biomarkers measured at sublethal concentrations are regarded as useful endpoints for ecological risk assessment, given their ability to manifest at levels below those causing mortality [22]. Biomarkers are defined as molecular, biochemical, cellular, physiological, and behavioral alterations caused by sublethal concentrations of stressors [23]. They thus serve as early indicators of pollutant exposure. This early detection facilitates the implementation of preventive measures prior to the occurrence of adverse effects at higher biological levels [24]. A reliable biomarker for environmental assessment is required to meet specific criteria. These include ease of measurement, cost-effectiveness, and sensitivity to the effects of pollutants [25]. The effective use of biomarkers has the potential to significantly enhance environmental monitoring and inform decision-making processes.

Biomarkers can be categorized into two broad classifications: exposure or effect biomarkers. Exposure biomarkers are defined as parameters that indicate the presence of xenobiotics, their metabolites, or substance-specific effects in organisms. These biomarkers serve a diagnostic function, specifically for the identification of particular chemical groups. For instance, metallothioneins (MTs) are utilized in the detection of heavy metals, while cholinesterases (ChE) are employed in the analysis of organophosphates and carbamates. Cytochrome CYP1A, on the other hand, is a marker for PAHs and PCBs [23,26]. Effect biomarkers, in turn, reflect biochemical, physiological, or behavioral alterations resulting from contaminant action [25]. Following exposure to xenobiotics, organisms initiate Phase I and II biotransformation reactions, which facilitate the metabolic processing of hydrophobic

compounds and subsequent excretion via Phase III [27]. Phase I reactions, catalyzed by mixed-function oxidases (MFOs), generate reactive oxygen species (ROS), thereby activating antioxidant defenses including non-enzymatic compounds (reduced glutathione—GSH, ascorbic acid, vitamin E) and enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) [24]. SOD is responsible for the conversion of O_2^- to H_2O_2, while CAT and GPX act as catalysts for its subsequent degradation. Glutathione S-transferase (GST), a pivotal Phase II enzyme, conjugates electrophiles with GSH for the purpose of detoxification and excretion [28,29]. When antioxidant defenses are overwhelmed, this is known as oxidative stress, which can lead to lipid peroxidation (LPO) and protein carbonylation (PC). These are well-established indicators of cellular damage in aquatic organisms exposed to organic pollutants [30]. Furthermore, the disruption of neutral lipid homeostasis may result in lipid accumulation (lipidosis). In addition, increased energy demands associated with detoxification render energy metabolism parameters informative biomarkers in aquatic invertebrates [31–33]. Genotoxic endpoints, including DNA strand breaks and adduct formation, are also ecotoxicologically relevant given their association with carcinogenesis and potential transgenerational effects [34].

The sensitivity of a biomarker is defined as its ability to detect biochemical or physiological alterations at contaminant concentrations that are lower than those that cause death. Therefore, the concept of sensitivity should not be confused with the concept of organism-level tolerance, which is conventionally assessed by lethal endpoints [35].

In view of the wide variety of pesticides and their diverse effects on organisms [36], a battery of biomarkers measuring complementary parameters is essential for accurately assessing environmental impact [25]. The execution of controlled laboratory toxicity tests has been demonstrated to facilitate the elucidation of the fundamental toxic mechanisms of contaminants.

This has given rise to the interest in conducting a comparative analysis of the biological responses exhibited by *P. argentinus* and *M. borellii* to a range of pesticides. The objective of this study is to identify sensitive parameters that can be utilised as biomarkers in freshwater environments where these species are present. The present study therefore aims to analyze and discuss the findings of previous research focusing on the impact of pesticides on these two prawn species and to compare their effectiveness as indicator organisms. Throughout this review, regulatory guideline values are included as reference benchmarks to provide context for reported environmental concentrations and experimental exposure levels, especially with regard to the protection of aquatic biota.

2. Materials and Methods

The bibliographic search was conducted using the ScienceDirect and Google Scholar databases, employing English keywords and combinations such as ("*Macrobrachium borellii*" OR "*Palaemon argentinus*") AND ("toxic effects" OR "biomarkers" OR "end point" OR "lethal concentration" OR "LC50") AND ("insecticides" OR "herbicides" OR "pesticides").

Articles published up to December 2025 were selected based on the following inclusion criteria: (a) original studies; (b) the presence of the target species' name in the title or abstract; and (c) toxicity assessments performed under controlled laboratory conditions, in situ assays, or using organisms collected from sites with different land uses. Any articles that did not meet these specific criteria were excluded from the review.

Characteristics of Organisms Selected

The typical adult size of *M. borellii* ranges from 45 to 62 mm from the tip of the rostrum to the telson [37] although it can exceptionally reach up to 10 cm (personal observation). The biology of this species is widely known, having been addressed in numerous studies.

It exhibits omnivorous–carnivorous feeding habits, preying on various benthic species [37]. The reproductive period begins in spring and ends in mid-summer (November to February). Reproductive females produce relatively large eggs (1.5–2.0 mm) with abundant yolk, which are carried in a brood chamber between the pleopods [38]. Clutches are small, ranging from 30 to 100 eggs, and take approximately 40–50 days to hatch directly as postlarvae [10]. In Argentina, *M. borellii* has been reported in turbid, temperate watercourses, including rivers, lakes, and reservoirs within alluvial plains [9,39–41]. It has been observed that *M. borellii* possesses a tolerance to temperature increases primarily driven by anthropogenic factors [42]. Furthermore, the species has shown sensitivity to variations in water quality caused by different land-use practices [8,43] and climatic conditions associated with the "La Niña" phenomenon [44].

The average body size of *P. argentinus* populations typically ranges between 26 and 38 mm, though this fluctuates seasonally [45]. This species is classified as an opportunistic omnivore, with a diet that shifts according to seasonal environmental availability. Consequently, it serves as a critical trophic link between primary producers and higher-level consumers, such as fish and birds [46]. The reproductive period for *P. argentinus* begins in the spring and concludes in mid-summer, spanning from October to February [47]. Notably, *P. argentinus* exhibits a higher reproductive output compared to *M. borellii*, with observed clutch sizes ranging between 136 and 174 embryos [48]. *P. argentinus* has proven to be a highly sensitive and suitable model organism for ecotoxicological research [13,14,49,50].

3. Results and Discussion

A total of 128 studies were identified in the initial search, of which 15 were ultimately selected. The remaining studies were excluded as they did not meet the specific selection criteria detailed in the previous section. Notably, all the selected studies were conducted in Argentina, specifically in the Pampas region. Despite their distribution in a few South American countries (Figure 1), studies involving these species were identified only in Argentina, highlighting an important geographic gap in the available literature.

Effects of Pesticides on Freshwater Prawn

The data set clearly shows differences in pesticide testing patterns between *M. borellii* and *P. argentinus* (Figure 2). Studies on *M. borellii* focused primarily on a limited number of insecticides, particularly chlorpyrifos (CPF), cypermethrin (CYP) and spirotetramat (STM), accounting for most experimental exposures (Figure 2A). By contrast, *P. argentinus* has been tested with a broader spectrum of compounds, including insecticides and herbicides such as atrazine (ATZ), glyphosate (GLY) and metolachlor (MET). Notably, no studies were found evaluating the effects of fungicides on these crustacean species. In terms of the type of compound tested, experiments with *M. borellii* predominantly used commercial formulations (approximately 90%), whereas studies with *P. argentinus* showed a higher proportion of assays with pure active ingredients (approximately 33%) (Figure 2B). Exposure concentrations also differed between the two species. For *M. borellii*, most studies employed concentrations in the ng/L range (50%), followed by µg/L (30%), while fewer experiments used pg/L (10%) or mg/L levels (10%) (Figure 2C). In contrast, studies with *P. argentinus* more frequently applied lower exposure levels, with 33% in the µg/L range, 25% in the ng/L range, and 42% in the pg/L range, while no studies reported exposures in the mg/L range. Overall, the compiled data indicate that experimental exposures for both species span a wide concentration gradient, although most assays were conducted within environmentally relevant ng–µg/L levels. Overall, these results highlight taxonomic and methodological biases in the available ecotoxicological literature on freshwater decapods.

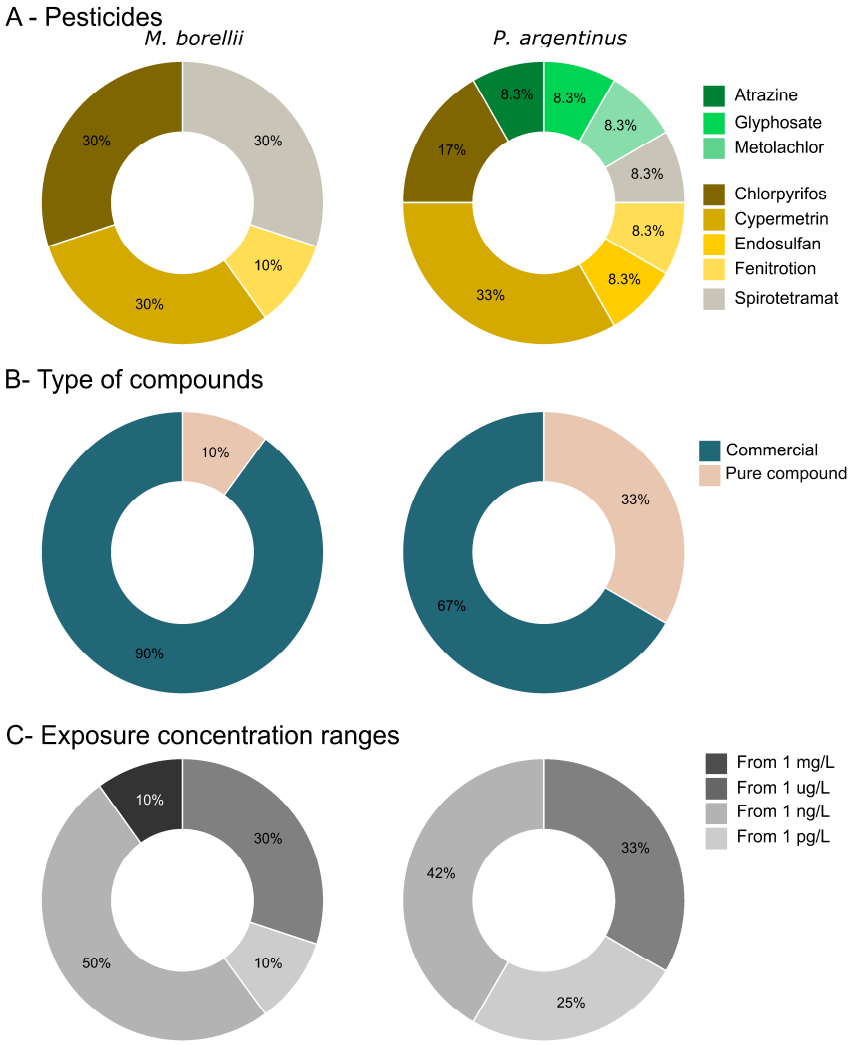


Figure 2. Pesticides, compound types (commercial and pure compounds), and exposure concentration ranges used in ecotoxicological studies with the freshwater crustaceans *Macrobrachium borellii* and *Palaemon argentinus*. (**A**) The proportion of pesticide active ingredients tested in experimental studies for each species. (**B**) The relative contribution of commercial formulations versus pure (parental) compounds used in toxicity assays. (**C**) Distribution of exposure concentration ranges categorized by order of magnitude (from 1 pg/L, from 1 ng/L, from 1 µg/L, and from 1 mg/L). Percentages represent the proportion of bioassays within each category for each species.

The available literature on *P. argentinus* and *M. borellii* includes studies evaluating pesticides from several chemical groups.

3.1. Chlorpyrifos

Chlorpyrifos (O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate; CPF), a widely used organophosphorus insecticide, has been employed in agriculture and urban pest control [51]. Its mechanism involves inhibiting cholinesterase (ChE) enzymes, leading to neurotransmitter accumulation, disrupted neurotransmission, and ultimately, organism death [52]. Due to its neurotoxic effects, CPF has been banned in several regions. In Argentina, CPF concentrations in surface waters have raised environmental concerns over the past decade, with levels significantly exceeding aquatic life protection guidelines, such as those set by the Canadian Council of Ministers of the Environment (0.002 µg/L) [53] and the Argentinean Environmental Water Quality Guidelines (0.006 µg/L) [54]. For example, peaks of 0.8 µg/L [55] and 10.8 µg/L [56] in the Pampean region far exceed safety thresholds. Even lower levels, such as those reported in the Suquía River basin (0.0006 µg/L) [57]

can induce sublethal effects [58]. CPF has contributed significantly to ecological risk in Pampean aquatic ecosystems, with macroinvertebrates showing high sensitivity to both acute and chronic toxicity [12,50,58,59]. Despite its historical use, the impact of CPF on non-target organisms [60] has led to severe restrictions, including a ban in California [61] and a prohibition on commercialization and use in Argentina from 2021 onwards [62].

In this context, research has utilized CPF concentrations of up to 3198.3 μg/L for *M. borellii* embryos and 10 μg/L for adults [63,64], whereas studies on *P. argentinus* have reached 0.022 μg/L for juveniles and 0.0945 μg/L for adults. These experimental levels, alongside detected environmental peaks, exceed the environmental quality guidelines in several cases, which are presented here as reference values to contextualize exposure concentrations and their relevance for aquatic biota protection. Regarding the sensitivity of *M. borellii* to CPF, Jergentz et al. [43] conducted in situ bioassays and observed 100% mortality in a prawn population in a Pampean stream during a period of high organophosphate contamination (64 μg/kg in suspended particles). Comparative evaluations of the sensitivity of *M. borellii* adults and embryos to CPF revealed that adults were several orders of magnitude more sensitive than embryos (96 h LC_{50} values of 1.8 μg/L and 31,983 μg/L CPF, respectively (Table 1) [64]. The authors proposed that embryonic envelopes play a protective role during development; however, this protection diminishes in embryos exposed to CPF at the time of hatching [64], a phenomenon also observed with hydrocarbons (HC) [65]. Furthermore, Ríos et al. [63] investigated the impact of CPF on ChE activity in adult *M. borellii* consuming diets with different lipid content. They observed a significant decrease in the activity of both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in organisms that were exposed, compared to those in the control group that were fed a very low-lipid diet. In contrast, the CPF did not affect these enzymes in prawn fed lipid-rich diets [63]. This tolerance in the latter treatments could be attributed to the hydrophobicity of CPF (log *Kow* 4.7), favoring its accumulation in neutral lipid deposits and thereby reducing its bioavailability to other susceptible tissues and organs [66] (Table 2).

Table 1. Summary of lethal effects reported for *Macrobrachium borellii* and *Palaemon argentinus* exposed to individual pesticide compounds under laboratory conditions.

Action Mode	Chemical Family	Parental Compound	Type of Compound Tested	Species	Life Stage	Lethal Concentration (LC) (µg/L)		References
Insecticide	Organophosphorus	Fenitrothion	Parental compound	*M. borellii*	Adult	96 h LC_{50}	4.24	[15]
				P. argentinus	Adult	96 h LC_{50}	1.12	[14]
		Chlorpyrifos	Commercial compound	*M. borellii*	Adult	96 h LC_{50}	1.8	[64]
				M. borellii	Embryo	96 h LC_{50}	31,983	[64]
				P. argentinus	Juvenile	96 h LC_{50}	0.49	[67]
	Organochlorine	Endosulfan	Commercial compound	*P. argentinus*	Juvenile	96 h LC_{50}	6.28	[67]
	Pyrethroid	Cypermethrin	Commercial compound	*M. borellii*	Adult	96 h LC_{50}	0.12	[16]
				P. argentinus	Adult	96 h LC_{50}	0.005	[13]
					Juvenile	96 h LC_{50}	0.002	[68]
	Tetramic acids	Spirotetramat	Commercial compound	*M. borellii*	Adult	96 h LC_{50}	8200	[16]
					Embryo	96 h LC_{50}	>150,000	[69]
					Larva	96 h LC_{50}	11	[69]
				P. argentinus	Adult	96 h LC_{50}	4430	[13]
Herbicide	Triazine	Atrazine	Parental compound	*P. argentinus*	Adult	96 h LC_{50}	8900	[70]
	Glycine derivative	Glyphosate	Commercial compound	*P. argentinus*	Juvenile	96 h LC_{50}	51,050 *	[71]

* The mL of commercial Roundup/L was converted to µg glyphosate/L; Composition: Glyphosate 36% (POTASSIUM SALT) [SL] *w*/*v* Authorization number: ES-00725 ROUNDUP ULTRA PLUS 5 L.

Table 2. Summary of sublethal effects reported for *Macrobrachium borellii* exposed to individual pesticide compounds under laboratory conditions.

Action Mode	Chemical Family	Active Ingredient	Compound	Life Stage	Tested Concentrations (µg/L)	Accumulation	Biochemical Biomarkers	Morphological Biomarkers	Behavioural Biomarkers	References
Insecticide	Organophosphorus	FS	P	Adult	0.2–1.4	Not evaluated	↓ ChE activity (Hm) ↑ LPO, SOD, CAT and GST (Hp)	Not evaluated	Not evaluated	[15]
		CPF	C	Adult	10	Not evaluated	↓ AChE, BChE and CbE, lipid level mitigates the inhibitory effect	Not evaluated	Not evaluated	[63]
				Adult	0.03–0.12	Not evaluated	Not evaluated	Not evaluated	Not evaluated	[64]
				Embryo	0.03–3198.3	Not evaluated	Not evaluated	↓ Effective hatching	Not evaluated	[64]
	Pyrethroid	CYP	C	Adult	0.006–0.02	Not evaluated	↑ LPO and PC levels (Hp)	↑ LF, NL, HL and ↑ IBR index (Hp)	Not evaluated	[16]
				Adult	0.006	Not evaluated	↓ Glyc (Ms)	Not evaluated	↑ Pleopod beat rate ↓ Locomotor activity and ↓ Food consumption	[72]
				Juvenile	0.0001–0.01	Not evaluated	Not evaluated	↑ HL (Hp)	Not evaluated	[8]
	Tetramic acids	STM	C	Adult	500–1700	Not evaluated	↑ GST, LPO and PC (Hp)	↑ LF, NL, HL and IBR index (Hp)	Not evaluated	[16]
				Embryo	1700	Not evaluated	↑ LPO levels	Not evaluated	Not evaluated	[69]
				Larva	5–15	Not evaluated	Not evaluated	↑ HL (Hp, Gi)	Not evaluated	[69]

Abbreviations: **AChE**, acetylcholinesterase; **BChE**, butyrylcholinesterase; **C**, commercial compound; **CAT**, catalase; **CbE**, carboxylesterase; **ChE**, cholinesterase; **CPF**, chlorpyrifos; **CYP**, cypermethrin; **FS**, Fenitrothion; **Gi**, gills; **Glyc**, glycogen; **GST**, glutathione S-transferase; **HL**, histopathological lesions; **Hm**, hemolymph; **Hp**, hepatopancreas; **IBR index**, integrated biomarker response index; **LF**, Lipofuscins; **LPO**, lipid peroxidation; **Ms**, muscle; **NL**, neutral lipids; **P**, parental compound; **PC**, protein carbonyl content; **SOD**, superoxide dismutase; **STM**, spirotetramat; ↑, increase in the biomarker response; ↓, decrease in the biomarker response.

The first study about the toxicological effects of CPF on *P. argentinus* was reported by Montagna and Collins [67]. The authors reported an LC_{50} of 0.49 µg/L after 96 h of exposure. Organisms exposed to CPF exhibited excitation and jerky movements, particularly in response to gentle stimulation. These findings highlight the immediate impact of CPF on the nervous system of *P. argentinus*, indicating that even short-term exposure can result in substantial physiological stress. In addition, Bertrand et al. [50] investigated the toxic effects of CPF on *P. argentinus* by exposing the organisms to environmentally relevant concentrations for 96 h. The results revealed that CPF exposure significantly inhibited microsomal AChE activity, with noticeable effects occurring at concentrations as low as 3.5 ng/L of CPF—below the Argentinean guidelines suggested for protecting aquatic biota. This finding corroborates previous reports and provides further support for the idea that *P. argentinus* is highly sensitive to CPF, even at concentrations that are considered environmentally safe. Furthermore, exposure for 96 h evidenced oxidative stress, as indicated by increased levels of H_2O_2, LPO, and PC (Table 3). Although antioxidant enzymes such as CAT and GST were induced, their activity was insufficient to prevent oxidative harm. Interestingly, the study documented the mobilization of alpha-tocopherol from the abdomen to the cephalothorax, as well as a significant decrease in MTs at lower CPF concentrations, suggesting a protective response. In most of the biomarkers analyzed, the cephalothorax exhibited greater sensitivity compared to the abdomen sector. The findings emphasized the sensitivity of *P. argentinus* to CPF, with Integrated Biomarker Response (IBR) values significantly correlating with exposure concentrations. Although these biomarkers indicate early responses at an individual level, sustained physiological disturbances may ultimately impact the performance of organisms, which could have consequences for population dynamics and ecosystem functioning.

3.2. Fenitrothion

The effects of organophosphates on crustaceans were first reported for fenitrothion (O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate; FS) [73], which has been widely used in agriculture and mosquito control in our region [74]. Although the use of FS has been banned in several countries, including Argentina in 2013 [75], it has recently been detected in Pampas freshwater ecosystems due to non-standard practices that pose environmental and health risks [59,76]. The 96 h LC_{50} for FS was 4.24 µg/L in adult *M. borellii* [15], while *P. argentinus* exhibited greater sensitivity with a 96 h LC_{50} of 1.12 µg/L [14] (Table 1). Both values exceeded the current Argentine water quality guideline for aquatic life protection (0.02 µg/L) [77], which is presented here as a reference for comparing species sensitivity and environmental exposure scenarios. Cholinesterase activity in the hemolymph proved to be an effective exposure biomarker. Significant inhibition was observed in *M. borellii* after seven days and in *P. argentinus* after four days of exposure to sublethal concentrations. A concentration-dependent response was observed in both species [14,15] (Tables 2 and 3). As observed with exposure to CPF, the behavior of this biomarker in both species reaffirms their status as a robust biomarker of exposure to organophosphate pesticides [78] (Figure 3A,B).

Table 3. Summary of sublethal effects reported for *Palaemon argentinus* exposed to individual pesticide compounds under laboratory conditions.

Action Mode	Chemical Family	Active Ingredient	Compound	Life Stage	Tested Concentrations (µg/L)	Accumulation	Biochemical Biomarkers	Morphological Biomarkers	Behavioral Biomarkers	References
Insecticide	Organophosphorus	FS	P	Adult	0.3–0.9	Not evaluated	↓ ChE (Hm) ↑ SOD, CAT, and GST (Hp)	Not evaluated	Not evaluated	[14]
		CPF	P	Adult	0.0035–0.0945	<LOD	↑ H_2O, ↓ AChE, MTs (Ceph) ↑ H_2O_2, TBARs, PC, and MTs (Abd) alpha–tocopherol (Abd → Ceph)	Not evaluated	Not evaluated	[50]
			C	Juvenile	0.005–0.022	Not evaluated	Not evaluated	↓ Intermolt period	Not evaluated	[67]
	Organo chlorine	END	C	Juvenile	0.122–0.488	Not evaluated	Not evaluated	↓ Intermolt period	Not evaluated	[67]
	Pyrethroid	CYP	C	Adult	0.0006	Not evaluated	↓ Glyc (Ms)	Not evaluated	↑ Pleopod beat rate ↓ Locomotor activity and food consumption	[72]
				Adult	0.001	Not evaluated	↑ LDH (Ms) and TAG (Hp)	Not evaluated	Not evaluated	[13]
				Juvenile	0.0002–0.0250	Not evaluated	↑ Oxygen uptake and ammonia-N excretion	↓ Size, pre- and postmolt length	Hyperactivity (swimming and walking behavior)	[68]
				Juvenile	0.0001–0.01	Not evaluated	Not evaluated	↑ frequency of F-cell and ↓ B-cell % (Hp)	Not evaluated	[8]
	Tetramic acids	STM	C	Adult	400	Not evaluated	↑ Pt, PC, CAT and GST (Hp) ↓ Lac (Ms)	Not evaluated	Not evaluated	[13]

Table 3. *Cont.*

Action Mode	Chemical Family	Active Ingredient	Compound	Life Stage	Tested Concentrations (µg/L)	Accumulation	Biochemical Biomarkers	Morphological Biomarkers	Behavioral Biomarkers	References
Herbicide	Chloroacetamide	MET	P	Adult	7.5	<LOD–2.75 ng/g ww (Ceph)	↓ CAT (Ceph and Abd) IBR index ↑	HL ↑ (Hp)	Not evaluated	[79]
	Triazine	ATZ	P	Adult	400	Not evaluated	↑ H_2O_2, SOD, GST, and GR ↓ Lipids content	Not evaluated	Not evaluated	[70]
	Glycine derivative	GLY	C	Juvenile	25,200–404,640 *	Not evaluated	↑ Oxygen consumption ↓ Ammonia-N excretion	High mortality and just two or three molts completed	Not evaluated	[71]

* The mL of commercial Roundup/L was converted to µg glyphosate/L; Composition: Glyphosate 36% (POTASSIUM SALT) [SL] *w*/*v* Authorization number: ES-00725 ROUNDUP ULTRA PLUS 5 L. Abbreviations: **AChE**, acetylcholinesterase; **Abd**, abdomen; **Ammonia-N,** ammonia nitrogen; **ATZ**, atrazine; **B-cell %**, B-cell percentage; **C**, commercial compound; **CAT**, catalase; **Ceph**, cephalothorax; **ChE**, cholinesterase; **CPF**, chlorpyrifos; **CYP**, cypermethrin; **FS**, Fenitrothion; **Glyc**, glycogen; **GLY**, Glyphosate; **GR**, glutathione reductase; **GST**, glutathione S-transferase; **H_2O_2**, hydrogen peroxide; **IBR index**, integrated biomarker response index; **Lac**, lactate; **LDH**, lactate dehydrogenase; **LOD**, Limit of detection; **LPO**, lipid peroxidation; **Ms**, Muscle; **MTs**, metallothionein content; **P**, parental compound; **PC**, protein carbonyl content; **Pt**, total protein content; **SOD**, superoxide dismutase; **STM**, spirotetramat; **TAG**, triacylglycerols; **TBARs**, thiobarbituric acid reactive substances; ↑, increase in the biomarker response; ↓, decrease in the biomarker response; →, mobilization.

The effects of FS on biomarkers were assessed in the hepatopancreas of adult prawns, which is crucial for food absorption, enzyme synthesis, lipids and glycogen storage, and biotransformation [80]. In *M. borellii*, LPO levels exhibited an acute response, increasing significantly after one day of exposure at all concentrations tested [15]. At two and four days, only the intermediate FS concentration (0.8 µg/L) resulted in a significant increase in LPO levels [15]. This indicates that high pesticide concentrations may mask oxidative damage due to the degradation and excretion of hydroperoxides [81]. By contrast, *P. argentinus* showed no significant differences in levels of oxidative damage between the control and FS treatments, which suggests that these biomarkers are insensitive to high pesticide concentrations, probably due to hydroperoxide instability [14]. Although LPO quantification in the hepatopancreas indicated low sensitivity, oxidative DNA damage in the hemocytes of *M. borellii* displayed concentration-dependent behavior after seven days of FS exposure [15].

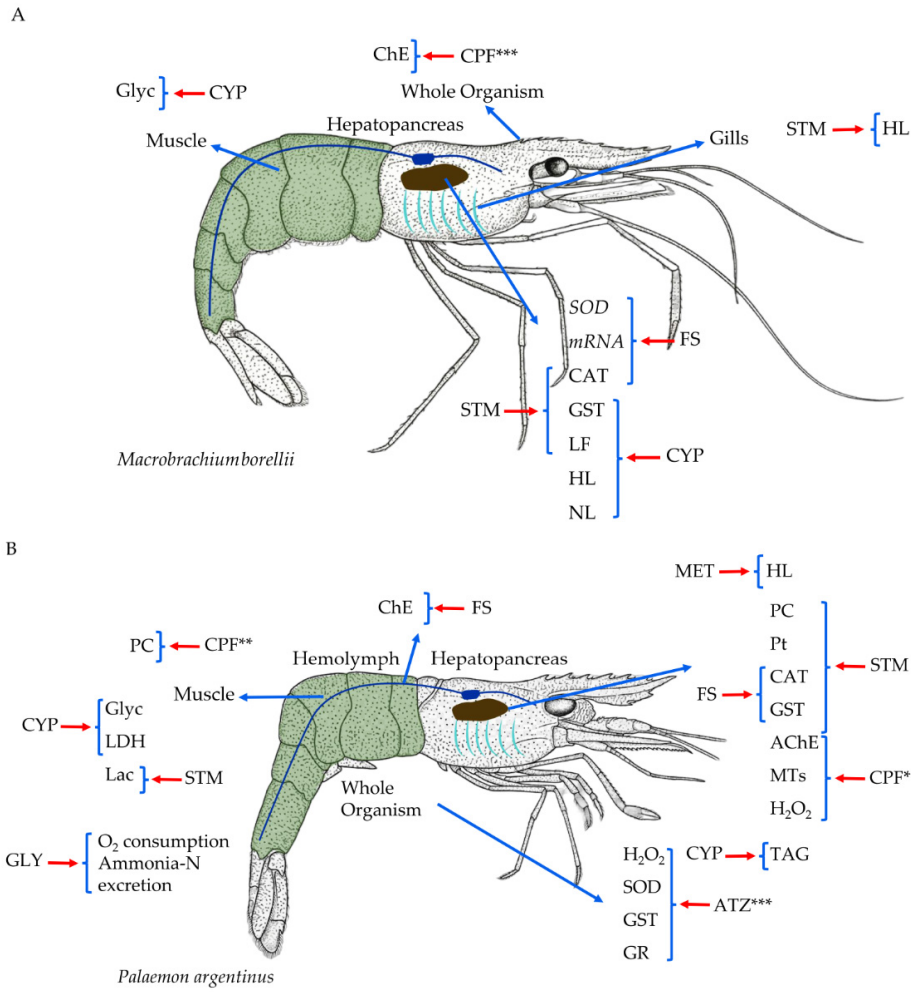


Figure 3. Biochemical and morphological biomarkers that responded robustly in *Macrobrachium borellii* (**A**) and *Palaemon argentinus* (**B**) to pesticide exposure. **AChE**, acetylcholinesterase Ammonia-N, ammonia nitrogen; **CAT**, catalase; **ChE**, cholinesterases; **CPF**, chlorpyrifos; **CYP**, cypermethrin; **FS**, Fenitrothion; **Glyc**, glycogen; **GLY**, Glyphosate; **GR**, glutathione reductase; **GST**, glutathione S-transferase; **H_2O_2**, hydrogen peroxide; **HL**, histopathological lesion; **Lac**, lactate; **LDH**, lactate dehydrogenase; **LF**, lipofuscins; **MTs**, metallothioneins; **NL**, neutral lipids; **PC**, protein carbonyl content; **Pt**, total protein content; **SOD**, superoxide dismutase; **SOD *m*RNA**, superoxide dismutase messenger RNA expression; **STM**, spirotetramat; **TAG**, triacylglycerols. * Biomarkers analyzed in the cephalothorax. ** Biomarkers analyzed in the abdomen. *** Biomarkers analyzed in the whole organism.

Exposure to FS significantly increased enzymatic activity in the hepatopancreas of both *M. borellii* and *P. argentinus* (Tables 2 and 3). The SOD-CAT system showed a marked response in *M. borellii*, with CAT activity depending on concentration and time. In contrast, SOD exhibited a limited response, indicating high sensitivity to oxidative stress. This was supported by the dose- and time-dependent induction of the SOD gene (*sod*) [15], which suggests that enzyme activity may mask actual stress levels. A similar pattern was observed in *P. argentinus*, where SOD activity increased only at low FS concentrations before returning to control levels at higher concentrations. Meanwhile, CAT activity displayed clear concentration dependence [14]. These results suggest that SOD may be less sensitive than CAT in response to stress demonstrating the robustness of the latter biomarker (Figure 3B). The ROS generated during Phase I reactions associated with detoxification pathways have been observed to induce antioxidant proteins expression. However, elevated ROS levels have also been demonstrated to cause oxidative damage to biomolecules [82]. This could provide a rationale for the biphasic behavior observed in both species, which may be indicative of the hormesis phenomenon, as evidenced by various biochemical biomarkers [35].

3.3. Endosulfan

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine 3-oxide; END) was an organochlorine insecticide and acaricide that was extensively used in Argentina. Due to concerns regarding its environmental impact, guideline values for the protection of aquatic life established surface water concentrations below 0.008 µg/L [54]. However, its high persistence, bioaccumulation potential, and toxicity to aquatic organisms ultimately led to its prohibition in Argentina in 2013 [83]. Although the environmental risk from END is expected to diminish over time, a recent study revealed its presence in freshwater environments in Argentina's Pampas region, with concentrations reaching 0.33 µg/L in surface water [84]. The only study on the effects of END on the selected crustaceans was conducted by Montagna and Collins [67]. The reported 96 h LC_{50} for juvenile *P. argentinus* was 6.28 µg/L (Table 1). In contrast, *M. malcolmsonii* juveniles and *M. rosenbergii* postlarvae were significantly more sensitive, with LC_{50} values of 0.16 µg/L [85] and 0.2 µg/L [86], respectively. Regarding sublethal effects, *P. argentinus* juveniles exposed to 0.122–0.488 µg/L for 90 days demonstrated no changes in growth rate [73]. However, their intermolt period was adversely affected, contrasting with the effects observed in prawns exposed to CYP [68] (Table 3).

3.4. Cypermethrin

Subsequent studies examined prawn sensitivity to relevant regional pesticides, particularly cypermethrin (α-cyano-3-phenoxybenzyl ester of 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropanecarboxylic acid; CYP), a pyrethroid insecticide [13,16,68]. This insecticide targets voltage-dependent sodium channels, leading to nerve depolarization and blockade in the peripheral nervous system [87]. The introduction of CYP in the 1980s was presented as a safer alternative to organophosphates, with the active compound being described as effective and less toxic to mammals [88]. However, recent findings indicate that aquatic organisms are highly sensitive to pyrethroids, which disrupt feeding behaviors and physiological functions related to development, growth, and reproduction [89]. Cypermethrin is frequently detected in environmental matrices worldwide, including in Argentina. Research has revealed that concentrations of up to 194 µg/L have been identified in surface water, while levels of up to 1075 µg/kg have been detected in sediments [56,84]. The reported concentrations in surface waters exceed both the national water quality guideline of 0.0006 µg/L [54] and the international threshold of 0.000053 µg/L. These thresholds have been established with the purpose of protecting aquatic biota [90].

Both *M. borellii* and *P. argentinus* exhibited high sensitivity to CYP compared to other decapods such as *M. pantanalense* [91], *M. lar* [92], and *P. pugio* [93]. In adult prawns, 96 h LC_{50} values were 0.12 µg/L for *M. borellii* and 0.005 µg/L for *P. argentinus* [13,16]. Collins and Cappello [68] reported a 96 h LC_{50} of 0.002 µg/L for juvenile *P. argentinus*, indicating heightened sensitivity in immature stages. These toxicity thresholds are substantially lower than some concentrations reported in regional surface waters. This comparison highlights the potential vulnerability of freshwater prawns and provides context for interpreting environmental exposure scenarios. (Table 1).

The effects of sublethal CYP concentrations were examined in *M. borellii* and *P. argentinus* (Tables 2 and 3)**.** Adult prawns exposed to sublethal concentrations (0.006 and 0.02 µg/L for *M. borellii* and 0.0005 µg/L for *P. argentinus*) for four days exhibited significant biochemical changes in the hepatopancreas [13,16]. In *M. borellii*, only the lowest CYP concentration caused a significant increase in LPO, as measured by TBARs accumulation [16], demonstrating a non-monotonic response. This pattern contrasts with the concentration-dependent TBARs increase reported in other crustaceans [94]. Conversely, lipofuscin levels in the hepatopancreas increased significantly at the highest CYP concentration [16]. This discrepancy may be due to the rapid degradation and excretion of hydrophilic TBARs under high oxidative stress [81], whereas lipofuscin pigments accumulate in neutral lipids and are a more stable indicator of oxidative damage [95]. As no studies have reported oxidative lipid damage from CYP in *P. argentinus*, further investigation of these biomarkers under varying conditions in this species is warranted for comparison with *M. borellii*.

Other oxidative damage biomarkers, such as PC, are sensitive indicators in crustaceans exposed to CYP [94]. In *M. borellii*, PC levels exhibited biphasic behavior, decreasing to levels comparable to the control group at the highest pyrethroid concentration [16]. However, PC levels in the hepatopancreas of *P. argentinus* demonstrated no sensitivity to the tested concentrations [13]. Contrary to expectations, the total protein content of both species did not differ significantly from the controls [13,16], despite the high amino acid demand for de novo enzymes synthesis involved in pesticide metabolism [27]. Furthermore, uric acid levels, which function as an antioxidant and represent the final product of nitrogenous compound catabolism, demonstrated no alterations [82]. GST activity also remained unchanged in the hepatopancreas of both species exposed to CYP [13,16], suggesting that GST may not be a sensitive biomarker for pyrethroid exposure. This is further supported by similar findings reported in other decapod species [96,97].

The analysis of neutral lipids is a valuable parameter for studying organisms exposed to xenobiotics, as many compounds promote their accumulation [31]. CYP exposure significantly increased neutral lipids in the hepatopancreas of *M. borellii* and triacylglycerols (TAG) in *P. argentinus*, indicating the sensitivity of these biomarkers [13,16] (Figure 3A,B). These changes may result from oxidative metabolism of CYP, which induces Phase I reactions that generate ROS [98]. The oxidative stress experienced in the endoplasmic reticulum can be mitigated by storing fatty acids, particularly polyunsaturated fatty acids that are sensitive to lipoperoxidation, in TAG and cholesterol esters [99]. This accumulation has the potential to enhance tolerance to lipophilic xenobiotics, such as CYP (log *Kow* 6.3), by reducing their bioavailability and protecting other organs or tissues [32].

It is noteworthy that both studies were conducted during the post-reproductive period [13,16], thus prompting a follow-up investigation on the effects of CYP on energy requirements and behavior in females of both species during the reproductive period, which is particularly vulnerable to stress [72]. The bioassay conditions were comparable, with vitellogenic and ovigerous females being exposed to sublethal concentrations of CYP (0.006 µg/L for *M. borellii* and 0.0006 µg/L for *P. argentinus*) for a period of four days. The pyrethroid exposure had a significant impact on the behavior of the female, as evidenced

by a marked increase in pleopod beating frequency, along with a substantial decrease in locomotor activity and food consumption when compared to the control group [99]. In contrast, juvenile *P. argentinus* exposed to CYP for mere of 10 min at concentrations ranging from 0.0002 to 0.025 µg/L exhibited signs of hyperactivity [68], suggesting the presence of neurotoxic effects associated with pyrethroid [87]. It has been demonstrated that behavioral parameters function as effective biomarkers of neurotoxic pesticides [100]. The augmented pleopod agitation may serve to enhance gas exchange by gills, thereby meeting the elevated oxygen demand that is associated with the process of detoxification [101]. Collins and Cappello [68] observed biphasic oxygen consumption in juvenile *P. argentinus* exposed to CYP for a period of three hours, indicative of increased metabolism. Furthermore, Mulreedy et al. [72] reported a significant decrease in glycogen content in the abdominal muscles of both vitellogenic and ovigerous females exposed to CYP, likely linked to the heightened energy demand during reproduction. In contrast, *P. argentinus* exposed to comparable CYP concentrations during the non-reproductive period exhibited no glycogen alterations in their abdominal muscles [13], thereby demonstrating that the reproductive stage is energetically vulnerable to stress [72].

Carbohydrate catabolism analysis revealed that lactate content in the abdominal muscle was unaffected by CYP exposure in females of both species during the reproductive stage [72] and in *P. argentinus* during the post-reproductive stage [13]. Although muscle lactate is not a suitable biomarker, the enzyme lactate dehydrogenase (LDH), which converts pyruvate to lactate, demonstrated sensitivity to CYP exposure [13] (Figure 3B). The observed discrepancy between lactate levels and LDH activity may be attributed to the rapid conversion of lactate back to pyruvate, thereby preventing intracellular acidification [102]. This could potentially obscure an escalation in anaerobic catabolism, which may furnish prompt energy for detoxification and oxidative damage mitigation in prawns [96].

The total protein content of the hepatopancreas in both species exhibited no significant differences between CYP treatments and controls [13,16]. The increased demand for amino acids in relation to de novo enzyme synthesis associated with pesticide metabolism [27] is likely to compensate for a decrease in non-essential proteins. In accordance with this observation, the levels of uric, the end product of nitrogen catabolism in crustaceans, remained constant, suggesting its function as both an excretory product and an antioxidant [82]. Conversely, juvenile *P. argentinus* demonstrated concentration-dependent ammonia-N excretion [68], underscoring their heightened sensitivity and elevated demand for essential components during the growth phase. The authors observed a decline in growth rates and an alteration in ecdysis cycles, resulting in diminished survival rates in *P. argentinus* juveniles exposed to CYP concentrations ranging from 0.0001 to 0.01 µg/L [68]. Consequently, an investigation into the effects of CYP on immature *M. borellii* stages would provide a valuable basis for comparing the impacts of this insecticide on both species in the Pampas region.

Finally, CYP exposure significantly affected morphological parameters, with histological alterations in the hepatopancreas of adult *M. borellii* showing a concentration-dependent pattern [16]. Notable anomalies included an increase in R cells and cytoplasmic vacuolization. R cells are involved in the process of detoxification and have been observed to accumulate contaminants until cell death [103]. Consequently, vacuolization may be indicative of sequestration and storage of the insecticide in this pivotal detoxification organ [8]. Furthermore, the accumulation of neutral lipids is likely to contribute to this mechanism by sequestering the highly lipophilic pyrethroid. In summary, the IBR index demonstrated that histological parameters were the most sensitive biomarkers in *M. borellii* exposed to the pyrethroid (Figure 3A).

3.5. Spirotetramat

Lavarías et al. [16,69] and Etcheverry et al. [13] investigated the effects of the next-generation pesticide spirotetramat (cis-4-(ethoxycarbonyloxy)-8-methoxy-3-(2,5-xylyl)-1-azaspiro [4.5]dec-3-en-2-one; STM) on *M. borellii* and *P. argentinus* respectively. This class of pesticides has emerged as an alternative to traditional chemical agents. The indiscriminate use of these agents has resulted in target pests developing resistance mechanisms. Thus, STM helps to circumvent cross-resistance with well-known active ingredients [104]. Spirotetramat acts by disrupting lipogenesis through the inhibition of acetyl-coenzyme A carboxylase (ACCase), which impairs the growth of immature stages and hinders reproduction by inhibiting vitellogenesis [105]. Although STM has only recently been used in Argentina, it has not yet been detected in the environment. However, in other countries, this pesticide has been detected in surface waters at concentrations of 0.5 µg/L [106]. STM is considered a low-toxicity insecticide for aquatic organisms [107], which may explain why environmental concentration thresholds for the protection of aquatic life have not yet been established. Though, the progressive increase in its application could pose a risk to such ecosystems in the short term, which must be prevented.

The first reports on the effects of STM on decapod crustaceans came from Lavarías et al. [16,69] (Table 1). They initially determined lethality as an endpoint at various stages of *M. borellii* development. Their results showed that larvae were considerably more sensitive than adults, highlighting the greater vulnerability of immature stages to this class of insecticide. However, when comparing the sensitivity of adult prawns to STM, *P. argentinus* exhibited a 96 h LC_{50} value of 4.4 mg/L, approximately half that recorded for *M. borellii* which was 8.2 mg/L. This pattern differed from that observed for the neurotoxic insecticides CYP and CPF, where the difference in sensitivity between the two species reached nearly two orders of magnitude. The LC_{50} could not be experimentally determined for *M. borellii* embryos and was estimated to exceed 150 mg/L of STM [69]. This high tolerance is likely to be related to the protective role of the embryonic envelopes, which act as a selective barrier, limiting the penetration of the insecticide into the embryo [65]. This finding is consistent with the stability of the enzymatic activities of the SOD-CAT and GST systems, and with the stability of total protein and PC levels. These remained unaffected in embryos exposed to a sublethal concentration of 1.7 mg/L of STM for four days [69]. However, in contrast to these findings, TBARs levels increased significantly relative to the control, indicating that the oxidative metabolism of STM in embryos induces lipid peroxidation [108]. This is a noteworthy observation given that stage 5 embryos undergo active lipogenesis [109] and have high levels of polyunsaturated fatty acids in their TAGs and phospholipids [110]. Consequently, a more exhaustive study of lipid metabolism would be instrumental in understanding the toxic mechanism of STM in *M. borellii*. In adult prawns exposed to sublethal concentrations of STM (0.5 and 1.7 mg/L), certain biochemical parameters were found to be more sensitive than in embryos [16]. In *M. borellii*, STM significantly altered GST activity in the hepatopancreas only at the highest concentration that was tested. Etcheverry et al. [13] also reported a significant increase in GST activity in the hepatopancreas of *P. argentinus* exposed to a sublethal concentration of STM (0.44 mg/L), compared to the control group. This increase indicates that this phase II detoxification enzyme may be implicated in the biotransformation and detoxification of STM in prawns. This finding is consistent with the paucity of extant studies on this mechanism [108]. In contrast, the levels of LPO and PC levels in *M. borellii* were found to be influenced by the lowest tetramic acid concentrations, exhibiting a response pattern analogous to that induced by pyrethroids [16]. The histochemical evaluation of LPO proved to be more sensitive than the TBARs assay, with a significant increase observed in comparison to the control group at both tested STM concentrations. In a similar manner, neutral lipid accumulation

increased with STM treatment, demonstrating that the effect of lipidosis prevailed over the lipogenesis-inhibiting effect of the insecticide [111]. However, *P. argentinus* did not exhibit this symptom in the hepatopancreas under the tested conditions [13].

With regard to oxidative stress, the induction of CAT activity in *P. argentinus* signifies elevated ROS production in response to STM exposure [13]. Corroborating this finding, the elevated levels of PC in *P. argentinus* suggest a heightened sensitivity to STM exposure. This observation indicates that, within the experimental parameters employed, the activation of the antioxidant defense system proved inadequate in preventing oxidative protein damage. Consequently, although total protein and uric acid levels remain unaffected in *M. borellii*, the sensitivity of PC levels and lipid markers in this species highlights its potential as an efficient biomarker for assessing STM-induced physiological stress (Figure 3A).

Furthermore, exposure to STM has been shown to induce histopathological alterations in the hepatopancreas that bears a resemblance to those caused by CYP in adult *M. borellii* [16]. In a similar manner, *M. borellii* larvae exposed to 0.005 and 0.015 mg/L of STM for a period of four days demonstrated an increase in the number of R cells and vacuolization [69]. Moreover, the morphology of the larvae's gills exhibited high sensitivity to STM, characterized by a concentration-dependent progression of epithelial cell hyperplasia. These lesions were also observed in other *Macrobrachium* species exposed to different pesticides [91]. Epithelial cell hyperplasia and inflammatory responses are typically the principal alterations associated with the basic defense reactions developed by the gills when exposed to contaminants [112]. Histological analysis is widely regarded as a valuable tool for providing information on the general health status of organisms as it reflects the morphological changes at cellular and subcellular levels that are caused by biochemical and physiological alterations [113]. Histopathological studies of *M. borellii* have demonstrated high sensitivity in laboratory investigations to date [16,69], suggesting their potential as robust biomarkers. In relation to this, prawns of the species *P. argentinus* and *M. borellii* collected in aquatic environments affected by pollutants such as pesticides, showed several histopathologic signs of stress [8]. The symptoms exhibited alterations in both the number and structure of F and R cells in the hepatopancreas of both species of prawn. The structural alterations encompassed mitochondrial swelling, endoplasmic reticulum reduction, microvilli border alteration, vacuolar membrane denaturation, and premature autolysis. Consequently, these morphological parameters serve as a valuable tool for monitoring the impact of a stressful environment on these organisms [8].

While the sensitivity concentrations of both prawn species are in the order of mg/L of STM, the concentrations detected in the environment are in the order of ug/L. However, a continuous increase in the use of this pesticide has been detected [113], which could endanger the health of freshwater environments.

3.6. Atrazine

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine; ATZ) is a systemic triazine herbicide that is used extensively in Argentina to control weeds in crops such as maize, sorghum, and sugarcane. It inhibits electron transport in photosystem II, thereby halting photosynthesis in target species [114]. Due to its moderate persistence in the environment and high mobility, ATZ has a high potential to leach and run off into aquifers and surface waters [115]. Consequently, it is frequently detected across various environmental matrices in the country's main agricultural regions [114]. In Argentina, ATZ concentrations in surface waters exhibit broad spatial variability. In regions such as Tucumán, Córdoba, and Buenos Aires, reported concentrations reached up to 2.3 µg/L [116]. However, in other areas, monitoring reports have indicated levels ranging from values near the limit of quantification [117] to peaks of 44 µg/L in the province of Santa Fe. International regula-

tions set a limit of 1.8 µg/L for ATZ to protect aquatic life [53], whereas national standards set a threshold of 2 µg/L [118]. According to Frau et al. [119], ATZ concentrations in Santa Fe exceeded the water quality guidelines established for the protection of aquatic biota in 40% of the samples, surpassing these reference limits by up to 22-fold. Griboff et al. [70] investigated the effects of ATZ on oxidative stress biomarkers in *P. argentinus* and assessed the potential protective role of dietary vitamin E (Table 3). Prawns were fed a commercial diet that was enriched with protein, or an identical diet to which vitamin E had been added. This dietary supplementation was administered for a period of 21 days. Subsequently, individuals from each dietary group were exposed to ATZ (400 µg/L) for a period of 24 h. Exposure to ATZ resulted in a marked oxidative stress response in the prawn, as evidenced by elevated H_2O_2 levels and enhanced activity of antioxidant enzymes, including SOD, GST, and glutathione reductase (GR) (Figure 3B). While this enzymatic response appeared to limit LPO, it was associated with an energetic cost, as indicated by reduced lipid reserves. It is noteworthy that vitamin E supplementation was able to mitigate the oxidative imbalance, resulting in the normalization of biomarker responses and a reduction in the requirement for antioxidant enzyme activation. These results demonstrate that ATZ can induce oxidative stress in this freshwater crustacean, and that dietary antioxidants may partially mitigate herbicide-induced toxicity.

Nevertheless, the evidence of the sublethal effects of ATZ on non-target organisms remains limited. In *P. argentinus*, the study referenced above evaluated a comparatively elevated exposure concentration (400 µg/L) [70], which significantly exceeds environmental quality guidelines. This mismatch highlights the need for future bioassays assessing a broader and more environmentally relevant range of concentrations.

3.7. Metolachlor

Metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-ethylethyl) acetamide; MET) is a systemic pre-emergent herbicide belonging to the chloroacetamide family. It is used globally on corn, soybean, sunflower, and peanut crops [120]. Metolachlor has been shown to inhibit the growth of germinating plants by blocking the synthesis of very long-chain fatty acids. This process prevents the formation of structural lipids and the cuticle, thereby inhibiting cell division and ultimately leading to the death of the weed before it emerges [121]. This compound has been found to have significant potential for leaching and runoff due to its high solubility and mobility. It frequently reaches water bodies in agricultural regions [122]. Several studies have reported variable concentrations of MET in surface waters in Argentina. In regions exhibiting high agricultural intensity, concentrations have been observed to reach peak levels of up to 24 µg/L [123].

International regulations set a MET limit of 7.8 µg/L to protect aquatic life [53], while water quality standards regarding this pesticide are absent at this time in Argentina. Despite its widespread use, knowledge regarding its sublethal effects on non-target organisms, such as crustaceans, remains limited. Recently, Bertrand et al. [79] evaluated the toxicity of MET in the native prawn *P. argentinus* (Table 3). Adult organisms were exposed to a concentration of 7.5 µg/L for a period of seven days. This concentration is considered environmentally relevant and aligns with concentration ranges that have been observed in natural aquatic systems. The results demonstrated that 33% of the cephalothorax samples exhibited a significant accumulation of MET, with concentrations ranging from below the limit of quantification (LLOQ) to 2.75 ng/g of wet weight. Concurrently, the concentration in the abdomen remained below the limit of detection. At the biochemical level, a significant decrease in CAT activity was observed in the organisms that were exposed, compared to the control group. Furthermore, an upward trend in PC levels was observed, indicating a state of oxidative stress. While AChE activity exhibited a downward trend, the levels of

other biomarkers, including GST and BChE activities and PC, remained consistent with those of the control group. This finding suggests that the tested concentration of MET had no effect at these subcellular levels. However, severe histopathological damage in the hepatopancreas was observed in organisms exposed to MET, reaching a histopathological condition index significantly greater than the control condition. The lesions observed included progressive damage, such as cellular hyperplasia and hypertrophy, and critical regressive alterations, such as detachment of the basal lamina, increased interstitial spaces, vacuolization, reduction in microvilli, and the presence of necrotic cells. The biochemical and tissular responses included in an Integrated Biomarker Response (IBR) index demonstrated that exposure to environmentally relevant concentrations of MET induced significant levels of stress in *P. argentinus*. Although these responses were measured at biochemical and tissue levels, persistent alterations of this nature could impact the fitness and ecological performance of organisms under long-term exposure conditions.

These findings indicate that exposure to environmentally relevant concentrations of MET can induce measurable biochemical alterations and severe histopathological damage in *P. argentinus* adults, even when responses at some subcellular biomarkers remain limited (Figure 3B). The results obtained demonstrate the sensitivity of tissue-level endpoints and integrated biomarker approaches for detecting sublethal effects in aquatic invertebrates. However, there is a paucity of data regarding the toxicity of MET to aquatic organisms, particularly in freshwater crustaceans. It is therefore evident that further studies are required in order to achieve a more comprehensive characterization of toxic effects across a range of biological levels. In addition, the underlying mechanisms of toxicity in aquatic ecosystems must be elucidated.

3.8. Glyphosate

Glyphosate (N-(phosphonomethyl)glycine; GLY) is the most widely used herbicide in South America, including Argentina. It inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, thereby blocking the synthesis of essential aromatic amino acids in plants and microorganisms [124]. Despite its high capacity for soil adsorption, its extensive utilization has led to its pervasive presence in the environment. It has been demonstrated that both GLY and its metabolite AMPA are compounds with a high sediment-affinity, but they are transported to surface waters through runoff and wind erosion [18].

In recent years, scientific research has revealed the presence of GLY in surface waters of Argentinean watersheds, suggesting a pervasive presence of the substance in the region's water sources. In the Pampean region, the presence of the herbicide was detected in 71% of samples with levels reaching 13.5 μg/L [84]. However, post-application runoff events have been shown to trigger peaks of up to 80 μg/L in the drainage channels of the Cuenca del Plata [18], and critical concentrations of 105 mg/L in freshwater environments [125], posing a high risk to aquatic life. According to the national guideline level in Argentina for protecting freshwater aquatic biota, the value of GLY is established at 240 μg/L [54]. Conversely, within the international context, the Canadian Council of Ministers of the Environment [53] establishes a more lenient limit of 800 μg/L for long-term aquatic life.

Montagna and Collins [71] conducted a study on the exposure of *P. argentinus* juveniles to the commercial formulation Roundup®, determining a 96 h LC_{50} value of 51.05 mg/L of GLY (Table 1). At the physiological level, a significant reduction in metabolic oxygen requirements and ammonia-N excretion was observed after 24 h of exposure to concentrations from 10,800 to 108,000 μg/L. This finding suggests a disruption to energy acquisition and protein catabolism. These metabolic alterations preceded severe impacts on development and individual fitness. The herbicide interfered with the molting process (ecdysis), thereby inhibiting growth and resulting in a 100% mortality within three days at the maximum

evaluated concentration. In sublethal treatments, surviving individuals only managed to complete two or three growth units (molting). These results indicate that GLY compromises not only immediate survival but also induces a state of metabolic stress that prevents the successful completion of the prawn's life cycle. These effects on growth, molting and survival could ultimately impact the recruitment of freshwater prawn populations and their ecological role in aquatic food webs.

4. Conclusions and Future Prospects

Freshwater environments are of significance due to their role in underpinning a wide array of ecological functions and ecosystem services. It has been demonstrated that these systems facilitate the assimilation and recycling of nutrients and carbon, thus contributing to the sustenance of biodiversity through the provision of essential habitats and food resources for both aquatic and terrestrial ecosystems [126]. However, the ecological integrity of these ecosystems is increasingly being compromised by intensified human activities, including agro-industrial expansion, inadequate management of industrial effluents, urban waste issues and rapid urbanization, all of which are exacerbated by the overarching effects of climate change [127]. The ongoing challenges associated with increasing food production and developing resistance mechanisms in pest organisms are driving the development of environmentally friendly compounds with novel modes of action [128]. While the intention behind high specificity is to discriminate between target and non-target organisms, the latter can be rendered highly vulnerable due to risk to ecosystems. This approach also fosters the emergence of more sensitive and specific biomarkers, which can assist in predicting the environmental risks posed by new compounds. Concurrently, the integration of "omics" technologies—including functional genomics, metagenomics, transcriptomics, proteomics, metabolomics and lipidomics—is imperative for comprehending the way chemical stressors disrupt an organism's physiology at multiple levels of biological organization. The integration of advanced techniques with traditional methods has been demonstrated to enhance the precision of aquatic health assessments. Consequently, this approach can facilitate the development of more sophisticated monitoring programmes for freshwater ecosystems [129].

Although biomarker responses cannot be directly extrapolated to effects at the ecosystem level, they provide an early warning of physiological disturbance, which may precede impacts on growth, reproduction, population dynamics and ecosystem processes. However, in some cases, these early individual-level responses are inadequate for fully capturing the complex toxicological effects on an organism. In the case of *M. borellii* and *P. argentinus*, the use of a battery of complementary biomarkers is highly recommended in order to gain a comprehensive understanding of the biological responses triggered by freshwater contamination [130]. This integrated approach, combining modern molecular tools with established bioindicators, is the most robust strategy for safeguarding the health of these vital aquatic resources.

A growing body of evidence suggests that *P. argentinus* and *M. borellii* exhibit a high degree of sensitivity to a broad spectrum of aquatic pollutants, including pesticides. The biological responses of these organisms are closely associated with exposure concentrations, duration, target tissues, and developmental stages. Across the studies reviewed, oxidative stress biomarkers and histopathological alterations emerged as some of the most consistent responses, although species- and pesticide-specific differences in sensitivity were also observed. This supports their use as reliable model organisms for ecotoxicological research not only within the Pampas region but also across their broader distributional range. Furthermore, the biomarker responses to sublethal concentrations of pesticides in both species provide valuable early-warning indicators for predicting ecotoxicological risks at

the population level. It is recommended that future research expands the range of pesticides evaluated in freshwater crustaceans by considering compounds with different modes of action, building on the evidence presented. It is particularly evident that there is a paucity of information regarding the toxic effects of fungicides, despite the extensive utilization of these chemicals and the increasing prevalence of detection in aquatic environments. Furthermore, given the multiple sources of contaminants reaching aquatic ecosystems, it is essential to evaluate the effects of chemical mixtures rather than individual compounds. Such mixtures, which may include different classes of pesticides, have the potential to induce additive, synergistic, or antagonistic interactions, thereby altering toxicity and ecological risk. Consequently, studies employing ecologically relevant native species such as *P. argentinus* and *M. borellii*, are imperative to enhance the characterization of mixture toxicity and to elucidate the mechanisms underlying the combined effects of contaminants in aquatic ecosystems.

Author Contributions: L.E.: Investigation, Formal analysis, Writing—Original draft preparation. N.C.Y.V.: Investigation, Formal analysis, Writing—Original draft preparation. L.B.: Investigation, Data curation, Formal analysis, Validation, Writing—Original draft preparation, Reviewing and Editing. S.M.L.L.: Investigation, Conceptualization, Methodology, Data curation, Formal analysis, Validation, Writing—Original draft preparation, Reviewing and Editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Universidad Nacional de La Plata (25/N1046), the Secretaría de Ciencia y Técnica (SECyT) of the Universidad Nacional de Córdoba (UNC, Argentina) (Formar 2023–00038CB), and the Agencia Nacional de Promoción Científica y Técnica (PICT2019-2892, PICT2020-0890, and PICT2021-0616).

Data Availability Statement: Not application. No new data were created or analyzed in this study.

Acknowledgments: The authors would like to acknowledge the CONICET (Argentina) for providing a doctoral fellowship (Beca doctoral de finalización) for N.C. Yacelga Villavicencio and the CICPBA (Argentina) for providing a doctoral fellowship (Beca doctoral) for L. Etcheverry. We gratefully acknowledge financial support from the Universidad Nacional de La Plata (25/N1046), the Secretaría de Ciencia y Técnica (SECyT) of the Universidad Nacional de Córdoba (UNC, Argentina) (Formar 2023–00038CB), and the Agencia Nacional de Promoción Científica y Técnica (PICT2019-2892, PICT2020-0890, and PICT2021-0616). The funding sources acknowledged above correspond to a period in which Argentina sustained active programs of investment in science and technology. Over the past two years, however, national research funding has undergone a substantial reduction, jeopardizing the continuity of the country's scientific system. The generation of new toxicological data requires sustained financial support, and the current situation does not guarantee the continuation of this or other lines of basic and applied research. In the interest of scientific transparency, this scenario must be acknowledged: it threatens not only scientific and technological development, but also the nation's capacity to produce knowledge and inform evidence-based decision-making in areas critical to sustainable development. We are grateful to L. de Tezanos for graphic design assistance. During the preparation of this manuscript, the authors used the free version of DeepL (https://www.deepl.com/es/translator, accessed on 18 June 2026) as a support tool for grammatical revision. They have reviewed and edited the output and take full responsibility for the content of this publication. This paper is Scientific Contribution No. 1298 of the Instituto de Limnología de La Plata "Dr. Raúl A. Ringuelet" (ILPLA, CCT-La Plata, CONICET, UNLP).

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

AChE, acetylcholinesterase; Ammonia-N, ammonia nitrogen; ATZ, atrazine; BChE, butyrylcholinesterase; CAT, catalase; CbE, carboxylesterase; ChE, cholinesterase; CPF, chlorpyrifos; CYP, cypermethrin; endosulfan; FS, fenitrothion; GLY, glyphosate; GR, glutathione reductase; GSH, glutathione; GST, glutathione S-transferase; GPX, glutathione peroxidase; H_2O_2, hydrogen peroxide; IBR index, integrated biomarker response index; LC_{50}, median lethal concentration; LDH, lactate dehydrogenase; LPO, lipid peroxidation; MET, metolachlor; MFOs, mixed-function oxidases; PC, protein carbonyl content; rER, rough endoplasmic reticulum; ROS, reactive oxygen species; SOD, superoxide dismutase; STM, spirotetramat; TAG, triacylglycerols; TBARs, thiobarbituric acid reactive substances.

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