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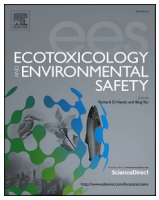

Sensitivity of embryos and larvae of the freshwater prawn *Macrobrachium borellii* to the latest generation pesticide spirotetramat

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ABSTRACT

The aim of this study was to evaluate the effects of the last generation insecticide spirotetramat (STM) on embryos and larvae of the freshwater prawn *Macrobrachium borellii.* Both embryos and larvae were exposed to serial dilutions of STM to determine the LC_{50} values. After 96-h of exposure, live larvae were fixed for histological analysis. In addition, ovigerous females were exposed to a sublethal concentration of STM (1.7 mg/L) for 96 h to evaluate the activity of the enzymes catalase, glutathione-S-transferase, and superoxide dismutase as well as the lipoperoxidation (LPO) and protein oxidation levels in embryos. The larvae showed a high sensitivity to STM evidenced by the LC_{50}-96 h value (0.011 mg/L). On the contrary, the embryos were highly resistant to STM exposure, and no lethal effect was observed in the treatments with high concentrations of this insecticide (LC_{50}-96 h > 150 mg/L). Among all the biochemical parameters evaluated in the embryos exposed to STM, only LPO showed a significant increase compared to controls. This was probably due to a restricted entry of the insecticide through the embryonic coat. Thus, a preliminary study of the structure and permeability of the embryonic coat was carried out in control embryos. The analysis by electron microscopy revealed that its structure is formed by four embryonic envelopes composed of multiple layers while the assay with a fluorescent probe revealed that the embryonic coat increases its permeability during development. STM caused significant histopathological alterations in the hepatopancreas and gills of larvae. This study showed that although the embryos of *M. borellii* could be protected by the embryonic coat, the larvae are very vulnerable to the STM toxicity. So, it is necessary to continue evaluating the effects of these new pesticides on non-target organisms, such as aquacultured species, to help predict their ecotoxicological risks derived from the increasing agricultural activity developed worldwide.

1. Introduction

Globally, the rapid expansion of agriculture over the past few decades has resulted in a dependency on the extensive use of pesticides and in huge destruction of the environment (Araújo and Shinn, 2017). One of the main reasons for the indiscriminate use of traditional chemical pesticides is the improvement of resistance mechanisms developed by pest organisms, which generates a growing public concern about the adverse effects on the impacted environments (Brevik et al., 2018). This situation has promoted the development of new pesticides that avoid cross-resistance with the active principles of some known chemical groups (Elizondo Silva and Morales, 2010). Thus, the new-generation pesticides are considered to be more environmentally benign than the traditional ones such as organophosphates and pyrethroids (Chen and Stark, 2010; Francesena et al., 2017). Among the new insecticides, it is the biorational spirotetramat (cis-3-(2,5-dimethylphenyl)− 8-methoxy-2-oxo-1-azaspiro [4.5] dec-3-en-4 yl-ethyl carbonate (STM)) with a two-way systemic action that enables translocation by the phloem and xylem of the plant (Brück et al., 2009). STM is a tetramic acid which toxic mechanism consists of the disruption of lipogenesis by inhibiting the acetyl-CoA carboxylase enzyme involved in the synthesis of fatty acids (Brück et al., 2009). Such alteration in the metabolic pathway impairs reproduction by the inhibition of vitellogenesis affecting growth and development in immature stages, mainly in sucking insects (Chen and Stark, 2010).

Even though little information is available on the consequences of STM on non-target organisms, some researchers predict that the wide application and residual issues of this new product will certainly be hazardous to organisms from both terrestrial and aquatic ecosystems (Wu et al., 2018). It has been observed that this pesticide has caused

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both oxidative damage in biomolecules, such as lipids and proteins, as well as alterations in the enzymatic antioxidant defense system (Zhang et al., 2015; Wu et al., 2018). Both processes are directly linked to the metabolic pathways of biotransformation that are carried out in organisms exposed to pollutants (Lushchak, 2011). Aquatic ecosystems are particularly vulnerable to pesticide contamination due to several factors such as run-off, which send the pesticides toward freshwater bodies near the application sites (Andrade et al., 2021). This problem is significantly noticeable in countries with great agricultural activity, like Argentina (Pérez et al., 2021), where STM is registered (https://www.argentina.gob.ar/senasa/programas-sanitarios/productosveterinarios-fitosanitarios-y-fertilizantes/registro-nacional-de-terapeutica-vegetal). Hence, non-target organisms could be useful toxicological indicators of environmental pollution caused by these new pesticides (Lushchak et al., 2018). Among the organisms proposed as indicators, crustaceans stand out for their high sensitivity to pesticides (Kumar et al., 2010; Daam and Rico, 2016). One of the most representative species of native crustaceans that inhabits in a region with high agricultural activity in the country (the Pampas region), is the freshwater decapod *Macrobrachium borellii* (Nobili), which represents a high trophic level within the community and is taxonomically related to aquacultured species (Carvalho and Collins, 2011; Stumpf et al., 2020). Previous studies carried out by our group have shown that *M. borellii* is an interesting model to evaluate the effects of different pollutants such as hydrocarbons and pesticides (Lavarías et al., 2011, 2013, 2022). Moreover, a recent study has been conducted on the effect of STM on adult prawns (Lavarías et al., 2022) although —to the best of our knowledge— there are no reports about the effects of STM on the early life stages of decapod crustaceans. Therefore, this study aimed to evaluate the sensitivity of embryos and larvae of *M. borellii* to this latest generation pesticide. For this purpose, bioassays were initially carried out to calculate the lethal doses as an endpoint to compare the sensitivity between the different developmental stages of *M. borellii* to STM. Then antioxidant status parameters were measured in embryos while histopathological alterations were analyzed in larvae.

2. Materials and methods

2.1. Sample collection

Adult females of *M. borellii* were sampled during the reproductive season (austral spring-summer) in a watercourse close to the Rio de La Plata river, Argentina (35°8′18.13"S 57°33'58.33"O). They were taken to the laboratory and kept in dechlorinated tap water ($CaCO_3$ hardness, 160 mg/L, pH between 6.6 and 6.9, and dissolved oxygen between 4.5 and 5 mg/L) at 22 ± 1 ºC, and 14 h: 10 h light/dark photoperiod for at least a week before the experiments. Prawns were fed ad libitum during the acclimation period with an optimum diet (Collins and Petriella, 1996).

2.2. Pesticide solutions

The main stock solution of STM (Movento® formulated, 15.3% of active principle, and purchased from Bayer S.A., Argentina) was prepared in acetone (grade p.a.) at a concentration of 15 g/L at the beginning of each test and kept in darkness at 4 ºC. The subsequent working stock solutions were obtained by diluting the main stock STM in acetone to nominal concentrations of exposure. The final solvent acetone concentration was always lower than 0.001% in all the exposure treatments and controls.

2.3. Embryo toxicity test

The static-renewal test was designed to determine the LC_{50} for STM in the early life stages of *M. borellii*. For the lethal embryonic bioassays, eggs were removed from the pleopods of ovigerous females carefully with forceps and the stage of embryonic development was identified under a stereoscopic microscope. Stage 5 (Lavarías et al., 2002) was selected for the bioassays since it has shown to present the highest metabolic rates and to be the most vulnerable stage to other toxicants (Lavarias et al., 2006; Lavarías et al., 2007). Eggs were placed individually into 24-well tissue-culture plastic plates with 2 mL of filtered water, as previously described by Lavarías et al. (2004). The dechlorinated tap water used for the embryo bioassays was filtered through a 0.22 mm membrane filter.

After 24-h acclimation, embryos were checked to observe the heartbeat through the transparent egg coat using a stereoscopic microscope (OLYMPUS SZ51). Dead embryos were removed (<5%) and the rest were exposed to different STM concentrations (0.6–150 mg/L) for 96 h to assess their LC_{50} value. In addition, a control group with acetone and another one with water were included. Every treatment, as well as the controls, were carried out in a single 24-well tissue-culture plate (n = 24 embryos per each treatment level). Before 24-h replacement medium, dead embryos were checked and removed. The experiment was conducted at 22 ± 1 °C and a 14 h: 10 h light/dark photoperiod.

To assess the sensitivity of *M. borellii* embryos to STM under more realistic biological conditions, ovigerous females of prawns carrying stage 5 eggs (Lavarías et al., 2002) were exposed to sublethal concentrations of the pesticide under similar conditions as previously described (Lavarías et al., 2022). An interesting feature to note is that ovigerous females, with a size range between 40 and 60 mm (total length from the tip of the rostrum to the telson), have between 40 and 100 eggs in their clutches, showing a positive correlation (unpublished data). Groups of four ovigerous females were kept into 3 L glass flasks containing 2.5 L of the test solution and at constant soft aeration. For each treatment, three flasks were used (n = 12 *per* experimental group). Initially, the prawns were left for 24 h to acclimatize and then were exposed to 1.7 mg/L of STM for 96 h. Such concentration was previously determined as 20% of the 96 h-LC_{50} value for adults, which corresponded to the highest sublethal concentration tested (Lavarías et al., 2022). A control group with acetone solvent was also included. For both, acclimatization and exposure period, ovigerous female prawns were maintained at 22 ± 1 °C under a 14 h: 10 h light /dark cycle, without feeding and a 24-h change of the test solution. Temperature, pH, and dissolved oxygen were measured with a portable water quality checker (HORIBA® U-10). All experiments were performed according to guidelines of the Institutional Animal Care and Use Committee of the National University of La Plata (UNLP).

2.3.1. Biochemical measurements

2.3.1.1. Preparation of tissue homogenate. At 96-h of bioassays, egg clutches from exposure-ovigerous females and their respective controls were carefully removed immediately. Then, two clutches were pooled, weighed, and cooled on ice for being homogenized in a glass potter with teflon plunger, in 1:9 (w:v) of 125 mM Tris-base buffer solution, pH 6.8. The egg homogenates (n = 6 *per* experimental group) were centrifuged at 10,000 xg at 4 °C for 10 min and the supernatants were collected and stored at − 20 °C for biochemical parameters determination. Antioxidant enzymatic activities, total protein and oxidative damage in lipids and proteins were measured in each supernatant. Total protein concentration was determined as described by Bradford (1976) using bovine serum albumin as standard.

2.3.1.2. Enzymatic activities. Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured as described by Marklund and Marklund (1974). The method is based on the inhibition of the auto-oxidation of pyrogallol (26 mM, pH 2) followed spectrophotometrically at 420 nm. The reaction was carried out in 50 mM Tris-cacodylate buffer (pH 8.8) in 1 mL final volume. One SOD unit was defined as the amount of enzyme necessary to inhibit 50% of autocatalytic pyrogallol oxidation/min. The specific activity was expressed as units of SOD per mg of total protein.

Catalase (CAT, EC 1.11.1.6) activity was determined by following the decrease in absorbance at 240 nm due to H_2O_2 (10 mM) decomposition (Aebi, 1984). The reaction mixture was 1 mL of 50 mM potassium phosphate buffer (pH 7). One CAT unit was defined as the amount of enzyme catalyzing 1 μmol of H_2O_2/min. The specific activity was expressed as units of CAT per mg of total protein.

Glutathione S-transferase (GST, EC 2.5.1.18) activity was measured as the method described by Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The final reaction mixture contained 0.1 M phosphate buffer (pH 6.5), 1 mM CDNB, and 1 mM GSH in 1 mL final volume. One GST unit was defined as the amount of enzyme required to conjugate GSH with 1 μmol of CDNB/min determined at 340 nm. The specific activity was expressed as units of GST per mg of total protein.

2.3.1.3. Oxidative damage in lipids and proteins. The lipid peroxidation level was measured spectrophotometrically using the formation of thiobarbituric acid-reactive substances (TBARs) according to Buege and Aust (1978). Samples were added to the reaction mixture containing trichloroacetic acid 15% (w/v), 2-thiobarbituric acid 0.375% (w/v), and 0.147 mM butylhydroxytoluene at a ratio of 1:5 (v/v). The mixture was vigorously shaken, maintained in boiling water for 60 min, and immediately cooled at 4 °C for 5 min (Ohkawa et al., 1979). After centrifugation at 5000 x*g* for 10 min, the supernatant was measured at 535 nm. LPO was expressed as μmol TBARs complexes per g of wet tissue weight.

Protein oxidation was assessed by the method described by Reznick and Packer (1994) with slight modifications. Briefly, protein carbonyl-groups concentration (PCs) was quantified by reaction with 2, 4-dinitrophenylhydrazine (DNPH). The sample was incubated with DNPH 10 mM in HCl 2 N for 15 min in darkness, followed by neutralization with NaOH 1 N, and then measured at 505 nm. Sodium pyruvate was used as standard. Results were expressed as μg protein carbonyl per mg of total protein.

2.3.2. Preliminary study of embryonic coat structure and permeability

To analyze the coat structure, eggs at 4–5 stages of development (1.6–1.8 mm maximum diameter) (Lavarías et al., 2002) were taken from the ovigerous females and fixed with 4% glutaraldehyde (n = 3 *per* experimental group). Next, three washes were carried out with buffer containing 5% sucrose, and then they were included in Spur resin. Microtome sections were cut and finally stained with uranyl acetate and lead citrate. Observations were made in a transmission electron microscope (JEOL®, JEM-1200 EXII) and images were captured.

To study the permeability of the egg coat, the hydrophobic fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) —excitation λ 361 nm— was selected. The control live embryos at different stages of development were placed in glass tubes (n = 4 *per* experimental group) and exposed to 10.8 μM of DPH concentration, in a final volume of 300 μL with filtered water. Then, they were incubated for 24 h in the darkness at room temperature. Observations were made using a fluorescence microscope (Zeiss®, Axiolab) coupled with a photographic camera for recording the embryos.

2.4. Larval toxicity test

One-two day-old post-hatching larvae (5–7 mm total length from the tip of the rostrum to the telson) were tested in groups of 12 organisms. They were placed in 450 mL glass flasks containing 250 mL of the test solution and they were randomly exposed to different concentrations of STM (0.005–5.4 mg/L) for 96 h. Control groups were also included, one with acetone and another one with water. Larvae were not fed from hatching nor during the exposure period. Bioassay conditions were at 22 ± 1 °C and a 14 h:10 h light/dark cycle with constant soft aeration. In all cases, the final concentration of acetone was always lower than 0.001%. Before each 24-h medium changed, mortality was recorded, and dead animals were removed. Larvae were considered dead when no evidence of movement was visible. At 96 h of bioassays, live larvae exposed to different STM concentrations were fixed for histological analyses to evaluate the dose-response relationship, as described below.

Table 1
LC_{50} values for larvae of *Macrobrachium borellii* exposed to spirotetramat (STM). (Confidence intervals) Equation of the Probit analysis for STM $y = 0.39e^{-0.04x}$ $R^2 = 0.99$.

LC_{50}	STM mg/L
24-h	0.16 (0.08–0.51)
48-h	0.07 (0.03–0.18)
72-h	0.02 (0.001–0.06)
96-h	0.01 (0.003–0.02)

2.5. Histological studies

Histological analysis of the hepatopancreas and gills were studied on control and exposed prawn larvae to STM. At 96 h of treatment, the entire live larvae were fixed in Bouin's solution for 6-h and then washed and stored in 70% ethanol. Samples were dehydrated using an increasing series of ethanol concentrations and then embedded in glycolmethacrylate resin (Leica Historesin®). Blocks were sectioned with tungsten knives at 5 μm using an automatic microtome (Leica® RM2155) and then mounted on microscope slides. The slides were stained with hematoxylin-eosin and observed using a light microscope (AXIOPLAN 2 Zeiss®). The degree of histopathology (slight, moderate, and severe) was scored according to the frequency of appearance of each histological alteration (Arrighetti et al., 2018).

2.6. Statistical analyses

Median lethal concentrations (LC_{50}) with 95% confidence limits were determined by PROBIT analysis program version 1.5 (US, EPA) as described by Finney (1971). In the sublethal pesticide bioassay, the results are shown as mean ± standard deviations (SD) and different treatments were compared using a Student's *t*-test after checking for normality and homogeneity of variances. Significant differences (P value < 0.05) were compared using the Tukey post hoc test.

3. Results and discussion

3.1. Determination of LC_{50} values

Initially, the sensitivity of the early life stages of *M. borellii* to STM was evaluated by mortality as the endpoint. In reference to the larval bioassays, no mortality was observed in the two control groups (with and without solvent). The LC_{50} values of STM determined for prawn larvae are shown in Table 1. These results indicate that the larvae of *M. borellii* are much more sensitive to STM than the adults, which 96 h-LC_{50} value was 8.2 mg/L for the pesticide (Lavarías et al., 2022). This biological response was different from the one shown by this species when exposed to the water-soluble fraction of the crude oil since both life stages had a sensitivity to hydrocarbons within the same order of magnitude (Lavarías et al., 2004). On the other hand, Houssou et al. (2018) also observed a similar response in the sensitivity of nauplii and adults of the freshwater copepod *Cyclops abyssorum* against STM, which 48 h-LC_{50} values were 34.85 and 37.42 mg/L, respectively. Likewise, neonates of the cladoceran *Ceriodaphnia dubia* had a 48 h-LC_{50} value of 23.77 mg/L when tested with the STM formulation Movento® showing a lower sensitivity than the decapod crustacean larvae of *M. borellii* (Chen and Stark, 2010) (Table 1). In reference to pesticide formulations, the commercial preparations of STM usually have adjuvants that increase their effectiveness (Salazar-López et al., 2016). Therefore, the toxicological evaluation of pesticide formulations is a more appropriate

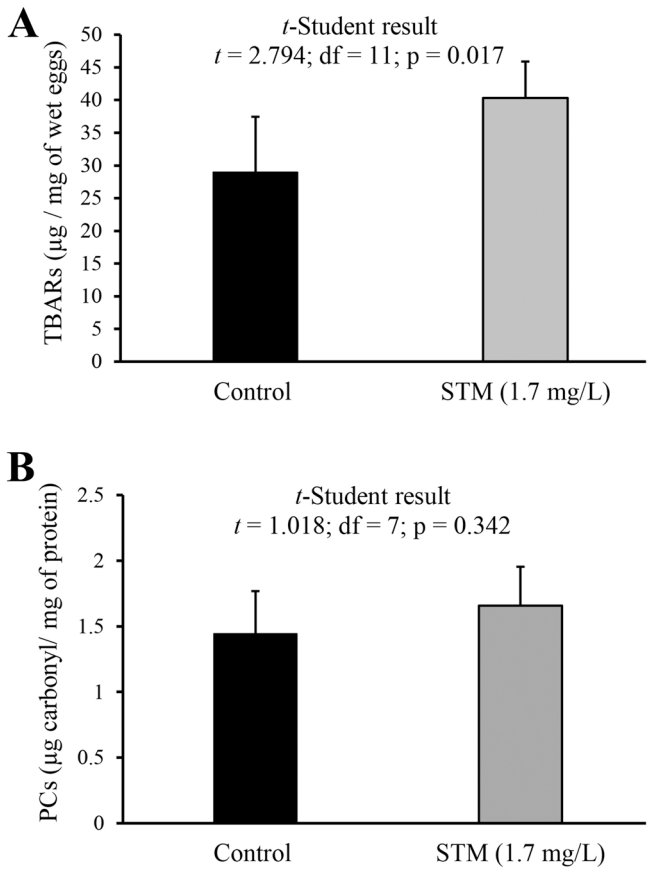

Fig. 1. Effect of spirotetramat (STM) on (A) lipid peroxidation levels (TBARs) and (B) protein oxidation damage (PCs) on *Macrobrachium borellii* embryos exposed to 1.7 mg/L. The columns represent the mean values ± standard deviation.

predictor of risk in non-target organisms that inhabit impacted ecosystems.

Among all the sensitivities reported so far for crustaceans, the larvae of the prawn *M. borellii* present the highest sensitivity to the STM pesticide (Table 1). In addition, it was also observed that the larval stage of the grass shrimp *Palaemonetes pugio* was the most sensitive life stage to the pyrethroid permethrin, being four times more sensitive than adults (DeLorenzo et al., 2006). The authors explained that larvae tend to have a higher metabolic rate than adults due to their faster growth and development, which would enable them to absorb more pollutants (DeLorenzo et al., 2006). Another reason is that larvae have a higher surface-area-to-volume ratio than adults, so they have faster uptake kinetics of the chemicals (Kefford et al., 2004). Consequently, the high sensitivity of early life stages of aquatic organisms makes them good research models for predicting environmental risks of many pollutants

Table 2
Effect of spirotetramat (STM) on total proteins and enzymatic activities of *Macrobrachium borellii* embryos.

	Control	STM (1.7 mg/L)	*t*-value	df	*p*-value
Total protein (mg/g of wet eggs)	109.6 ± 21.7	131.1 ± 14.2	1.696	7	0.133
SOD (Units/mg of protein)	0.77 ± 0.17	0.73 ± 0.15	0.404	6	0.70
CAT (Units/mg of protein)	0.43 ± 0.11	0.56 ± 0.21	1.294	7	0.237
GST (Units/mg of protein)	1.94 ± 1.20	1.45 ± 0.40	0.78	6	0.467

(Sucahyo et al., 2008). However, several reports show that the embryonic stage of several crustacean species is much more resistant to toxicants than the adult stage (DeLorenzo et al., 2006; Lavarías et al., 2004). This response was also observed in this study, as in any embryo bioassays no significant differences in mortality were observed between the control and insecticide-treated eggs and mortality was never high enough to calculate any LC_{50} value in the tested ranges, (96 h-LC_{50} > 150 mg/L for STM). On the contrary, when Zhang et al. (2019) evaluated the toxicity of STM in embryos of the zebrafish *Danio rerio*, the 96 h-LC_{50} value for fish embryos was 5.94 mg/L of STM (Zhang et al., 2019). Besides, it was observed that the embryos of the insect pest *Frankliniella occidentalis* showed great sensitivity to STM, which causes structural damage in their embryonic (Yang et al., 2021). The differences between these two species and the crustacean *M. borellii* could be due to the ability of the embryonic coat to serve as an efficient barrier to the entry of toxicants into the eggs (DeLorenzo et al., 2006). Therefore, the study of the embryonic structures would help to understand the sensitivity to environmental stressors (see below).

3.2. Biochemical parameters measured in embryos

Pesticide exposure induces oxidative stress in organisms and represents one of the most studied toxicological mechanisms in aquatic invertebrates (Gonçalves et al., 2021). Such induction is linked with pesticide metabolism through phase I reactions that release reactive oxygen species (ROS) and cause damage to lipids and proteins (Lushchak et al., 2018; Silvestre, 2020). Regarding the vulnerability of these biomolecules in *M. borellii* embryos, it is noticeable that embryos at stages 5/6 —the latest stage reached by the embryos at the end of the bioassay— have about 25% of lipid content (dry weight) (Heras et al., 2000). Besides, this high lipid content is related to a progressive enrichment of polyunsaturated fatty acids (Heras et al., 2000), which are ROS targets by the lipid peroxidation pathway (Storey, 1996). The lipid peroxidation products are usually measured as TBARs and influence the membrane fluidity as well as the integrity of its associated biomolecules like proteins and cholesterol (Wei and Yang, 2015). TBARs levels in embryos exposed to 1.7 mg/L of STM for 96 h showed a significant increase ($p < 0.02$) compared to controls (Fig. 1A). So, this fact would be indicating that ROS produced by STM metabolism would be causing the peroxidation of membrane polyunsaturated fatty acids in embryos (Zhang et al., 2015). However, in adult prawns of *M. borellii* exposed to the same STM concentration, TBARs values did not show a significant increase compared to the control, possibly due to their rapid degradation and excretion (Lavarías et al., 2022). Thus, adult prawns may have a greater capacity to metabolize lipid peroxidation products compared to embryos, which detoxification systems could be immature (DeLorenzo et al., 2006). On the other hand, TBARs retention could result from the selective permeability of the egg's coat, which inhibits the lipoperoxidation products generated in the embryos exposed to STM from easily migrating from the egg to the surrounding aquatic environment.

Furthermore, lipid peroxidation products can react with amino acids promoting protein oxidation by forming carbonyl groups (PCs) (Lushchak, 2011). The consequence of protein oxidation could be its structural and functional alteration through the cleavage of the peptide backbone and modification of the amino acid side chain (Wei and Yang, 2015). Such alterations in the protein integrity are crucial for a developing embryo that is under active organogenesis (Heras et al., 2000). However, no significant differences ($p > 0.05$) were observed in the PCs concentration between *M. borellii* control and exposed embryos to STM (Fig. 1B). The increase in PCs content is an indication that the normal protein metabolism has been altered by pesticide exposition (Lushchak, 2011). Hence, the results of this study may suggest that —under the tested conditions— the embryo proteins would be more resistant to oxidative damage than lipids. Alternatively, it could be that the characteristic high lipid content of *M. borellii* embryos, which is a result of their abundant yolk (Heras et al., 2000), may act as a "buffer" to the

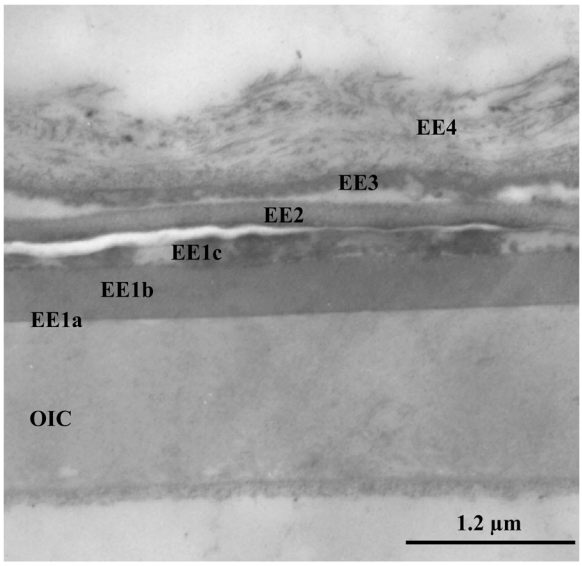

Fig. 2. Transmission electron micrograph of the egg coat composition of *Macrobrachium borellii* embryos at stage 5.
The outer layer is the outer investment coat (OIC). The first embryonic envelope (EE1) is composed of three layers: a thin electron-dense layer (EE1a), a thinker less electron-dense layer (EE1b), and a flocculent loosely defined layer (EE1c). The second embryonic envelope (EE2) is between the EE1c and the third embryonic envelope (EE3). The fourth embryonic envelope (EE4) is the envelope closest to the embryo and is formed by large folds.

oxidative damage by ROS, preserving essential proteins. In any case, it must be considered that ROS are normally generated during aerobic metabolism (Lushchak, 2011). Therefore, *M. borellii* embryos that primarily rely on lipids for energy supply (González-Baró et al., 2000), probably have a high generation of ROS that needs to be neutralized with an efficient antioxidant defense system. Among the different components of the antioxidant system, the enzymes SOD and CAT represent the first defense line against ROS. The SOD/CAT system has the function to convert $O_2^{\cdot}$ in H_2O and O_2 under prooxidant conditions (Hermes-Lima and Zenteno-Savín, 2002). Although exposure to STM can cause the induction in the activity of both antioxidant enzymes (Wu et al., 2018), no significant alterations ($p > 0.05$) were observed between controls and embryos of *M. borellii* exposed to the pesticide (Table 2).

On the other hand, the phase II detoxification enzyme GST catalyzes the conjugation of reduced glutathione to diverse electrophilic substrates facilitating the excretion of several xenobiotics (Rauch and Nauen, 2004). It has been shown that GST can metabolize STM in invertebrates (Zhang et al., 2015). In fact, adult prawns of *M. borellii* showed a significant increase in their activity when they were exposed to 1.7 mg/L of STM for 96 h (Lavarías et al., 2022). However, in *M. borellii* embryos, GST activity was not induced by pesticide exposure (Table 2). Although it is evident that the activity of GST in embryos was not sensitive to the pesticide under the conditions tested in this study, its activity values were an order of magnitude higher than in adults (Lavarías et al., 2022). Thus, these results might indicate that *M. borellii* embryos would have a basal detoxifying activity high enough to metabolize the pesticide (Table 2). In contrast, *Macrobrachium malcolmsonii* embryos have a gradual increase of GST activity during embryogenesis but it does not reach the adults' values and the larvae are the ones that show the highest activity of this enzyme (Arun and Subramanian, 1998). These results would indicate that some immature life stages could have sufficiently active defense mechanisms to cope with stressful situations that substantially alter their metabolism. Therefore, it would be interesting to continue studying different types of defense systems in these animal models as it will help predict risks at different stages of development.

3.3. Preliminary study of embryonic coat structure and permeability

During the period in which female decapods incubate their eggs, the embryonic envelopes protect the embryos from physical and chemical stress and keep the internal environment constant (Fisher and Clark, 1983). In *M. borellii* embryos, the egg coat composition was analysed at stage 5 (29–32 days of development) by transmission electron microscopy (Fig. 2). Although little information is available about egg coat structure/composition in decapods, six envelopes similar to those described for *Palaemonetes pugio* were identified (Glas et al., 1997). As explained by the authors (Glas et al., 1997), the outermost layer of the embryonic coat, i.e, the outer investment coat or OIC, forms the funiculus that fixes the eggs to the female's pleopods, to where they remain attached until hatching. Additionally, this OIC would represent the first external defense for the embryos controlling the permeability of different substances and/or toxins from the aquatic environment into the eggs. Below the OIC, several layers or envelopes are found. The first embryonic envelope —EE1— is made up of a thin outer layer (EE1a), a thick inner layer (EE1b), and a layer of filamentous material with a disorganized structure (EE1c) (Fig. 2). In direct contact with it the EE1, the EE2 comprises a dense outer thin layer and a less dense inner layer. The third layer —EE3— is made up of a thin electron-dense layer and a broad heterogeneous band with clear regions. The last envelope, EE4, is similar in structure to EE3 and remains close to the embryonic surface during embryonic development. These four envelopes (EE1-EE4) have an embryonic origin, and they are synthesized during development (Glas et al., 1997).

According to DeLorenzo et al. (2006), the embryonic coat of the grass shrimp *P. pugio* may also protect the embryo from insecticide exposition. As a first approximation to the study of the barrier function of the egg

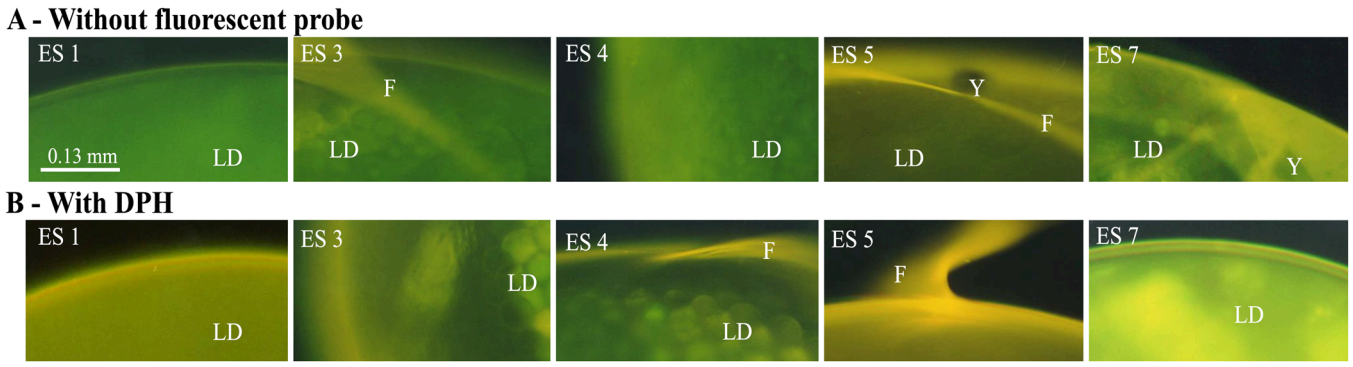

Fig. 3. Fluorescence micrographs of the egg coat permeability test in embryos at different stages of development (A) without the fluorescent probe and (B) exposed to the fluorescent probe DPH for 24 h. Embryonic stage (ES); Lipid droplets (LD); Funiculus (F); Eyes (Y).

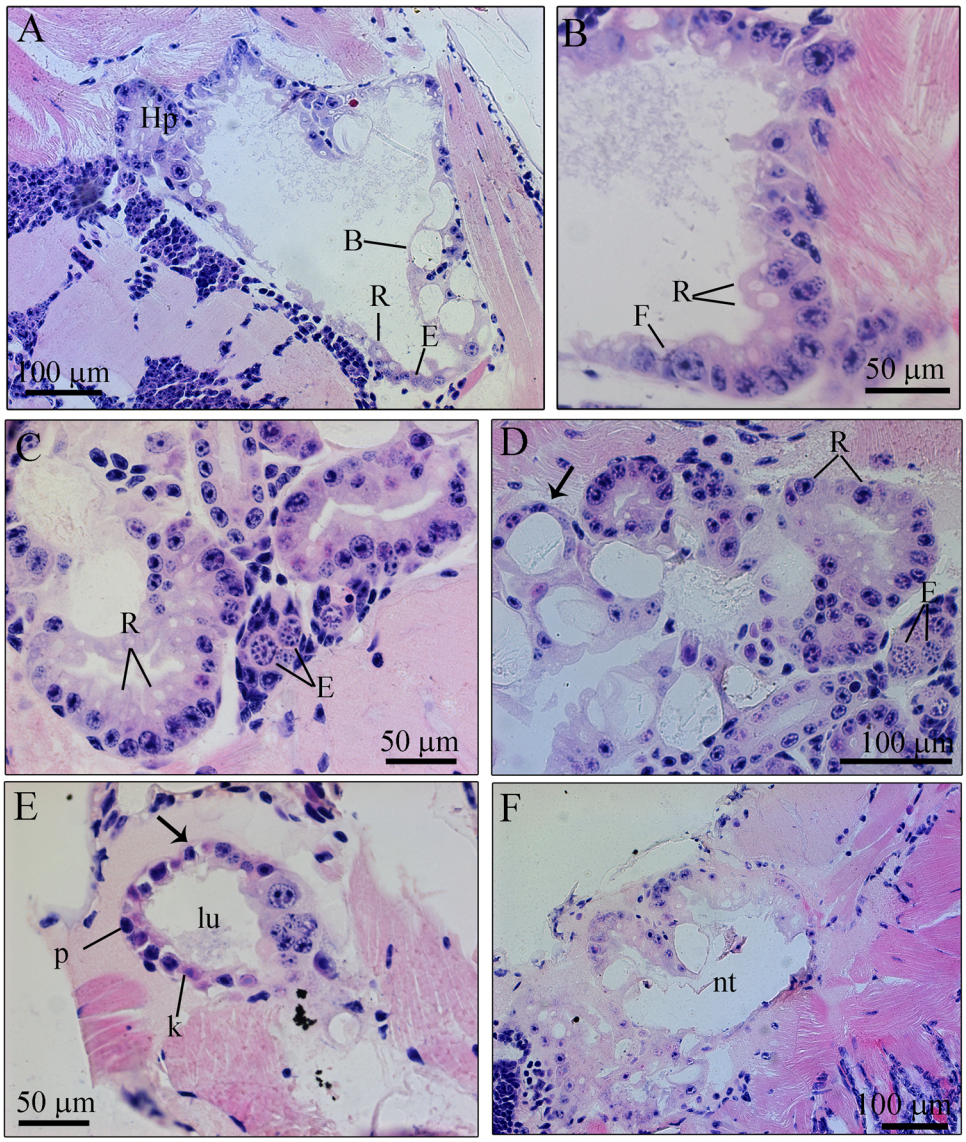

Fig. 4. A and B. Histological sections of the hepatopancreas of control *Macrobrachium borellii* larvae. A. Overview of a longitudinal section of the normal structure of one of the paired lateral lobes of the hepatopancreas (Hp) showing B-cells (B), R-cells (R), and E-cells (E). B. Detail of a section showing F-cells (F), and R-cells (R). C and D. Hepatopancreas from larvae exposed to 0.005 mg/L of STM. C. Histological section showing the epithelium with numerous R-cells (R) and E-cells (E) in the tip of a tubule. D. Overview of the hepatopancreas showing the atrophied epithelium (arrow) and tubules with R-cells (R) and E-cells (E). E and F. Hepatopancreas from larvae exposed to 0.015 mg/L of STM. E. Detail of a hepatopancreatic tubule showing a reduction of the epithelial thickness (arrow), wide lumen (lu), and some cells with pyknosis (p) and karyolysis (k). F. Detail of a necrotic tubule (nt).

coat, permeability tests were carried out with the hydrophobic probe DPH in control embryos at different stages of development. It was observed that the different embryonic envelopes allowed the passage of DPH in the exposed stages, and the intensity of fluorescence was increased approaching hatching (stage 7) (Fig. 3). The same change in egg coat permeability was also observed in *P. pugio* embryos (Glas et al., 1997). In fact, such an increase in permeability could have facilitated the pesticide uptake (Lund et al., 2000). Although the *M. borellii* embryos did not reach the last stage of development (stage 7) under the pesticide-exposure conditions tested in this study, it was previously observed that embryos exposed to hydrocarbons until hatching showed a significant increase in sensitivity to these toxicants (Lavarías et al., 2004). Such a situation could be related to the increase in the volume of the egg that reaches its maximum value in the last stage probably causing a stretching of the egg coat and enabling the break during hatching (Lavarías et al., 2002).

All things considered, it would be worthwhile to continue studying the reduction/increase of toxicants uptake by the egg coat and/or the resistance mechanisms associated with it in crustaceans such as the ABC (ATP-binding cassette) proteins responsible of resistance to numerous insecticides (Dermauw and Van Leeuwen, 2014). Especially because it would preserve a very vulnerable life stage of a taxonomic group that is more sensitive than other aquatic invertebrates to several environmental stressors (Bae and Park, 2014).

3.4. Histological alterations in larvae

The morphology of the hepatopancreas and gills of *M. borellii* larvae exposed for 96 h to 0.005 and 0.015 mg/L of STM were analyzed. In crustaceans, both organs are particularly sensitive to oxidative stress caused by ROS (Romero et al., 2011).

The hepatopancreas of crustaceans is involved in the digestion and absorption of nutrients, excretion, and storage as well as in the detoxification of pollutants. (Collins, 2010; Vogt, 2019). These characteristics make this organ very sensitive to physiological and environmental conditions, being a very suitable control organ to evaluate the impact of xenobiotics (Saravana Bahavan and Geraldine, 2000; Wei and Yang, 2015; Lavarías et al., 2022).

Histological sections of the hepatopancreas of control larvae showed a simple columnar epithelium with different cell types (Fig. 4A, B). The R cells (resorptive cells) with a medium-sized nucleus and lightly stained cytoplasm, B cells (blister-like cells) with distinct supranuclear vacuoles, E cells (embryonic cells) characterized by a high nucleus to cytoplasm ratio, and F cells (fibrous cells) with large nucleus and strongly stained

Table 3
Histological alterations observed in the hepatopancreas and gills of *Macrobrachium borellii* larvae exposed to different spirotetramat (STM) concentrations. (−) no histopathology in any field, (+) mid histopathology present in < 25% of the slides, (++) moderate histopathology present in 25%− 75% of the slides and (+++) severe histopathology present in > 75% of the slides.

			STM (mg/L)	
	Histological alteration	Control	0.005	0.015
Hepatopancreas	Epithelial disorganization	–	+	+ +
	Increased R-cells	–	+ +	–
	Atrophied epithelium	–	+	+ +
	Necrosis	–	–	+ +
Gills	Hemocytes accumulation	–	+	+ ++
	Hyperplasia of the epithelial cells	–	+ +	+ +
	Disorganization of gill lamellae	–	–	+ +

cytoplasm. The hepatopancreas of *M. borellii* larvae include the same type of cells as the adults (Lavarías et al., 2022), in agreement with that observed for other penaeid larvae (Vogt, 2008, 2019).

The microscopic and histopathological analysis clearly revealed that STM damaged the hepatopancreas of *M. borellii* larvae (Table 3, Fig. 4C-F). Larvae exposed to STM showed evident disorganization and rupture of the epithelial cell layer (Fig. 4C-E) and at the highest concentration the entire hepatopancreas was damaged (Fig. 4F). At 0.005 mg/L of STM exposure, the hepatopancreas showed an increment in the number of R-cells in concomitance with the absence of B-cells (Fig. 4C). E-cells were present in the tip of the tubules (Fig. 4C). Also, the digestive tubules showed a moderate atrophied epithelium, characterized by a notable reduction in the epithelial height (Fig. 4D). Larvae exposed to 0.015 mg/L of STM showed the epithelium of the hepatopancreas atrophied (Fig. 4E). At this STM concentration, the first evidence of acute cell injury appeared, with the presence of nuclear abnormalities such as shrinkage of the nucleus (pyknosis) and dissolution of the chromatin (karyolysis) (Fig. 4E). Besides, the hepatopancreas showed severe necrosis in some larvae (Fig. 4F). At the highest pesticide concentration, there was no evidence of the presence of B-cells in this study.

In decapods, the hepatopancreas is regenerated from the E-cells located at the tip of the tubules. The reduction in the number of this cell type might impair the normal phase of each digestive cycle. According to the evidence presented in this work at a low STM concentration, the high number of R-cells could indicate that a detoxification mechanism would be activated. However, at 0.015 mg/L STM, several cellular damages were observed indicating a failure in the detoxification process and a point-of-no-return, as was observed in adults of *M. borellii* exposed to cypermethrin (Collins, 2010).

Regarding the structure of the gill filaments, the control larvae showed a normal arrangement, with small hemocoel spaces and conserved lamellae (Fig. 5A). Several progressive alterations were observed in prawns exposed to different STM concentrations (Table 3). Hyperplasia of the epithelial cells were commonly observed in prawns exposed to 0.005 mg/L of STM (Fig. 5B). In larvae exposed to 0.015 mg/L of STM, hyperplasia of the epithelium was also observed along with the accumulation of intercellular hemocytes (Fig. 5C). In addition, gills filaments lost their normal structure. It is possible that hyperplasia and hemocyte infiltration induced the formation of disorganized tissue in the gills. Similar lesions were observed in other *Macrobrachium* species exposed to other pesticides (Saravana Bahavan and Geraldine, 2000; Pereira Soares et al., 2019).

In freshwater crustaceans, gills are vital organs involved in gas exchange, salt absorption, acid-base equilibrium, and excretion of nitrogen compounds (Dutra et al., 2017). As gills are in close contact with water, when they are exposed to xenobiotics, lesions such as hyperplasia of the epithelial cells and inflammatory responses could be interpreted as basic defense reactions (Dutra et al., 2017). Both types of histological damage may reflect an adaptive change to oxidative stress induced by STM exposure, leading to a thickening of the lamellae and thus, increasing the distance between the central hemolymphatic space and the surrounding freshwater. Histopathological damage may compromise physiological gill functions causing hypoxia as well as xenobiotic metabolism and osmoregulation alterations, which finally may lead to the death of the organism (Wei and Yang, 2015).

STM detection in aquatic environments has received very little attention so far (Babic et al., 2018; Sjerps et al., 2019); and although the concentrations measured in this study are lower than those predicted for natural environments (Houssou et al., 2020), the negative impact and damage caused on the larvae prawns are clear. Therefore, it would be interesting to evaluate other sensitive parameters in vulnerable stages of development that may aid in predicting damage from emerging pesticides.

4. Conclusions

This study showed that larvae of *M. borellii* are very sensitive to STM compared to embryos. It is likely that the high resistance of embryos to pesticide is due to the protective effect of the egg coat, restricting the bioavailability of the toxicant to an immature and susceptible life stage.

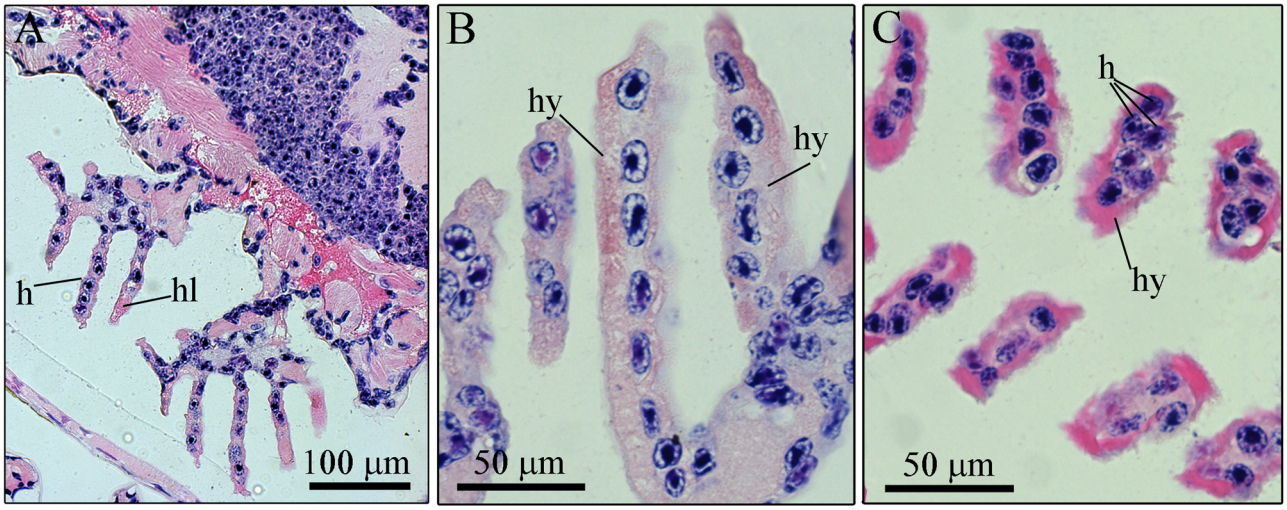

Fig. 5. A. Histological sections of the gill filaments of control *Macrobrachium borellii* larvae with a reduced hemolymphatic space (hl) and a low number of hemocytes (h). B-C. Histological section of the gills from larvae exposed to 0.005 mg/L STM showing hyperplasia (hy) of the epithelial cells (B) and 0.0015 mg/L STM (C) showing hyperplasia (hy) of the epithelial cells and accumulation of hemocytes (h).

The pesticide caused histopathological alterations in the gills and hepatopancreas of the larvae, while in the embryos it only caused a significant increase in lipid peroxidation. Therefore, the results of this study could represent the starting point to continue evaluating additional parameters at different levels of organization to relate the toxic effect of STM and biological responses in non-target organisms.

CRediT authorship contribution statement

Lavarías, S.M.L.: Investigation, Conceptualization, Resources, Methodology, Data curation, Validation, Writing – original draft preparation, Reviewing and editing. Arrighetti, F.: Resources, Methodology, Formal analysis, Writing – original draft preparation, Graphic design, Reviewing and editing Landro, S.M.: Methodology, Reviewing and Editing Colpo, K.D.: Methodology, Data curation, Formal analysis, Graphic design.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Aebi, H., 1984. Catalase in vitro. Meth. Enzym. 105, 121–126.
Andrade, V.S., Gutierrez, M.F., Regaldo, L., Paira, A.R., Repetti, M.R., Gagneten, A.M., 2021. Influence of rainfall and seasonal crop practices on nutrient and pesticide runoff from soybean dominated agricultural areas in Pampean streams, Argentina. Sci. Total Environ. 788, 147676.
Araújo, C.V.M., Shinn, C., 2017. Ecotoxicology in Latin America. Nova Science Publishers, USA, pp. 1–623.
Arrighetti, F., Ambrosio, E., Astiz, M., Rodrigues Capítulo, A., Lavarías, S., 2018. Differential response between histological and biochemical biomarkers in the apple snail *Pomacea canaliculata* (Gasteropoda: Amullariidae) exposed to cypermethrin. Aquat. Toxicol. 194, 140-15.
Arun, S., Subramanian, P., 1998. Comp. Biochem. Physiol. Part B 121, 273–277.
Babic, S., Barisic, J., Stipanicev, D., Repec, S., Lovric, M., Malev, O., Martinovic-Weigelt, D., Coz-Rakovac, R., Klobucar, G., 2018. Assessment of river sediment toxicity: combining empirical zebrafish embryotoxicity testing with in silico toxicity characterization. Sci. Total Environ. 643, 435–450.
Bae, M.J., Park, Y.S., 2014. Biological early warning system based on the responses of aquatic organisms to disturbances: a review. Sci. Total Environ. 466–467, 635–649.
Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Brevik, K., Lindström, L., McKay, S.D., Chen, Y.H., 2018. Transgenerational effects of insecticides — implications for rapid pest evolution in agroecosystems. Curr. Opin. Insect Sci. 26, 34–40.
Brück, A., Elbert, A., Fischer, R., Krueger, S., Kuhnhold, J., Klueken, A.M., Nauen, R., Niebes, J.F., Reckmann, U., Schnorbach, H.J., Steffens, R., van Waetermeulen, X., 2009. Movento, an innovative ambimobile insecticide for sucking insect pest control in agriculture: biological profile and field performance. Crop Prot. 28, 838–844.
Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzym. 52, 302–309.
Carvalho, D.A., Collins, P.A., 2011. Ontogenetic predation capacity of *Macrobrachium borellii* (Caridea: Palaemonidae) on prey from littoral-benthic communities. Nauplius 19, 71–77.
Chen, X.D., Stark, J.D., 2010. Individual and population level toxicity of the insecticide, spirotetramat and the agricultural adjuvant, Destiny to the Cladoceran, *Ceriodaphnia dubia*. Ecotoxicol 19, 1124–1129.
Collins, P., 2010. Environmental stress upon hepatopancreatic cells of freshwater prawns (Decapoda: Caridea) from the floodplain of Paraná River. Nat. Sci. 2, 748–759.
Collins, P.A., Petriella, A.M., 1996. Crecimiento y supervivencia del camarón *Macrobracbium borellii* (Decapoda: Palaemonidae) alimentado con dietas artificiales. Neotrópica 42, 3–7.
Daam, M.A., Rico, A., 2016. Freshwater shrimps as sensitive test species for the risk assessment of pesticides in the tropics. Environ. Sci. Poll. Res. 25, 13235–13243.
DeLorenzo, M.E., Serrano, L., Chung, K.W., Hoguet, J., Key, P.B., 2006. Effects of the insecticide permethrin on three life stages of the grass shrimp, *Palaemonetes pugio*. Ecotoxicol. Environ. Saf. 64, 122–127.
Dermauw, W., Leeuwen, T, Van, 2014. The ABC gene family in arthropods: comparative genomics and role in insecticide transport and resistance. Insect Biochem. Mol. Biol. 45, 89–110.
Dutra, F.M., Ronnau, M., Sponchiado, D., Forneck, S.C., Ballester, C.A.F., Cupertino, E.L., 2017. Histological alterations in gills of *Macrobrachium amazonicum* juveniles exposed to ammonia and nitrite. Aquat. Toxicol. 187, 115–123.
Elizondo Silva, A.I., Murguido Morales, C.A., 2010. Spirotetramat, nuevo insecticida para el control de insectos chupadores en el cultivo de la papa. Fitosanidad 14, 229–234.
Finney, D.J., 1971. Probit. Analysis, Third edition.,. Cambridge University Press, London, UK.
Fisher, W.S., Clark Jr., W.H., 1983. Eggs of *Palaemon macrodactylus*: I. Attachment to the pleopods and formation of the outer investment coat. Biol. Bull.. 164, 189–200.
Francesena, N., Desneux, N., de Campos, M.R., Schneider, M.I., 2017. Side effects of spirotetramat on pupae and adults of a Neotropical strain of *Eretmocerus mundus* (Hymenoptera: Aphelinidae): effects on the life parameters and demography. Environ. Sci. Pollut. Res. 24, 17719–17730.
Glas, P., Courtney, L., Rayburn, J., Fisher, W., 1997. Embryonic coat of the grass shrimp *Palaemonetes pugio*. Biol. Bull.. 192, 231–242.
Gonçalves, A.M.M., Rocha, C.P., Marques, J.C., Gonçalves, F.J.M., 2021. Enzymes as useful biomarkers to assess the response of freshwater communities to pesticide exposure – A review. Ecol. Indic. 122, 107303.
González-Baró, M.R., Heras, H., Pollero, R.J., 2000. Enzyme activities involved in lipid metabolism during embryonic development of *Macrobrachium borellii*. J. Exp. Zool. 286, 231–237.
Habig, W., Pabst, M.J., Jakoby, W.B., 1974. Glutathione-S-Transferases. J. Biol. Chem. 22, 7130–7139.
Heras, H., González-Baró, M.R., Pollero, R.J., 2000. Lipid and fatty acid composition and energy partitioning during embryo development in the shrimp *Macrobrachium borellii*. Lipids 35, 645–651.
Hermes-Lima, M., Zenteno-Savín, T., 2002. Animal response to drastic changes in oxygen availability and physiological oxidative stress. Comp. Biochem. Physiol. C. 133, 537–556.
Houssou, A.M., Cocan, D., Bonou, C.A., Miresan, V., Montchowui, E., 2018. Survival and reproduction of *Cyclops abyssorum* (freshwater copepod) exposed to spirotetramat and 2,4-D. Rom. Biotechnol. Lett. 23, 13761–13770.
Houssou, A.M., Cocan, D., Miresan, V., Bonou, C.A., Montchowui, E., 2020. Effect of spirotetramat and 2,4-dichlorophenoxyacetic pesticides on two phytoplankton species (*Monoraphidium griffithii* and *Coscinodiscus* sp.) in a single and compound mixture laboratory exposure. Cogent. Environ. Sci. 6, 1724064.
Kefford, B.J., Dalton, A., Palmer, C.G., Nugegoda, D., 2004. The salinity tolerance of eggs and hatchlings of selected aquatic macroinvertebrates in southeast Australia and South Africa. Hydrobiologia 517, 179–192.
Kumar, A., Correll, R., Grocke, S., Bajet, C., 2010. Toxicity of selected pesticides to freshwater shrimp, Paratya australiensis (Decapoda: Atyidae): Use of time series acute toxicity data to predict chronic lethality. Ecotoxicol. Environ. Saf. 73, 360–369.
Lavarias, S., Pollero, R., Heras, H., 2006. Activation of lipid catabolism by the water-soluble fraction of petroleum in the crustacean *Macrobrachium borellii*. Aquat. Toxicol. 77, 190–196.
Lavarías, S., Heras, H., Demichelis, S., Portiansky, E., Pollero, R.J., 2002. Morphometric study of embryonic development of *Macrobrachium borellii* (Arthropoda: Crustacea). Invertebr. Reprod. Dev. 41, 157–163.
Lavarías, S., Heras, H., Pollero, R.J., 2004. Toxicity, uptake, and release of the water-soluble fraction of crude oil in different developing stages of the prawn *Macrobrachium borellii*. Arch. Environ. Contam. Toxicol. 47, 215–222.
Lavarías, S., García, F., Pollero, R.J., Heras, H., 2007. Effect of the water-soluble fraction of petroleum on microsomal lipid metabolism of *Macrobrachium borellii* (Arthropoda: Crustacea). Aquat. Toxicol. 82, 265–271.
Lavarías, S., Heras, H., Pedrini, N., Tournier, H., Ansaldo, M., 2011. Antioxidant response and oxidative stress levels in *Macrobrachium borellii* (Crustacea: Palaemonidae) exposed to the water-soluble fraction of petroleum. Comp. Biochem. Physiol. C. 153, 415–421.
Lavarías, S., García, C., Crespo, R., Pedrini, N., Heras, H., 2013. Study of biochemical biomarkers in freshwater prawn *Macrobrachium borellii* (Crustacea: Palaemonidae) exposed to organophosphate fenitrothion. Ecotoxicol. Environ. Saf. 96, 10–16.
Lavarías, S.M.L., Colpo, K.D., Landro, S.M., Ambrosio, E.S., Rodrigues Capítulo, A., Arrighetti, F., 2022. Deleterious effects of two pesticide formulations with different toxicological mechanisms in the hepatopancreas of a freshwater prawn. Chemosphere 286, 131920. https://doi.org/10.1016/j.chemosphere.2021.131920.

Lund, S.A., Fulton, M.H., Key, P.B., 2000. The sensitivity of grass shrimp, *Palaemonetes pugio*, embryos to organophosphate pesticide induced acetylcholinesterase inhibition. Aquat. Toxicol. 48, 127–134.
Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13–30.
Lushchak, V.I., Matviishyn, T.M., Husak, V.V., Storey, J.M., Storey, K.B., 2018. Pesticide toxicity: a mechanistic approach. EXCLI J. 17, 1101–1136.
Marklund, S., Marklund, G., 1974. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47, 469–474.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.
Pereira Soares, M., Oliveira, N., Rebelo, D., Marcondes, S.F., Fernandes, C.E., Domingues, I., Soares, A., Hayd, L., 2019. Cypermethrin-based formulation Barrage® induces histological changes in gills of the Pantanal endemic shrimp *Macrobrachium pantanalense*. Environ. Toxicol. Pharmacol. 67, 66–72.
Pérez, D.J., Iturburu, F.G., Calderón, G., Oyesqui, L.A.E., de Geronimo, E., Aparicio, V.C., 2021. Ecological risk assessment of current-use pesticides and biocides in soils, sediments and surface water of a mixed land-use basin of the pampas region. Argent. Chemosphere 263, 128061.
Rauch, N., Nauen, R., 2004. Characterization and molecular cloning of a glutathione S-transferase from the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae). Insect Biochem. Mol. Biol. 34, 321–329.
Reznick, A.Z., Packer, L., 1994. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Method. Enzym. 233, 357–363.
Romero, M.C., Tapella, F., Sotelano, M.P., Ansaldo, M., Lovrich, G.A., 2011. Oxidative stress in the subantarctic false king crab *Paralomis granulosa* during air exposure and subsequent re-submersion. Aquacul 319, 205–210.
Salazar-López, N., Aldana-Madrid, M., Silveira-Gramont, M., Aguiar, J., 2016. Spirotetramat - An Alternative for the Control of Parasitic Sucking Insects and its Fate in the Environment. In (Ed.), Insecticides Resistance. IntechOpen. https://doi.org/10.5772/61322.
Saravana Bhavan, P., Geraldine, P., 2000. Histopathology of the hepatopancreas and gills of the prawn *Macrobrachium malcolmsonii* exposed to endosulfan P. Aquat. Toxicol. 50, 331–339.
Silvestre, F., 2020. Signaling pathways of oxidative stress in aquatic organisms exposed to xenobiotics. J. Exp. Zool. Part A: Ecol. Integr. Physiol. https://doi.org/10.1002/jez.2356.
Sjerps, R.M.A., Kooij, P.J.F., van Loon, A., Van Wezel, A.P., 2019. Occurrence of pesticides in Dutch drinking water sources. Chemosphere 235, 510–518.
Storey, K.B., 1996. Oxidative stress: animal adaptations in nature. Braz. J. Med. Biol. Res. 29, 1715–1733.
Sucahyo, D., van Straalen, N.M., Krave, A., van Gestel, C.A.M., 2008. Acute toxicity of pesticides to the tropical freshwater shrimp *Caridina laevis*. Ecotoxicol. Environ. Saf. 69, 421–427.
Vogt, G., 2008. Investigation of hatching and early post-embryonic life of freshwater crayfish by in vitro culture, behavioral analysis, and light and electron microscopy. J. Morphol. 269, 790–811.
Vogt, G., 2019. Functional cytology of the hepatopancreas of decapod crustaceans. J. Morphol. 280, 1405–1444.
Wei, K., Yang, J., 2015. Oxidative damage induced by copper and beta-cypermethrin in gill of the freshwater crayfish *Procambarus clarkii*. Ecotoxicol. Environ. Saf. 113, 446–453.
Wu, H., Rao, Q., Zheng, J., Mao, C., Sun, Y., Gu, D., Wang, M., Liu, X., 2018. Biochemical and histological alterations in adult zebrafish (*Danio rerio*) ovary following exposure to the tetronic acid insecticide spirotetramat. Ecotoxicol. Environ. Saf. 164, 149–154.
Yang, X., Zhou, G., Sun, L., Zheng, C., 2021. Ovicidal activity of spirotetramat and its effect on hatching, development and formation of *Frankliniella occidentalis* egg. Sci. Rep. 11, 20751. https://doi.org/10.1038/s41598-021-00160-6.
Zhang, J., Qian, L., Teng, M., Mu, X., Qi, S., Chen, X., Zhou, Y., Cheng, Y., Pang, S., Li, X., Wang, C., 2019. The lipid metabolism alteration of three spirocyclic tetramic acids on zebrafish (*Danio rerio*) embryos. Environ. Poll 248, 715–725.
Zhang, Q., Zhang, G., Yin, P., Lv, Y., Yuan, S., Chen, J., Wei, B., Wang, C., 2015. Toxicological effects of soil contaminated with spirotetramat to the earthworm *Eisenia fetida*. Chemosphere 139, 138–145.