Science of the Total Environment 942 (2024) 173685

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Assessing the impact of imidacloprid, glyphosate, and their mixtures on multiple biomarkers in *Corbicula largillierti*

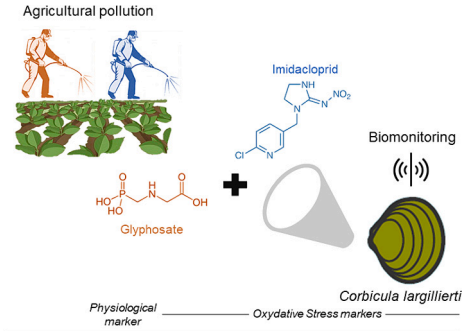
V.L. Lozano [a,b,c,1], E.M. Paolucci [d,1], S.E. Sabatini [e,f], T. Noya Abad [e], C. Muñoz [b,c], F. Liquin [a], H. Hollert [g], F. Sylvester [a,c,g,*]

[a] Instituto para el Estudio de la Biodiversidad de Invertebrados (IEBI), Facultad de Ciencias Naturales, Universidad Nacional de Salta, Salta, Argentina
[b] Cátedra de Biología y Diversidad de Protistas Autótrofos y Fungi, Universidad Nacional de Salta, Salta, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CCT CONICET Salta-Jujuy, Salta, Argentina
[d] Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", CONICET, Buenos Aires, Argentina
[e] Laboratorio de Enzimología, Estrés Oxidativo y Metabolismo (LEEM), Departamento de Química Biológica, IQUIBICEN-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
[f] Departamento de Biodiversidad y Biología Experimental (DBBE), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
[g] Faculty of Biological Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany

HIGHLIGHTS

- Pesticides impact ecosystems in emerging agricultural regions worldwide.
- Invasive bivalves can be used for environmental monitoring in freshwater habitats.
- *Corbicula largillierti* show additive effects of glyphosate and imidacloprid.
- Combined biomarkers offer a more robust toxicity assessment than individual tests.

GRAPHICAL ABSTRACT



	SMR	Proteins	Glycogen	GSH	GST	TBARS
Glyphosate	-	-	-	-	-	-
Imidacloprid	↓	-	-	↓	-	-
Mixture	↓	-	↓	↓	-	↑

ARTICLE INFO

Editor: Damià Barceló

Keywords:
Invasive bivalves
Pesticides
Chemical mixtures

ABSTRACT

Pesticide mixtures are frequently utilized in agriculture, yet their cumulative effects on aquatic organisms remain poorly understood. Aquatic animals can be effective bioindicators and invasive bivalves, owing to their widespread distribution, provide an opportunity to assess these impacts. Glyphosate and imidacloprid, among the most prevalent pesticides globally, are frequently detected in freshwater systems in South America. This study aims to understand the cumulative effects of pesticide mixtures on aquatic organisms, using invasive *Corbicula largillierti* clams from a natural stream in northwestern Argentina. We conducted 48-hour exposure experiments

* Corresponding author at: Max-von-Laue Str. 13, 60438 Frankfurt am Main, Germany.
E-mail address: sylvester@bio.uni-frankfurt.de (F. Sylvester).
[1] Both authors contributed equally.

https://doi.org/10.1016/j.scitotenv.2024.173685
Received 22 February 2024; Received in revised form 3 May 2024; Accepted 30 May 2024
Available online 31 May 2024

Metabolism
Oxidative stress

using two concentrations of imidacloprid (20 and 200 μg L^{-1} a.i), two concentrations of glyphosate (0.3 and 3 mg L^{-1} a.i), and two combinations of these pesticides (both at low and high concentrations, respectively), simulating the direct contamination of both pesticides based on their agronomic recipe and observed values in Argentine aquatic environments. Clam metabolism was assessed through the examination of multiple oxidative stress parameters and measuring oxygen consumption rate as a proxy for standard metabolic rate (SMR). Our findings revealed that imidacloprid has a more pronounced effect compared to glyphosate. Imidacloprid significantly decreased clam SMR and cellular levels of reduced glutathione (GSH). However, when both pesticides were present, also cellular glycogen and thiobarbituric acid-reactive substances (TBARS) were affected. Proteins and glutathione S-Transferase (GST) activity were unaffected by either pesticide or their mixture at the assayed concentrations, highlighting the need to test several stress parameters to detect toxicological impacts. Our results indicated additive effects of imidacloprid and glyphosate across all measured parameters. The combination of multiple physiological and cytological biomarkers in invasive bivalves offers significant potential to enhance biomonitoring sensitivity and obtain insights into the origins and cellular mechanisms of chemical impacts. These studies can improve pollution regulatory policies and pesticide management.

1. Introduction

The health of aquatic ecosystems can be compromised by a variety of stressors arising from the numerous uses of water and the common lack of government controls and environmental protection (Ormerod et al., 2010). The massive use of pesticides has been identified as the primary source of contamination associated with the expansion of agricultural areas, impacting ecosystem and human health globally (Lykogianni et al., 2021; Nicolopoulou-Stamati et al., 2016). From 2011 to 2021, annual global pesticide usage consistently exceeded 3 million tons, with a peak of 3.5 million tons in 2021 (FAOSTAT, 2024). Studies on freshwater bodies' contamination reveal widespread and multiple chemical pollution, with herbicides being the most prevalent in the environmental mixtures (Navarro et al., 2024). Biological invasions are another growing threat to aquatic ecosystems, often occurring alongside chemical pollution. Despite impacts, some introduced species are potentially useful for ecotoxicological testing of chemical pollution due to their high abundance and biological characteristics (Sousa et al., 2008). Unfortunately, few studies simultaneously address both stressors (Sylvester et al., 2023).

In Argentina, the advance of industrial agriculture in recent decades has been accompanied by an increasing use of agrochemicals that can contaminate freshwater systems directly through air spraying and by containers washing in water bodies or indirectly by drift or runoff (De Gerónimo et al., 2014; López et al., 2006; Pérez et al., 2017). Glyphosate is known to impact freshwater Argentinian ecosystems promoting eutrophication (Pérez et al., 2007; Pizarro et al., 2016a; Vera et al., 2010; Vera et al., 2012) and pico-cyanobacteria growth (Castro Berman et al., 2020; Pizarro et al., 2016b; Zagarese et al., 2022). The use of imidacloprid has been increasing in extensive crops and horticulture (Iturburu et al., 2019), and their use particularly in combination with other agrochemical such as glyphosate represent a high ecological risk for local aquatic organisms (Pérez et al., 2021). In recent years, multiple studies have demonstrated the negative ecotoxicological impacts of both pesticides in freshwater ecosystems (Bonmatin et al., 2015; Bragança et al., 2018; Nicolopoulou-Stamati et al., 2016). Argentina holds the record for the highest environmental concentration of glyphosate in freshwater, at 105 mg L^{-1} (Brovini et al., 2021). The maximum reported concentration of imidacloprid in freshwater in the country were 18.50 μg kg^{-1} in sediments and 0.56 μg L^{-1} in surface water (Pérez et al., 2017). However, most of these studies have focused on the effects of these pesticides when applied separately or combined on terrestrial organisms (e.g., bees and humans; Mocellin Conte et al., 2022; Pal et al., 2022), while their combined effects on aquatic ecosystems are poorly understood.

Corbicula spp. is a genus of clams that originated in Southeast Asia. In association with human transport vectors such as transoceanic vessel ballast water, bilge water in fishing and recreational boats, live bait, and through its use as food by Asian communities, it has been introduced to and became invasive in freshwater habitats worldwide (McMahon, 2002; Sousa et al., 2008). It was introduced to Argentina ~50 years ago and has since become a dominant element in benthic communities (Ituarte, 1994). In the province of Salta, *Corbicula largillierti* (Phillipi 1844) was first detected in 1994 (Davies and Ramírez, 1997), and today it is found in many freshwater bodies in the province (Hünicken, 2020). Consequently, it provides an opportunity to being used as a biomonitoring tool. Unfortunately, despite its potential as an indicator of aquatic pollution, studies on *C. largillierti* are rare (but see Reyna et al., 2019, 2021), and attempts to assess the effects of pesticides on this species are altogether lacking. The congener *Corbicula fluminea* (Müller 1774) has previously been used for the ecotoxicological assessment of pesticides in different continents (e.g., dos Santos and Martínez, 2014; Shan et al., 2020). Yet, this better studied clam is minoritarian or altogether lacking across extensive agricultural regions of South America (Ludwig et al., 2024; Hünicken et al., in prep.), where *C. largillierti* provides an opportunity for biomonitoring.

Biomarkers allow the spotting of chemical toxic effects well before more obvious morphological changes or population declines become apparent (Lomartire et al., 2021; Chahouri et al., 2023). For example, stress enzymes and physiological responses have often been used to identify aquatic toxicity (dos Santos and Martínez, 2014; Iummato et al., 2018). In the case of the exotic mussel *Limnoperna fortunei* (Dunker 1857), morphological variations, such as changes in the relative gill area and shell morphology, have been linked to the presence of sediment and chemical pollution (Paolucci et al., 2014). Physiological variables, including metabolic and feeding rates in introduced bivalves, have served as biomarkers for assessing environmental stress and pollution in both marine and freshwater habitats (Barrera-Escorcia et al., 2010; Xiao et al., 2014; Collins et al., 2020). While the activation of cellular detoxification mechanisms offers an early warning of toxicity, effects at higher levels of organization, such as individual physiology, morphology, or behavior, have greater potential to translate into population and ecosystem impacts (Sureda et al., 2018; Lionetto et al., 2021). An applied challenge is that estimated toxicity can be highly dependent of the standards and methods used, and approaches to reconcile conclusions are needed (Hollert and Backhaus, 2019). One possible way to enhance the informativeness and sensitivity of monitoring is using a battery of biomarkers instead of single measurements (Potet et al., 2016).

This study aimed to experimentally evaluate, for the first time, the individual and combined effects of commercial formulations of glyphosate and imidacloprid on the freshwater invasive clam *C. largillierti*. To elucidate the ecotoxicological sublethal concentrations of these pesticides on aquatic organisms, we utilized glycogen content as an indicator of cellular energy status and multiple oxidative stress parameters as indicators of cellular redox status. In order to assess responses at the organism level, we measured respiration rates as a proxy for metabolic rates. Further, we hypothesized that, considering the different modes of action of the active ingredients, the combined effects would follow additive patterns on studied biomarkers of the clams. To test this, we

compared the effects of the mixtures to the algebraic sum of the effects from the clams treated with single doses.

2. Material and methods

2.1. Field methods and organism acclimation

Specimens of *C. largillierti* were sampled in an irrigation channel (length ca. 10 km, average width 1.5 m) in the town of Coronel Moldes, Salta, Argentina (25°16′25.4″S 65°28′31.2″W; see detailed map in Liquin et al., 2023) in August 2021. Individuals were collected manually. In-situ water characterization using a Hanna Edge multiparameter meter was 12.3 °C, conductivity 450 μS, pH 8.67. For dissolved nutrients, immediate determinations were done using Hach kits. Dissolved nitrogen was 3.7 mg L^{-1} and dissolved phosphorus 0.04 mg L^{-1}. Turbidity 6.99 NTU and total alkalinity 48.5 mg L^{-1} $CaCO_3$. Ca. 60 clams were transported within 2 h to the laboratory at the University of Salta, placed into small holding aquaria (10 L) supplied with dechlorinated tap water permanently aerated, and fed once per day with *Chlorella vulgaris* monoculture. Water was partially replaced, and temperature was adjusted daily at a rate of 1 °C per day until reaching the experimental temperature (18 °C).

2.2. Exposure experiments

A total of 35 clams were exposed for 48 h to two concentrations per pesticide and two pesticide combinations: low and high imidacloprid (LI and HI, respectively); low and high glyphosate (LG and HG, respectively); and low and high mixtures treatments (LM and HM). In addition, a control treatment (C) was applied using dechlorinated and filtered tap water. For that, five days after collection, each clam was randomly assigned individually to an experimental unit with 450 mL of filtered and dechlorinated tap water without pesticides (C), 20 (LI) or 200 μg L^{-1} (HI) of active ingredient (a.i) imidacloprid, 0.3 (LG) or 3 mg L^{-1} (HG) of a.i glyphosate and their mixtures 20 μg L^{-1} I + 0.3 mg L^{-1} G (LM) and 200 μg L^{-1} I + 3 G mg L^{-1} (HM) (Fig. 1). Pesticide concentrations were chosen to be representative of probable situations in natural aquatic ecosystems in Argentina using glyphosate environmental reports (Brovini et al., 2021) and within the range of previous studies for comparative purposes (e.g., Iummato et al., 2013; dos Santos and Martínez,

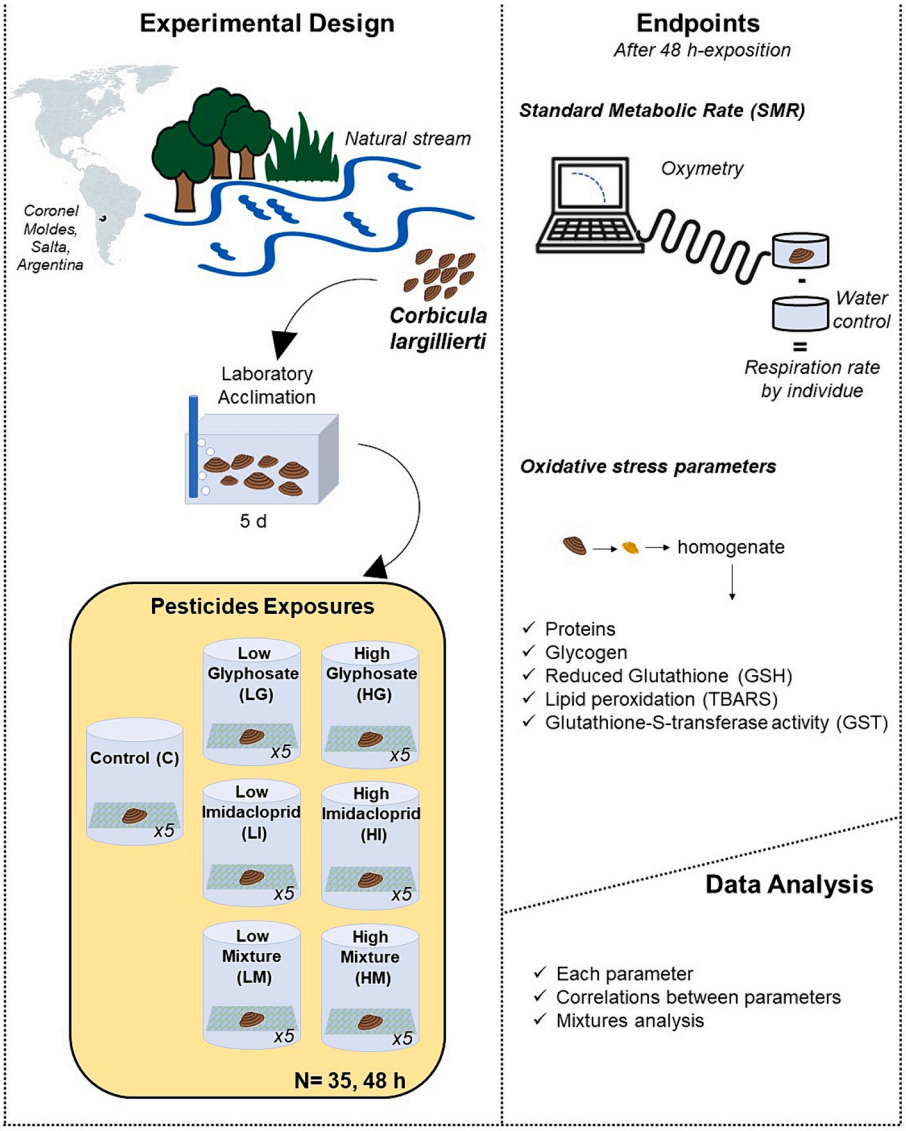


Fig. 1. General scheme of the experimental design and studied endpoints. Pesticide treatments were C (without pesticides), LG (0.3 mg L^{-1} glyphosate), HG (3 mg L^{-1} glyphosate), LI (20 μg L^{-1} imidacloprid), HI (200 μg L^{-1} imidacloprid), LM (0.3 mg L^{-1} glyphosate +20 μg L^{-1} imidacloprid) and HM (3 mg L^{-1} glyphosate +200 μg L^{-1} imidacloprid). Experimental units had 450 mL of filtered and dechlorinated tap water.

2014; Lozano et al., 2018; Lozano et al., 2020). For imidacloprid, the concentration in its mixture with glyphosate was estimated based on both: agronomic recipes for horticultural crops (at a ratio of 15:1 glyphosate to imidacloprid) and previous studies (Shan et al., 2020). This approach ensures relevance to real-world scenarios and enhances the ecological validity of our experimental design. Constant temperature of 18 °C, permanent aeration and natural day-night cycle were maintained throughout the experiments. The number of replicates per treatment was $n = 5$.

Stock solutions for each treatment were prepared using the appropriate volume of chemical into dechlorinated and filtered tap water. Exposure concentrations were prepared by pipetting the appropriate amount of the stock solutions into test chambers containing the additional volume of dechlorinated and filtered tap water. Each treatment had 5 replicates, a single individual per chamber was used (total 35 clams). Experiments were conducted in controlled temperature chambers at 18 °C and a natural 11.5–12.5 h day-night cycle. Clams were not fed during the exposure time. No dead individuals were recorded during or after the experiments (open valves and response to tactile stimuli were checked daily during the experiments).

2.3. Measurement of oxygen consumption and calculation of standard metabolic rates

Oxygen consumption in the experimental chambers was measured on individual specimens using planar oxygen-sensitive spot sensors and fiber optic oxygen sensitive coating (type PSt7-10) connected to an OXY-1 ST temperature-compensated single channel device (PreSens®, Regensburg, Germany). Sensor spots were glued in the internal glass face of the respiration chambers, following factory instructions, which allowed to record oxygen concentrations inside the sealed chambers approaching the fiber optic to the external face (Watson et al., 2013; Liquin et al., 2021). The combination of fiber optic and contactless oxygen sensors has proved to be a reliable method to measure oxygen concentration due to it reduces error variation, reducing drift over time, does not consume oxygen, and provides faster response time as a compared to polarographic oxygen sensors or even to chemical methods as Winkler (Papkovsky, 2004; Drown et al., 2020; Sukigara et al., 2021). Sensors were calibrated at 0 and 100 % oxygen saturation points using a 5 % sodium sulfite solution and gently stirred filtered water at 18 °C, respectively (Paolucci et al., 2022). Specimens were carefully transferred from the experimental units into the individual 30 mL glass chambers filled with treatment water at 18 °C, sealed and incubated for ~4 h, or until oxygen saturation reached a minimum of 50 %. The use of a sealed respiration chamber during a single period allows to yield an average metabolic rate based on the oxygen consumption at multiple time points for a single organism as the oxygen declines in the chamber, integrating changes on respiration activity along with natural metabolic variability (Auer et al., 2018). Measures of fourteen respiration chambers, seven with one clam each and seven using only experimental water (respiration controls) were recorded simultaneously on a personal computer through a serial connector until totalizing 35 individuals (fiver per treatment) and 35 water respiration control measurements. The measure of respiration in water was conducted to discard possible effects of the treatments in the water column. The use of antibiotics was avoided due to possible effects on clams' bacterial microfilms (Vargas et al., 2019), each clam's oxygen consumption rate was corrected by the respective water respiration control measurement. Oxygen concentrations were measured every 30 min in each chamber. Linear regressions of oxygen concentrations against the time were performed and the rate of oxygen consumption was estimated from the 50–80 % oxygen concentration interval.

After each respiration session, shell length (L), width (W), and height (H) were measured to the nearest 0.1 mm using a caliper in each clam (precision 0.01 mm) to estimate maximum anterior–posterior, lateral, and dorsal–ventral dimensions, respectively. These dimensions were used to estimate shell free dry weight (SFDW) and clam's volume following equations in Coughlan et al. (2021) and Novack-Gottshall (2008), respectively. Clam standard metabolic rates (SMR) were calculated as the consumed oxygen (μmol) of each clam from the chamber and relativized to each specimen using its SFDW in grams. All clams were conserved at −80 °C after respiration and kept for further oxidative stress analyses.

2.4. Oxidative stress parameters

Soft tissue body of each individual was homogenized separately with an agitator machine (Precytec, 1500 rpm) in 0.154-M ice-cold KCl solution (1:6 weight:volume ratio) with protease inhibitors (0.2-mM benzamidine and 0.5-mM phenylmethylsulfonul fluoride). Homogenates were used for determining the total glycogen content (Van Handel, 1965). The rest of each homogenate was further centrifuged for 20 min at 11,000 g and 4 °C. The supernatant was used for enzyme assays, and determination of proteins and reduced glutathione contents and acid-reactive substances by the means of molecular spectrophotometric methods (UV-160A Shimadzu Corporation).

Proteins were determined following Bradford (1976). The technique used to measure reduced glutathione (GSH) was the Anderson (1985) method. This procedure involved the deproteinization of the samples using 10 % sulfosalicylic acid. Next, GSH concentration was oxidized by 5.5-dithiobis-2-nitrobenzoic acid (DTNB) in a 0.134-M sodium phosphate buffer (pH 7.5, 6.3 mM EDTA) and monitored at 412 nm. The results were expressed as nanomoles of GSH per milligram of protein. The levels of lipid peroxidation were determined using the modified method of Buege and Aust (1978) by estimating the concentration of thiobarbituric acid-reactive substances (TBARS). Samples were incubated with 0.37 % thiobarbituric acid, 15 % trichloroacetic acid, and 0.68-mM butylated hydroxytoluene at 100 °C for 15 min. After cooling, the samples were centrifuged at 11,000*g* for 10 min at 4 °C, and the concentration of TBARS was measured at 535 nm. The results were expressed as micromoles of TBARS per milligram of protein. Finally, glutathione S-Transferase (GST) activity was determined following the method described by Habig et al. (1974). The assay involved monitoring changes in the absorbance of 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm for a duration of 90 s. A single GST unit was defined as the enzyme quantity required to catalyze the formation of 1 μmol of DNP-SG per minute at 25 °C. The results were expressed in units of GST per milligram of protein.

2.5. Data analysis

To assess the treatment effects on *Corbicula largillierti*'*s* metabolic parameters, ANOVA and Holm-Sidak comparisons with the control were conducted in SigmaPlot v.14. Where the ANOVA assumptions of normality (Shapiro-Wilk test) and homoscedasticity (Brown-Forsythe test) were not met, various transformations were tested until they were satisfied. Only SMR data was transformed using the square root. Principal Component Analysis classified by treatments was runned in InfoStat v.2020 using standardized values. Mixture effects analyses were employed to identify any antagonistic, additive, or synergistic interactions resulting from the mixture treatments (LM and HM) on all metabolic parameters. Specifically, observed effects were compared to additive expected effects using Student's *t*-test, and the 95 % confidence intervals (CI95) of the expected additivity effects were compared to the obtained and normalized CI95 for the graphical resume, following Lozano et al. (2018).

3. Results

The clams used in this study ranged 12.6–19.1 mm length (average 14.4 mm) and 0.012–0.027 g_{SFDW} (average 0.019 g_{SFDW}), no significant differences in SFDW were found among pesticide treatments (ANOVA, p

= 0.8007).

The average SMR (±SD) for the control clams was 31.36 ± 13.05 μmol O_2 g_{SFDW}^{-1} h^{-1}. SMR was significantly reduced in HI, LM and HM treatments (ANOVA, $p < 0.001$, $p = 0.029$ and $p < 0.001$ respectively) (Fig. 2A). The highest and significant decreases in the metabolic rates were observed when the imidacloprid treatment was applied at the highest concentration, in which metabolic rates decreased until average values of 10.30 ± 6.04 μmol O_2 g_{SFDW}^{-1} h^{-1}. At low imidacloprid concentrations, standard metabolic rates decreased to 18.02 ± 16.40 and 16.59 ± 15.20 μmol O_2 g_{SFDW}^{-1} h^{-1} for the LI and LM, respectively. The oxygen variations from the water without specimens (respiration controls) did not show significant variations between treatments (ANOVA, $p = 0.951$), average was 0.05 μmol O_2 h^{-1} with a range from −0.19 to 0.18. When glyphosate was applied as a single treatment it did not produce variations of metabolic rates values with respect to non-pesticide controls regardless of the used concentration.

Protein content showed no significant differences among any of the treatments (ANOVA, $p = 0.585$; Fig. 2B) while glycogen concentration was significantly reduced by 37–40 % in the LM and HM treatments compared to the control (ANOVA, $p = 0.0017$ and $p = 0.024$ respectively; Fig. 2C). GSH was reduced by 28–37 % in LI, LM, and HM treatments (ANOVA, $p < 0.001$) while GST activity showed no significant differences in any of the treatments (Fig. 2D and E). Finally, TBARS almost doubled in the HM treatment compared with the control (ANOVA, $p = 0.0013$) (Fig. 2E).

The ordination of treatments using all biomarkers accounted for 76.9 % of the total variance (Fig. 3). Principal Component 1 (CP1) was

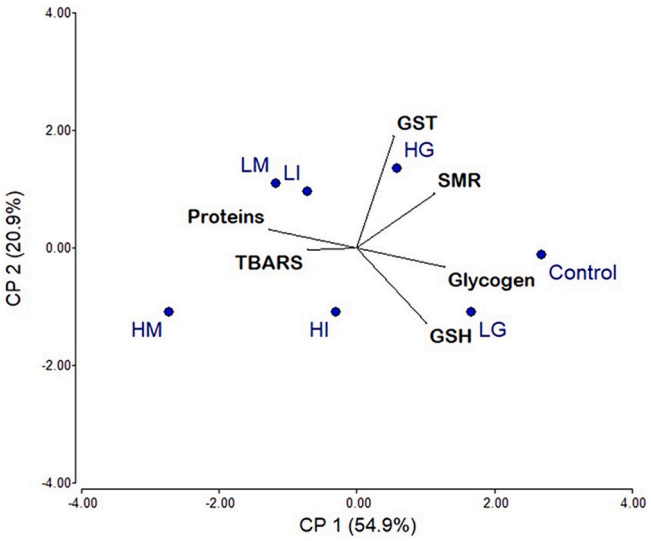


Fig. 3. Principal component analysis of all the studied biomarkers classified by treatments (5 replicates by treatment).

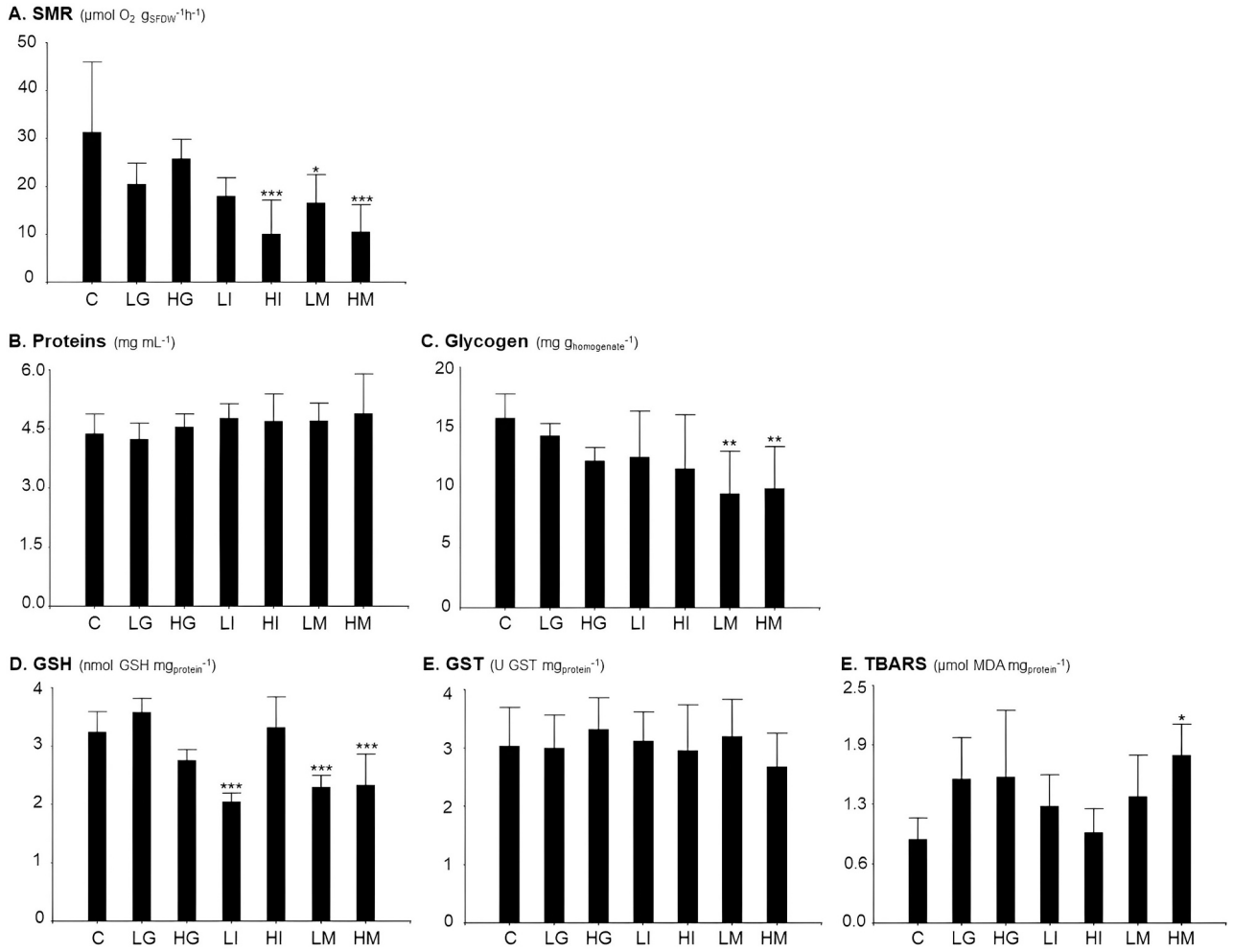


Fig. 2. Metabolic and oxidative stress parameters of *C. largillierti* after a 48-h exposure period. C (without pesticides), LG (0.3 mg L^{-1} glyphosate), HG (3 mg L^{-1} glyphosate), LI (20 μg L^{-1} imidacloprid), HI (200 μg L^{-1} imidacloprid), LM (0.3 mg L^{-1} glyphosate +20 μg L^{-1} imidacloprid) and HM (3 mg L^{-1} glyphosate +200 μg L^{-1} imidacloprid). Asterisks show significant differences to controls (ANOVA, Holm-Sidak comparisons, *p<0.05, **p <0.01, ***p <0.001).

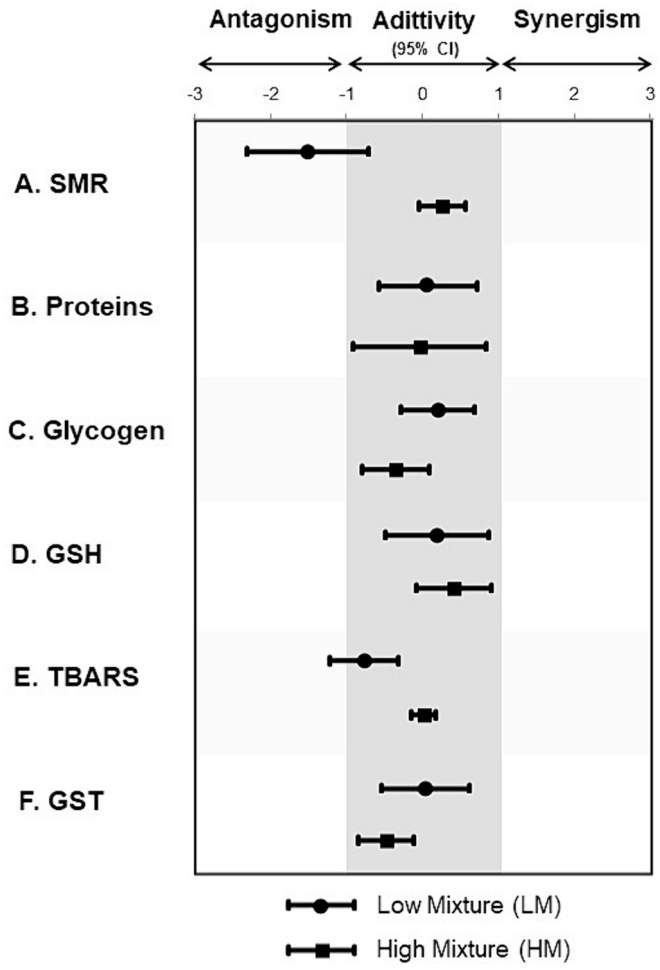


Fig. 4. Mixture's effects analysis on all the parameters. Confidence intervals inside the grey-zone are not statically significant for t-student when obtained and expected additivity results of the mixtures are compared.

positively correlated with glycogen and GSH, and inversely correlated with TBARS, explaining 54.9 % of the variance. Meanwhile, Principal Component 2 (CP2) was directly correlated with GST, explaining 20.9 % of the variance. The distribution of treatments was linked to the control group exhibiting high glycogen levels, while the LG and HI groups were associated with elevated GSH content. Treatments LI, LM, and HG were associated with GST. On the other hand, HM was linked to high TBARS values.

Mixture analysis shows that glyphosate and imidacloprid, at low and high concentrations, acts additively on all the parameters (t-student, $p>0.05$ in all cases, Fig. 4).

4. Discussion

The present results represent the first bioassays conducted using *C. largillierti* and the first available assessment of the effects of pesticides on bivalve metabolic rates in combination with other stress biomarkers. Our experiments shows that when applied individually, imidacloprid has a significant impact on *C. largillierti* metabolism, particularly on standard metabolic rate, whereas this effect was not observed in glyphosate treatment. However, when glyphosate and imidacloprid are combined, they exhibit additive effects in all the analyzed endpoints. These combined effects account for most of the significant results, which include not only a decrease in respiration rate but also a reduction in glycogen content and changes in several indicators of non-enzymatic responses to stressful conditions.

4.1. Effects of pesticides on metabolic rates

Prior to this study, the metabolic rates of *C. largillierti* have been measured only twice. The respiration rates measured in our study fell in the range of previously reported values for unexposed *C. largillierti* (Hünicken et al., 2022a; Rodríguez et al., 2020), as well as for *Corbicula* spp. in general (reviewed in Hünicken et al., 2022b). We did not observe any significant decrease in these rates when the clams were exposed to low or high concentrations of glyphosate. However, we did observe significant decreases up to 68 % in respiration rate after a two-day exposure to imidacloprid alone (high concentration) or in combination with glyphosate (any concentration). Previous reports have shown reduced oxygen consumption rates after exposure to neonicotinoid (Thiamethoxam) and organophosphate insecticides (Chlorpyrifos, Dimethoate) in freshwater bivalves (Kumar et al., 2012; Minakshi and Mahajan, 2013; Yancheva et al., 2017). Imidacloprid has been shown to have effects on gill morphology and filtration rates in several freshwater (Ahire and Dethe, 2023; Ewere et al., 2021) and marine mollusks (Ewere et al., 2019a; Ewere et al., 2019b; Malhotra et al., 2021). In the case of *Corbicula fluminea*, significant histopathological alterations of the gills and reductions in filtration rates were observed after exposure to imidacloprid concentrations ranging 20–2000 μg L^{-1} for 30 days (Shan et al., 2020). To the best of our knowledge, our results for *C. largillierti* constitute the first direct observation of impact of imidacloprid on respiration rates, confirming the swift and significant impacts of this pesticide on non-target aquatic organisms.

4.2. Effects of pesticides on oxidative stress parameters

Our analyses of oxidative stress parameters also suggest significant impacts of pesticides on aquatic organisms. We observed significant decreases in GSH levels in clams exposed to imidacloprid alone or in combination with glyphosate. GSH is a non-enzymatic antioxidant that protects organisms against the effects of oxidative stress (Cnubben et al., 2001). GSH levels have been observed to change (either increase or decrease, depending on the substance and organism) as a response to oxidative stress induced by pesticides (Sule et al., 2022). For example, GSH levels were found to increase in the gills of the freshwater mussel *Unio mancus* when exposed to concentrations of imidacloprid at 1000 μg L^{-1}, with lower concentrations having no effects (Yoloğlu, 2019). An increase in GSH levels can be interpreted as a cellular protective response when oxidative stress agents are detected, whereas decreases could be seen as a depletion of resources as stress agents increase further and defense mechanisms get overwhelmed. GSH decreases in *C. largillierti* exposed to imidacloprid, even at concentrations that did not produce any changes in *Unio mancus*, might indicate a higher sensitivity in the former.

TBARS and glycogen also provide evidence of oxidative stress in *C. largillierti*. TBARS is one of the earlier markers developed for detecting oxidative damage to lipids (Dasgupta and Klein, 2014; Sule et al., 2022), which was significant under high-mixture conditions. Both mixture conditions showed decreases in glycogen levels. It is conceivable that reduced siphoning or valve closure occurred in these treatments, as inhibition siphoning behavior has been observed in *Corbicula* sp. clams exposed to imidacloprid at concentrations ranging 20–2000 μg L^{-1} for 30 days (Shan et al., 2020). Glycogen is a form of cellular energy storage. Anaerobic metabolic pathways used during anoxia are less efficient than aerobic pathways and can lead to its depletion.

GST is enzyme protecting against xenobiotics that has been shown to play a role in imidacloprid and glyphosate detoxification several freshwater invasive bivalves including *C. fluminea* (Yoloğlu, 2019; Shan et al., 2020; Iummato et al., 2013, 2018, 2024). The fact that it was not affected by any pesticide treatment in our study could be attribute to the short exposure time (48 h) of our experiments. Previous studies have observed increases in GST in *C. fluminea* clams exposed to imidacloprid concentrations of 20–2000 μg L^{-1} after 30 d (Shan et al., 2020). GST

increases have been observed for other bivalve and gastropod mollusks onlyy after 4–14 d of exposure (Yoloğlu, 2019; Cossi et al., 2020). Iummato et al. (2013, 2018) have observed GST increases in invasive *Limnoperna fortunei* golden mussels exposed to glyphosate supplied dietarily and directly at concentrations between 1 and 6 mg L^{-1} in Buenos Aires. Yet these effects were not seen before 21–26 d. Similar results were obtained for the freshwater bivalve *Diplodon chilensis* in Patagonia exposed to 4–6 mg L^{-1}, where significant GST increases occurred after 7 d in dietary exposures and 14 d in direct exposures (Iummato et al., 2024). By contrasts, shorter (96-h) exposures at 2–10 mg L^{-1} did not have any GST effects on *C. fluminea* (dos Santos and Martínez, 2014). These comparisons suggest that our 48 h exposures were too short to observe and effect of either pesticide on GST. The delay in the effects is likely related to time needed by the cellular machinery to express and synthesize GST, which can be enhanced by the specific metabolic detoxification pathway followed for each compound. GST is primarily involved in phase II metabolism. The prime detoxification pathway for imidacloprid is phase I metabolism while phase II metabolism might be activated secondarily, after phase one has been overwhelmed (Lu et al., 2016).

4.3. Mixture effects

This is the first study on the combined action of glyphosate and imidacloprid on freshwater bivalves. Our results provide evidence of additive effects on *C. largillierti*, as mixtures of imidacloprid and glyphosate produced effects on stress markers (TBARS and glycogen) that were not observed when the individual pesticides were applied separately. Recent research has shown synergistic effects these pesticides on human liver (HepG2) cells (Conte et al., 2022) and either synergistic or additive effects on bees, depending on pesticide concentration (Pal et al., 2022). In concordance with our own results, these studies suggest that intermediate concentrations have higher effects than high concentrations (Pal et al., 2022). In aquatic environments, it has recently been observed that pesticides mixtures including glyphosate and imidacloprid induce both oxidative stress and genotoxicity in caimans in Argentina, although the action of the two compounds appears to be independent (Odetti et al., 2023). In that study, toxicity was primarily led by imidacloprid, which is in coincidence with our results (SMR and GSH) for Asian clams.

The effects of pesticides mixtures may not be permanent, as reported in other bivalves where a reduction of imidacloprid residuals and other pesticides was observed after a depuration period in pesticide-free water (Beltran and Pocsidio, 2010; Siu et al., 2004), or even after 2–3 days of continuous exposure in bivalves (Ewere et al., 2019a). However, considering the widespread use and persistence of these chemicals and their transformation products in the water (Thuyet et al., 2011), it is expected that exposure in natural ecosystems is likely to be longer and involve a greater variety of chemical compounds than those considered in the present study.

The complexities highlighted above underscore the difficulty of predicting the effects of pesticides on aquatic ecosystems. Our results suggest on the one hand that a strongly regulatory focus on imidacloprid should be placed. On the other hand, however, it is increasingly clear that overall toxicity is not provided by one component or the other but the mixture. Even individual components behave sometimes unexpectedly when, for example, lower concentrations yield higher toxicity than higher concentrations (Yoloğlu, 2019; Pal et al., 2022; Fig. 2D). Further research on pesticides mixtures and their interaction with other stressors (e.g., invasive species, habitat destruction, and climate change) is clearly needed before we can understand these effects sufficiently to inform policy and management. This need is highlighted by the fact that chemical mixtures and multiple stressors enhancing aquatic toxicity and ecosystem impacts (Pizarro et al., 2016a) likely represent common scenarios in agroecosystems (Bonmatin et al., 2015; Ewere et al., 2019a; Ewere et al., 2019b; LeBlanc et al., 2012).

4.4. Multiple biomarker approach

Our results demonstrate the advantages of adopting a combined and multilevel approach for detecting stressors or pollution effects (Lionetto et al., 2021). Cellular markers are potentially fast and easy to measure. Yet, unless organism physiology is affected, effects at the cellular level do not necessarily translate into consequences for fitness that can scale up to impact populations, communities, and ecosystems. Here, we have established direct links between changes on oxidative stress parameters and metabolic effects in *C. largillierti*. Furthermore, while physiological endpoints provide valuable information at the organism level, oxidative stress parameters can enhance sensitivity and offer precise insights into the source and cellular mechanisms of chemical impacts. Thus, the combination of multiple endpoints significantly enhances our understanding and predictive capabilities. Our results also underscore the potential for using invasive bivalves as bioindicators to monitor pollution in freshwater ecosystems.

A potential limitation of our approach is related to the existence of multiple commercial forms of the tested pesticides in the market. These forms may have different formulations, sometimes not precisely described. They can exert distinct effects on ecotoxicological biomarkers among themselves and differ from the active ingredients due to added toxicity from additives (Maletz et al., 2015). Moreover, once released into the environment, both the active ingredients and the additives can follow various transformation pathways and interact in unexpected ways, depending on the application procedures and ambient physicochemical conditions. These conditions can substantially differ from laboratory settings (Maletz et al., 2015) and their influence cannot be adequately assessed without the use of more sophisticated and realistic experimental settings, which are not universally available in every research center (e.g., Van den Brink et al., 2005). A further complexity arises from the potential impact of the ecotoxicological markers used and the specific exposure scenario assessed. A more recent study by Benbrook (2019) clearly exposed the vastly different conclusions that can be drawn regarding the toxicity of glyphosate to humans, depending on how these factors were considered by two different regulatory frameworks. These studies underscore the challenges associated with assessing the ecological impacts of even common agrochemicals (Hollert and Backhaus, 2019).

4.5. Implications for invasion ecology

Our study holds potential implications in the field of invasion ecology, particularly concerning the prediction of invasive potential. According to our present results, chemical pollution might impose limits on the spread of freshwater invasive bivalves in some situations. It can also influence the replacement of invasive species based on their differing physiological responses to chemical stressors (Potet et al., 2016).

In the case of *C. largillierti* and *C. fluminea*, two closely related invasive species typically introduced to the same environments, various factors, such as competition (Pereira et al., 2014), differences in tolerance to environmental conditions (Rodríguez et al., 2020), and physiological differences (Hünicken et al., 2022a), have been proposed to explain coexistence in Argentina. *Corbicula largillierti* tends to prevail in low-order rivers and lakes in the northern and central part of the country, where *C. fluminea* is relatively rare (Pereira et al., 2014; Rodríguez et al., 2020; Rumi et al., 2008). *Corbicula fluminea* is usually considered the stronger competitor and replaces *C. largillierti* in more productive habitats (Hünicken et al., 2022a). However, further studies are necessary to compare the response of these species to agrochemical exposure and analyze the role of chemical pollution in species substitutions.

4.6. Conclusions and next steps

Physiological endpoints allow us to detect the effects of chemical

pollution well before more obvious morphological changes or population declines become apparent. When combined with enzymatic markers, this approach not only enhances our ability to detect these effects but also help us trace their causes. It highlights the potential use of widespread and abundant invasive bivalves as potential bioindicators to monitor the degradation of aquatic ecosystems. Contaminants in chemical mixtures can potentiate and behave in unpredictably. Future research steps should include the assessment of condition index and morphological variables to further improve the use of this species as biomarker. Since exposure times vary among studies, conducting experiments at different exposure times would be a valuable next research step to gain deeper understanding of cellular stress pathways in *C. largillierti* exposed to imidacloprid. Moreover, experimentation under more realistic conditions, which test different commercial forms and incorporate the use of increasingly complex chemical mixtures, exposure to multiple stressors, and the utilization of mesocosms, should be considered.

Compliance with ethical standards

The authors declare that she has no conflict of interest. This article does not involve any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

V.L. Lozano: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **E.M. Paolucci:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review & editing. **S.E. Sabatini:** Methodology, Supervision, Writing – review & editing. **T. Noya Abad:** Formal analysis, Methodology, Writing – review & editing. **C. Muñoz:** Formal analysis, Methodology, Writing – review & editing. **F. Liquin:** Formal analysis, Methodology, Writing – review & editing. **H. Hollert:** Supervision, Writing – review & editing. **F. Sylvester:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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.

Acknowledgments

The authors would like to thank CONICET and the Public Universities (UBA and UNSa) where they work for their support. H.H. and F.S. have been supported by the RobustNature Excellence Initiative (internal prefunding of the Goethe University Frankfurt am Main). This research received funding through research grants from the Argentine Agencia Nacional de Promoción Científica y Tecnológica (PICT 2016–0631, PICT-2020-SERIE A-03802, CIUNSa 2621/0 to F.S., PIP 0252 2021-2023, and PICT 2020-0629 to E.M.P.). We thank Dr. Liliana Moraña for laboratory assistance, and Fabian G. Weichert and two anonymous reviewers for useful comments on an earlier version of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.173685.

References

Ahire, P.B., Dethe, V.D., 2023. Evaluation of Imidacloprid-induced toxicity and lipid peroxidation in the freshwater bivalve, *Lamellidens marginalis*. Int. J. Aquat. Biol. 11, 124–130. https://doi.org/10.22034/ijab.v11i2.1902.

Anderson, M.E., 1985. Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol. 113, 548–553. https://doi.org/10.1016/S0076-6879(85)13073-9.

Auer, S.K., Salin, K., Anderson, G.J., Metcalfe, N.B., 2018. Individuals exhibit consistent differences in their metabolic rates across changing thermal conditions. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 217, 1–6. https://doi.org/10.1016/j.cbpa.2017.11.021.

Barrera-Escorcia, G., Vanegas-Perez, C., Wong-Chang, I., 2010. Filtration rate, assimilation and assimilation efficiency in *Crassostrea virginica* (Gmelin) fed with *Tetraselmis suecica* under cadmium exposure. J. Environ. Sci. Health A 45 (1), 14–22. https://doi.org/10.1080/10934520903388533.

Beltran, K.S., Pocsidio, G.N., 2010. Acetylcholinesterase activity in *Corbicula fluminea* Mull., as a biomarker of organophosphate pesticide pollution in Pinacanauan River, Philippines. Environ. Monit. Assess. 165, 331–340. https://doi.org/10.1007/s10661-009-0949-y.

Benbrook, C.M., 2019. How did the US EPA and IARC reach diametrically opposed conclusions on the genotoxicity of glyphosate-based herbicides? Environ. Sci. Eur. 31 https://doi.org/10.1186/s12302-018-0184-7.

Bonmatin, J.M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A.D., Noome, D.A., Simon-Delso, N., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environ. Sci. Pollut. Res. 22, 35–67. https://doi.org/10.1007/s11356-014-3332-7.

Bradford, M.M., 1976. A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1006/abio.1976.9999.

Bragança, I., Grosso, C., Rede, D., Sousa, S.R., Lemos, P., Domingues, V.F., Delerue-Matos, C., 2018. Ecotoxicological effects of insecticides in plants assessed by germination and other phytotoxicity tools. In: Biotic and Abiotic Stress Tolerance in Plants, pp. 47–76. https://doi.org/10.1007/978-981-10-9029-5_3.

Brovini, E.M., Cardoso, S.J., Quadra, G.R., Vilas-Boas, J.A., Paranaíba, J.R., Pereira, R.D. O., Mendonça, R.F., 2021. Glyphosate concentrations in global freshwaters: are aquatic organisms at risk? Environ. Sci. Pollut. Res. 28 (43), 60635–60648. https://doi.org/10.1007/s11356-021-14609-8.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302–310. https://doi.org/10.1016/S0076-6879(78)52032-6.

Castro Berman, M., Llames, M.E., Minotti, P., Fermani, P., Quiroga, M.V., Ferraro, M.A., Metz, S., Zagarese, H.E., 2020. Field evidence supports former experimental claims on the stimulatory effect of glyphosate on picocyanobacteria communities. Sci. Total Environ. 701, 134601 https://doi.org/10.1016/j.scitotenv.2019.134601.

Chahouri, A., Yacoubi, B., Moukrim, A., Banaoui, A., 2023. Bivalve molluscs as bioindicators of multiple stressors in the marine environment: recent advances. Cont. Shelf Res. 264, 105056 https://doi.org/10.1016/j.csr.2023.105056.

Cnubben, N.H., Rietjens, J.M., Wortelboer, H., van Zanden, J., van Bladeren, P.J., 2001. The interplay of glutathione-related processes in antioxidant defense. Environ. Toxicol. Pharmacol. 10, 141–152. https://doi.org/10.1016/s1382-6689(01)00077-1.

Collins, C.L., Burnett, N.P., Ramsey, M.J., Wagner, K., Zippay, M.L., 2020. Physiological responses to heat stress in an invasive mussel *Mytilus galloprovincialis* depend on tidal habitat. Mar. Environ. Res. 154, 104849 https://doi.org/10.1016/j.marenvres.2019.104849.

Conte, F.M., Cestonaro, L.V., Piton, Y.V., Guimarães, N., Garcia, S.C., da Silva, D.D., Arbo, M.D., 2022. Toxicity of pesticides widely applied on soybean cultivation: synergistic effects of fipronil, glyphosate and imidacloprid in HepG2 cells. Toxicol. in Vitro 84, 105446. https://doi.org/10.1016/j.tiv.2022.105446.

Cossi, P.F., Herbert, L.T., Yusseppone, M.S., Pérez, A.F., Kristoff, G., 2020. Toxicity evaluation of the active ingredient acetamiprid and a commercial formulation (Assail® 70) on the non-target gastropod *Biomphalaria straminea* (Mollusca: Planorbidae). Ecotoxicol. Environ. Saf. 192, 110248 https://doi.org/10.1016/j.ecoenv.2020.110248.

Coughlan, N.E., Cunningham, E.M., Cuthbert, R.N., Joyce, P.W.S., Anastácio, P., Banha, F., Bonel, N., Bradbeer, S.J., Briski, E., Butitta, V.L., Čadková, Z., Dick, J.T.A., Douda, K., Eagling, L.E., Ferreira-Rodríguez, N., Hünicken, L.A., Johansson, M.L., Kregting, L., Labecka, A.M., Li, D., Liquin, F., Marescaux, J., Morris, T.J., Nowakowska, P., Ożgo, M., Paolucci, E.M., Peribáñez, M.A., Riccardi, N., Smith, E.R. C., Spear, M.J., Steffen, G.T., Tiemann, J.S., Urbańska, M., Van Doninck, K., Vastrade, M., Vong, G.Y.W., Wawrzyniak-Wydrowska, B., Xia, Z., Zeng, C., Zhan, A., Sylvester, F., 2021. Biometric conversion factors as a unifying platform for comparative assessment of invasive freshwater bivalves. J. Appl. Ecol. 58 (9), 1945–1956. https://doi.org/10.1111/1365-2664.13941.

Dasgupta, A., Klein, K., 2014. Methods for measuring oxidative stress in the laboratory. In: Antioxidants Food, Vitam. Suppl, pp. 19–40. https://doi.org/10.1016/B978-0-12-405872-9.00002-1.

Davies, D.A., Ramírez, V.G., 1997. Hallazgo de *Corbicula largillierti* (Philippi, 1844) en la Provincia de Salta (Mollusca, Corbiculidae). VI J. Cienc. Nat. Litoral 52, 5–8.

De Gerónimo, E., Aparicio, V.C., Bárbaro, S., Portocarrero, R., Jaime, S., Costa, J.L., 2014. Presence of pesticides in surface water from four sub-basins in Argentina. Chemosphere 107, 423–431. https://doi.org/10.1016/j.chemosphere.2014.01.039.

dos Santos, K.C., Martínez, C.B.R., 2014. Genotoxic and biochemical effects of atrazine and Roundup®, alone and in combination, on the Asian clam *Corbicula fluminea*. Ecotoxicol. Environ. Saf. 100 (1), 7–14. https://doi.org/10.1016/j.ecoenv.2013.11.014.

Drown, M.K., DeLiberto, A.N., Crawford, D.L., Oleksiak, M.F., 2020. An innovative setup for high-throughput respirometry of small aquatic animals. Front. Mar. Sci. 7 https://doi.org/10.3389/fmars.2020.581104.

Ewere, E.E., Powell, D., Rudd, D., Reichelt-Brushett, A., Mouatt, P., Voelcker, N.H., Benkendorff, K., 2019a. Uptake, depuration and sublethal effects of the neonicotinoid, imidacloprid, exposure in Sydney rock oysters. Chemosphere 230, 1–13. https://doi.org/10.1016/j.chemosphere.2019.05.045.

Ewere, E.E., Reichelt-Brushett, A., Benkendorff, K., 2019b. Imidacloprid and formulated product impacts the fatty acids and enzymatic activities in tissues of Sydney rock oysters, *Saccostrea glomerata*. Mar. Environ. Res. 151, 104765 https://doi.org/10.1016/j.marenvres.2019.104765.

Ewere, E.E., Reichelt-Brushett, A., Benkendorff, K., 2021. Impacts of neonicotinoids on molluscs: what we know and what we need to know. Toxics 9 (2), 21. https://doi.org/10.3390/toxics9020021.

FAOSTAT, Food and Agriculture Organization of the United Nations, 2024. United Nations. https://www.fao.org/faostat/en/#home.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249 (22), 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8.

Hollert, H., Backhaus, T., 2019. Some food for thought: a short comment on Charles Benbrook's paper "How did the US EPA and IARC reach diametrically opposed conclusions on the genotoxicity of glyphosate-based herbicides?" and its implications. Environ. Sci. Eur. 31 https://doi.org/10.1186/s12302-019-0187-z.

Hünicken, L., 2020. Invasión de *Corbicula* spp. en Argentina: distribución, variaciones morfológicas y metabolismo de un bivalvo invasor global. PhD Thesis. Universidad de Buenos Aires (143 pp.).

Hünicken, L.A., Sylvester, F., Paolucci, E.M., 2022a. Physiological and morphological assessments suggest opposite structural allocation strategies between closely related invasive clams. Hydrobiologia 849, 2859–2875. https://doi.org/10.1007/s10750-022-04906-9.

Hünicken, L.A., Sylvester, F., Bonel, N., 2022b. Fitness-related traits are maximized in recently introduced, slow-growing populations of a global invasive clam. Invertebr. Biol. 141 (1), e12364 https://doi.org/10.1111/ivb.12364.

Hünicken, L.A., Paolucci, E.M., Lavinia, P.D., Sylvester, F.. Morphological and Genetic Assessment of Invasive Corbicula Lineages in Southern South America: A Case Study in Argentina (Preprints). https://www.preprints.org/manuscript/202405.0457/v1.

Ituarte, C.F., 1994. *Corbicula* and *Neocorbicula* (Bivalvia: Corbiculidae) in the Paraná, Uruguay and Río de La Plata basins. Nautilus 107, 129–135.

Iturburu, F.G., Calderon, G., Amé, M.V., Menone, M.L., 2019. Ecological Risk Assessment (ERA) of pesticides from freshwater ecosystems in the Pampas region of Argentina: legacy and current use chemicals contribution. Sci. Total Environ. 691, 476–482. https://doi.org/10.1016/j.scitotenv.2019.07.044.

Iummato, M.M., Di Fiori, E., Sabatini, S.E., Cacciatore, L.C., Cochón, A.C., Ríos de Molina, M.D.C., Juárez, A., 2013. Evaluation of biochemical markers in the golden mussel *Limnoperna fortunei* exposed to glyphosate acid in outdoor microcosms. Ecotoxicol. Environ. Saf. 95, 123–129. https://doi.org/10.1016/j.ecoenv.2013.05.024.

Iummato, M.M., Sabatini, S.E., Cacciatore, L.C., Cochón, A.C., Cataldo, D., Ríos de Molina, M.D.C., Juárez, A., 2018. Biochemical responses of the golden mussel *Limnoperna fortunei* under dietary glyphosate exposure. Ecotoxicol. Environ. Saf. 163, 69–75. https://doi.org/10.1016/j.ecoenv.2018.07.046.

Iummato, M.M., Sabatini, S.E., Rocchetta, I., Yusseppone, M.S., del Carmen Ríos de Molina, M., Juárez, Á.B., 2024. Oxidative stress in the bivalve *Diplodon chilensis* under direct and dietary glyphosate-based formulation exposure. Environ. Sci. Pollut. Res. 1-13 https://doi.org/10.1007/s11356-024-32639-w.

Kumar, S., Pandey, R.K., Das, S., Das, V.K., 2012. Dimehoate alters respiratory rate and gill histopathology in freshwater mussel *Lamellidens Marginalis* (Lamarck). J. Appl. Biosci. 38, 154–158.

LeBlanc, H.M.K., Culp, J.M., Baird, D.J., Alexander, A.C., Cessna, A.J., 2012. Single versus combined lethal effects of three agricultural insecticides on larvae of the freshwater insect *Chironomus dilutus*. Arch. Environ. Contam. Toxicol. 63 (3), 378–390. https://doi.org/10.1007/s00244-012-9777-0.

Lionetto, M.G., Caricato, R., Giordano, M.E., 2021. Pollution biomarkers in the framework of marine biodiversity conservation: state of art and perspectives. Water 13 (13), 1847. https://doi.org/10.3390/w13131847.

Liquin, F., Hünicken, L.A., Arrighetti, F., Davies, D., Paolucci, E.M., Sylvester, F., 2021. Parasitism and fitness of invaders: oligochaete *Chaetogaster limnaei* produces gill damage and increased respiration rates in freshwater Asian clams. Hydrobiologia 848 (9), 2213–2223. https://doi.org/10.1007/s10750-020-04424-6.

Liquin, F., Sylvester, F., Davies, D., 2023. Susceptibility of invasive Asian clams to *Chaetogaster limnaei*: effect of parasite density and host size on infection dynamics. Biol. Invasions 25, 1087–1099. https://doi.org/10.1007/s10530-022-02964-7.

Lomartire, S., Marques, J.C., Gonçalves, A.M.M., 2021. Biomarkers based tools to assess environmental and chemical stressors in aquatic systems. Ecol. Indic. 122, 107207 https://doi.org/10.1016/j.ecolind.2020.107207.

López, L., Pozo, C., Rodelas, B., Calvo, C., González-López, J., 2006. Influence of pesticides and herbicides presence on phosphatase activity and selected bacterial microbiota of a natural lake system. Ecotoxicology 15 (5), 487–493. https://doi.org/10.1007/s10646-006-0084-2.

Lozano, V.L., Vinocur, A., Sabio y García, C., Allende, L., Cristos, D.S., Rojas, D., Wolansky, M., Pizarro, H.N., 2018. Effects of glyphosate and 2,4-D mixture on freshwater phytoplankton and periphyton communities: a microcosms approach. Ecotoxicol. Environ. Saf. 148, 1010–1019. https://doi.org/10.1016/j.ecoenv.2017.12.006.

Lozano, V.L., Dohle, S.A., Vera, M.S., Torremorell, A., Pizarro, H.N., 2020. Primary production of freshwater microbial communities is affected by a cocktail of herbicides in an outdoor experiment. Ecotoxicol. Environ. Saf. 201, 110821 https://doi.org/10.1016/j.ecoenv.2020.110821.

Lu, T.Q., Mao, S.Y., Sun, S.L., Yang, W.L., Ge, F., Dai, Y.J., 2016. Regulation of hydroxylation and nitroreduction pathways during metabolism of the neonicotinoid insecticide imidacloprid by *Pseudomonas putida*. J. Agric. Food Chem. 64 (24), 4866–4875. https://doi.org/10.1021/acs.jafc.6b01376.

Ludwig, S., Darrigran, G., Boeger, W.A., 2024. Opening the black box of the invasion of *Corbicula* clams (Bivalvia, Corbiculidae) in South America: a genetic and morphological evaluation. Hydrobiologia 851, 1203–1217. https://doi.org/10.1007/s10750-023-05378-1.

Lykogianni, M., Bempelou, E., Karamaouna, F., Aliferis, K.A., 2021. Do pesticides promote or hinder sustainability in agriculture? The challenge of sustainable use of pesticides in modern agriculture. Sci. Total Environ. 795, 148625 https://doi.org/10.1016/j.scitotenv.2021.148625.

Maletz, S., Wollenweber, M., Kubiak, K., Müller, A., Schmitz, S., Maier, D., Hecker, M., Hollert, H., 2015. Investigation of potential endocrine disrupting effects of mosquito larvicidal *Bacillus thuringiensis israelensis* (Bti) formulations. Sci. Total Environ. 536, 729–738. https://doi.org/10.1016/j.scitotenv.2015.07.053.

Malhotra, N., Chen, K.H.C., Huang, J.C., Lai, H.T., Uapipatanakul, B., Roldan, M.J.M., Hsiao, C.D., 2021. Physiological effects of neonicotinoid insecticides on non-target aquatic animals—an updated review. Int. J. Mol. Sci. 22 (17), 9591. https://doi.org/10.3390/ijms22179591.

McMahon, R.F., 2002. Evolutionary and physiological adaptations of aquatic invasive animals: r selection versus resistance. Can. J. Fish. Aquat. Sci. 59, 1235–1244. https://doi.org/10.1139/f02-105.

Minakshi, R., Mahajan, A.Y., 2013. Effect of thiamethoxam on oxygen consumption of the freshwater bivalve, *Lamellidens Marginalis* (Lamarck). Bioscan 8, 469–472.

Navarro, I., De la Torre, A., Sanz, P., Abrantes, N., Campos, I., Alaoui, A., Christ, F., Alcon, F., Contreras, J., Glavan, Paskovich, I., Paskovich, M.P., Nørgaard, T., Mandrioli, D., Sgargi, D., Hofman, J., Aparicio, V., Baldi, I., Bureau, M., Vested, A., Harkes, P., Huerta-Lwanga, E., Mol, H., Geissen, V., Silva, V., Martínez, M.Á., 2024. Assessing pesticide residues occurrence and risks in water systems: a Pan-European and Argentina perspective. Water Res. 254, 121419 https://doi.org/10.1016/j.watres.2024.121419.

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L., 2016. Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front. Public Health 4, 1–8. https://doi.org/10.3389/fpubh.2016.00148.

Novack-Gottshall, P.M., 2008. Using simple body-size metrics to estimate fossil body volume: empirical validation using diverse Paleozoic invertebrates. Palaios 23 (3/4), 163–173. https://doi.org/10.2110/palo.2007.p07-017r.

Odetti, L.M., González, E.C.L., Siroski, P.A., Simoniello, M.F., Poletta, G.L., 2023. How the exposure to environmentally relevant pesticide formulations affects the expression of stress response genes and its relation to oxidative damage and genotoxicity in *Caiman latirostris*. Environ. Toxicol. Pharmacol. 97, 104014 https://doi.org/10.1016/j.etap.2022.104014.

Ormerod, S.J., Dobson, M., Hildrew, A.G., Townsend, C., 2010. Multiple stressors in freshwater ecosystems. Freshw. Biol. 55, 1–4. https://doi.org/10.1111/j.1365-2427.2009.02395.x.

Pal, E., Almasri, H., Paris, L., Diogon, M., Pioz, M., Cousin, M., Sené, D., Tchamitchian, S., Tavares, D.A., Delbac, F., Blot, N., Brunet, J.L., Belzunces, L.P., 2022. Toxicity of the pesticides imidacloprid, difenoconazole and glyphosate alone and in binary and ternary mixtures to winter honey bees: effects on survival and antioxidative defenses. Toxics 10 (3), 104. https://doi.org/10.3390/toxics10030104.

Paolucci, E.M., Sardiña, P., Sylvester, F., Perepelizin, P.V., Zhan, A., Ghabooli, S., Cristescu, M.E., Oliveira, M.D., MacIsaac, H.J., 2014. Morphological and genetic variability in an alien invasive mussel across an environmental gradient in South America. Limnol. Oceanogr. 59 (2), 400–412. https://doi.org/10.4319/lo.2014.59.2.0400.

Paolucci, E.M., Ron, L., Thuesen, E.V., 2022. Metabolic response to increasing environmental temperature in the invasive mussel *Limnoperna fortunei*. Austral J. Ecol. 47 (4), 818–827. https://doi.org/10.1111/aec.13161.

Papkovsky, D., 2004. Methods in optical oxygen sensing: protocols and critical analyses. Methods Enzymol. 381, 715–735. https://doi.org/10.1016/s0076-6879(04)81046-2.

Pereira, D., Mansur, M.C.D., Duarte, L.D.S., de Oliveira, A.S., Pimpão, D.M., Callil, C.T., Ituarte, C., Parada, E., Peredo, S., Darrigran, G., Scarabino, F., Clavijo, C., Lara, G., Miyahira, I.C., Rodriguez, M.T.R., Lasso, C., 2014. Bivalve distribution in hydrographic regions in South America: historical overview and conservation. Hydrobiologia 735 (1), 15–44. https://doi.org/10.1007/s10750-013-1639-x.

Pérez, G.L., Torremorell, A., Mugni, H., Rodríguez, P., Vera, M.S., Nascimento, M.D., Allende, L., Bustingorry, J., Escaray, R., Ferraro, M., Izaguirre, I., Pizarro, H., Bonetto, C., Morris, D.P., Zagarese, H., 2007. Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study. Ecol. Appl. 17 (8), 2310–2322. https://doi.org/10.1890/07-0499.1.

Pérez, D., Okada, E., De Gerónimo, E., Menone, M., Aparicio, V., Costa, J., 2017. Spatial and temporal trends and flow dynamics of glyphosate and other pesticides within an agricultural watershed in Argentina. Environ. Toxicol. Chem. 36 https://doi.org/10.1002/etc.3897.

Pérez, D.J., Iturburu, F.G., Calderon, G., Oyesqui, L.A.E., De Gerónimo, 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, Argentina. Chemosphere 263, 128061. https://doi.org/10.1016/j.chemosphere.2020.128061.

Pizarro, H., Di Fiori, E., Sinistro, R., Ramírez, M., Rodríguez, P., Vinocur, A., Cataldo, D., 2016a. Impact of multiple anthropogenic stressors on freshwater: how do glyphosate and the invasive mussel *Limnoperna fortunei* affect microbial communities and water quality? Ecotoxicology 25, 56–68. https://doi.org/10.1007/s10646-015-1566-x.

Pizarro, H., Vera, M.S., Vinocur, A., Pérez, G., Ferraro, M., Menéndez Helman, R.J., dos Santos Afonso, M., 2016b. Glyphosate input modifies microbial community structure in clear and turbid freshwater systems. Environ. Sci. Pollut. Res. 23 (6), 5143–5153. https://doi.org/10.1007/s11356-015-5748-0.

Potet, M., Devin, S., Pain-Devin, S., Rousselle, P., Giambérini, L., 2016. Integrated multi-biomarker responses in two dreissenid species following metal and thermal cross-stress. Environ. Pollut. 218, 39–49. https://doi.org/10.1016/j.envpol.2016.08.025.

Reyna, P.B., Ballesteros, M.L., Albá, M.L., Bertrand, L., González, M., Miglioranza, K.S.B., Tatián, M., Hued, A.C., 2019. A multilevel response approach reveals the Asian clam *Corbicula largillierti* as a mirror of aquatic pollution. Sci. Total Environ. 692, 175–187. https://doi.org/10.1016/j.scitotenv.2019.07.194.

Reyna, P.B., Albá, M.L., Rodríguez, F.A., González, M., Pegoraro, C., Hued, A.C., Tatián, M., Ballesteros, M.L., 2021. What does the freshwater clam, *Corbicula largillierti*, have to tell us about chlorothalonil effects? Ecotoxicol. Environ. Saf. 208, 111603 https://doi.org/10.1016/j.ecoenv.2020.111603.

Rodríguez, F.A., Reyna, P.B., Maggioni, T., Giménez, D.R., Torre, L., 2020. The role of temperature and oxygen availability on the distribution of *Corbicula largillierti*. Invertebr. Biol. 139 (4), e12305 https://doi.org/10.1111/ivb.12305.

Rumi, A., Gutiérrez Gregoric, D.E., Núñez, V., Darrigran, G.A., 2008. Malacología Latinoamericana. Moluscos de agua dulce de Argentina. Rev. Biol. Trop. 56, 77–111.

Shan, Y., Yan, S., Hong, X., Zha, J., Qin, J., 2020. Effect of imidacloprid on the behavior, antioxidant system, multixenobiotic resistance, and histopathology of Asian freshwater clams (*Corbicula fluminea*). Aquat. Toxicol. 218, 105333 https://doi.org/10.1016/j.aquatox.2019.105333.

Siu, W.H.L., Mak, E., Cao, J., De Luca-Abbott, S.B., Richardson, B.J., Lam, P.K.S., 2004. Micronucleus induction in gill cells of green-lipped mussels (*Perna viridis*) exposed to mixtures of polycyclic aromatic hydrocarbons and chlorinated pesticides. Environ. Toxicol. Chem. 23 (5), 1317–1325. https://doi.org/10.1897/03-225.

Sousa, R., Antunes, C., Guilhermino, L., 2008. Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview. Ann. Limnol. Int. J. Limnol. 44 (2), 85–94. https://doi.org/10.1051/limn:2008017.

Sukigara, C., Mino, Y., Yasuda, A., Morimoto, A., Buranaprattheprat, A., Ishizaka, J., 2021. Measurement of oxygen concentrations and oxygen consumption rates using an optical oxygen sensor, and its application in hypoxia-related research in highly eutrophic coastal regions. Cont. Shelf Res. 229, 104551 https://doi.org/10.1016/j.csr.2021.104551.

Sule, R.O., Condon, L., Gomes, A.V., 2022. A common feature of pesticides: oxidative stress - the role of oxidative stress in pesticide-induced toxicity. Oxidative Med. Cell. Longev. 2022 (5), 1–31. https://doi.org/10.1155/2022/5563759.

Sureda, A., Capó, X., Busquets-Cortés, C., Tejada, S., 2018. Acute exposure to sunscreen containing titanium induces an adaptive response and oxidative stress in *Mytillus galloprovincialis*. Ecotoxicol. Environ. Saf. 149, 58–63. https://doi.org/10.1016/j.ecoenv.2017.11.014.

Sylvester, F., Weichert, F.G., Lozano, V.L., Groh, K.J., Bálint, M., Baumann, L., Bässler, C., Brack, W., Brandl, B., Curtius, J., Dierkes, P., Döll, P., Ebersberger, I., Fragkostefanakis, S., Helfrich, E.J.N., Hickler, T., Johann, S., Jourdan, J., Klimpel, S., Kminek, H., Liquin, F., Möllendorf, D., Mueller, T., Oehlmann, J., Ottermanns, R., Pauls, S.U., Piepenbring, M., Pfefferle, J., Schenk, G.J., Scheepens, J.F., Scheringer, M., Schiwy, S., Schlottmann, A., Schneider, F., Schulte, L.M., Schulze-Sylvester, M., Stelzer, E., Strobl, F., Sundermann, A., Tockner, K., Tröger, T., Vilcinskas, A., Völker, C., Winkelmann, R., Hollert, H., 2023. Better integration of chemical pollution research will further our understanding of biodiversity loss. Nat. Ecol. Evol. 7, 1552–1555. https://doi.org/10.1038/s41559-023-02117-6.

Thuyet, D.Q., Watanabe, H., Yamazaki, K., Takagi, K., 2011. Photodegradation of imidacloprid and fipronil in rice-paddy water. Bull. Environ. Contam. Toxicol. 86 (5), 548–553. https://doi.org/10.1007/s00128-011-0243-x.

Van den Brink, P.J., Tarazona, J.V., Solomon, K.R., Knacker, T., Van den Brink, N.W., Brock, T.C.M., Hoogland, J.P. (Hans), 2005. The use of terrestrial and aquatic microcosms and mesocosms for the the ecological risk assessment of veterinary medicinal products. Environ. Toxicol. Chem. 24, 820–829. https://doi.org/10.1897/04-268R.1.

Van Handel, E., 1965. Estimation of glycogen in small amounts of tissue. Anal. Biochem. 11 (2), 256–265. https://doi.org/10.1016/0003-2697(65)90013-8.

Vargas, R.P.F., Saad, J.F., Graziano, M., dos Santos Afonso, M., Izaguirre, I., Cataldo, D., 2019. Bacterial composition of the biofilm on valves of *Limnoperna fortunei* and its role in glyphosate degradation in water. Aquat. Microb. Ecol. 83 (1), 83–94. https://doi.org/10.3354/ame01907.

Vera, M.S., Lagomarsino, L., Sylvester, M., Pérez, G.L., Rodríguez, P., Mugni, H., Sinistro, R., Ferraro, M., Bonetto, C., Zagarese, H., Pizarro, H., 2010. New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. Ecotoxicology 19 (4), 710–721. https://doi.org/10.1007/s10646-009-0446-7.

Vera, M.S., Di Fiori, E., Lagomarsino, L., Sinistro, R., Escaray, R., Iummato, M.M., Juárez, A., Ríos de Molina, M.d.C., Tell, G., Pizarro, H., 2012. Direct and indirect effects of the glyphosate formulation Glifosato Atanor® on freshwater microbial communities. Ecotoxicology 21 (7), 1805–1816. https://doi.org/10.1007/s10646-012-0915-2.

Watson, S.A., Morley, S.A., Bates, A.E., Clark, M.S., Day, R.W., Lamare, M., Martin, S.M., Southgate, P.C., Tan, K.S., Tyler, P.A., Peck, L.S., 2013. Low global sensitivity of metabolic rate to temperature in calcified marine invertebrates. Oecologia 174 (1), 45–54. https://doi.org/10.1007/s00442-013-2767-8.

Xiao, B.C., Li, E.C., Du, Z.Y., Jiang, R.L., Chen, L.Q., Yu, N., 2014. Effects of temperature and salinity on metabolic rate of the Asiatic clam *Corbicula fluminea* (Müller, 1774). SpringerPlus 3, 1–9. https://doi.org/10.1186/2193-1801-3-455.

Yancheva, V., Mollov, I., Georgieva, E., Stoyanova, S., Tsvetanova, V., Velcheva, I., 2017. Ex situ effects of chlorpyrifos on the lysosomal membrane stability and respiration rate in Zebra mussel (*Dreissena polymorpha* Pallas, 1771). Acta Zool. Bulg. 8, 85–90.

Yologlu, E., 2019. Alterations in some biochemical responses of freshwater mussels in acute imidacloprid exposure. Adiyaman Univ. J. Sci. 9, 213–229. https://doi.org/10.37094/adyujsci.563239.

Zagarese, H.E., Diovisalvi, N.R., González Sagrario, M.Á., Izaguirre, I., Fermani, P., Unrein, F., Castro Berman, M., Pérez, G.L., 2022. Increases in Picocyanobacteria abundance in agriculturally eutrophic Pampean Lakes inferred from historical records of Secchi Depth and Chlorophyll-a. Water 2022 (14), 159. https://doi.org/10.3390/w14020159.