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The impact of oxybenzone on the neurophysiological and oxidative stress response, dispersal and aggressiveness of the Invasive Red Swamp Crayfish (*Procambarus clarkii*)

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ABSTRACT

Oxibenzone (BP-3) is a widely used UV filter and an emerging aquatic pollutant. The red swamp crayfish, *Procambarus clarkii*, a globally widespread species recognized both as food and as an invasive species, may be affected. However, the effects of BP-3 on its physiology, dispersal, aggressiveness, remain unclear. Throughout a holistic experimental trial, this study evaluated biochemical, physiological, and behavioral responses of *P. clarkii* after 15 days of exposure to 10, 100, and 1000 μg/L BP-3. Behavioral assays assessing dispersal and aggressiveness showed no significant effects. However, biochemical results revealed sex-dependent impacts. Muscle tissue showed oxidative damage through increased lipid peroxidation at higher concentrations. A decrease in acetylcholinesterase activity in the eyes of larger individuals seemed linked to growth-related physiological changes. Metabolic biomarkers also indicated altered energy storage, with elevated lipid and protein content in muscle. Overall, findings highlight *P. clarkii* capacities to cope with the ecological risks of sunscreen-derived pollutants.

1. Introduction

Oxybenzone (BP-3) is currently the most common organic ultraviolet radiation (UV) filter used by humans due to its low cost and high effectiveness in absorbing UVB and UVA radiation. This UV filter has been identified as an environmental pollutant, but so far, only banned in Hawaii and Florida, due to its impact on coral bleaching (He et al., 2021; Scheele et al., 2023). In addition, BP-3 is also active in textiles, plastics, colorants (Moreira et al., 2023), and personal care products, being present in almost all aquatic ecosystems due to its various forms of use (García-Márquez et al., 2024). Indeed, concentrations in nature vary considerably, with the highest values in the most contaminated locations reaching 223–1400 μg L^{-1} (Downs et al., 2016; Mao et al., 2019).

UV filters are known to bioaccumulate in the tissues of aquatic organisms, potentially exerting various negative effects (Scheele et al., 2023). Studies conducted with the Indian major carp, *Labeo rohita* (Riaz et al., 2025) exposed to BP-3 demonstrated histopathological damage in the gills and muscles, leading to a consequent reduction in growth, reproduction, and survival of the species (Riaz et al., 2025). In turn, studies with the zebrafish, *Danio rerio*, reported oxidative stress (Moreira et al., 2023) and behavioral alterations such as reduced learning and memory, as well as diminished social interactions, locomotion, and risk

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perception (Moreira et al., 2023; Moreira and Luchiari, 2022). Apart from fish, for instance, a study conducted with the red swamp crayfish, *Procambarus clarkii* identified BP-3 as capable of bioaccumulating in the tail tissues (He et al., 2021), giving way to future studies concerning the impact of BP-3 on this model species.

P. clarkii, native to the central/southern United States and northeastern Mexico, is currently used as a food resource in various parts of the world and has gained significant popularity in aquaculture (Zeng et al., 2024). Due to its short cultivation period, high commercial/nutritional value, and the growth of the industry in this sector, these organisms are frequently used in aquaculture (Zeng et al., 2024). Its taste, high protein content, excellent fatty acid composition, and low fat and cholesterol content are also factors contributing to the widespread expansion of this industry (Chen et al., 2022). In China, around 2.89 million tons of crayfish are produced (Zeng et al., 2024), while in Spain, approximately 20 million euros are generated annually from its sales (Resolution of the Council of Ministers No. 133/2021 of September 17, 2021).

Most notably, *P. clarkii* is an example of a worldwide successful invasive species due to intrinsic characteristics such as rapid maturation, high number of eggs, fast growth, diverse feeding strategies, but mainly due to its great tolerance to varied and extreme environments and high dispersal rate (Souty-Grosset et al., 2016). The implications of environmental factors on *P. clarkii* dispersal have been studied, including relative humidity, temperature, light conditions, food availability, and time of day (Marques et al., 2015; Oliveira et al., 2025a; Ramalho and Anastácio, 2015). Additionally, individual traits such as size, glucose levels, social status, and social context have also been examined (Galib et al., 2022; Oliveira et al., 2025b; Soares et al., 2022).

Moreover, *P. clarkii* has been extensively employed as a model organism to investigate the impacts of a wide range of environmental stressors and pollutants, which can trigger oxidative stress and lead to immunological, neurological, and intestinal alterations (El-Bakary and Sayed, 2011; Faria et al., 2010; Fernández-Cisnal et al., 2018; Hamed et al., 2025; Mokhtar et al., 2026; Said et al., 2025). However, the implications of these stressors for dispersal remain poorly understood. Only one study has examined a pharmaceutical contaminant, fluoxetine, frequently detected in wastewater, and found minimal effects on dispersal, except for a temporary decrease in velocity at the lowest dose (Oliveira et al., 2025b).

Although the importance of *P. clarkii* as both a food resource and an invasive species is well established, there is still a lack of studies addressing the effects of emerging contaminants such as BP-3 on its physiology, dispersal capacity, and aggressiveness. These traits are crucial in determining its impacts on biodiversity, its catchability as a food resource, and its potential implications for human health.

In this study, we tested holistically the effects of BP-3 exposure on *P. clarkii* by examining its biochemical, physiological, and behavioral responses in controlled conditions in laboratory. Considering the exposure concentrations tested, the study primarily contributes to hazard characterization rather than a direct environmental risk assessment. Biochemical responses focused on oxidative stress, a condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidant defenses to neutralize them. Physiological responses were assessed through energy metabolism biomarkers collected from muscle, gills, eyes, and hepatopancreas. Finaly, behavioral responses were evaluated by measuring dispersion velocity and aggressiveness using the mirror test. To achieve this, we exposed *P. clarkii* individuals to three concentrations of BP-3 for 15 days, measuring behavioral outcomes on the 8th and 15th day, and collecting biological tissue for biochemical and physiological analysis. Considering previous studies on teleost fish species (Moreira et al., 2023; Moreira and Luchiari, 2022), we hypothesized that exposure to BP-3 would lead to a redox imbalance and energy metabolism change, which could mean a reduction in dispersal speed, and aggressive response, reflected in an increase in “shyness” and a consequent increase in the duration of time spent still and away from the mirror.

2. Materials and methods

2.1. Sampling and acclimatization of P. clarkii

In July 2023, a total of 95 adult *P. clarkii* were captured from a stream near the rice fields of Cabrela (38.58561 N, 8.528580 W) using crayfish traps baited with sardines (*Sardina pilchardus*). Due to trap selectivity, only individuals with cephalothorax lengths between 3.9 cm and 5.3 cm were captured. After capture, crayfish were transported to the laboratory at the University of Évora and acclimatized for 15 days under controlled room temperature conditions, around 20 °C. Each individual was maintained separately in plastic containers (300 mL capacity, containing 250 mL of water) to prevent interactions. Water was renewed every three days, and a slice of carrot was provided as food.

2.2. Preparation of BP-3 stock solution

A stock solution of BP-3 was prepared by dissolving 100 mg of BP-3 (purity 98%; Sigma-Aldrich, CAS number: 131–57–7, catalog code: PHR1074) in 200 mL of Dimethyl Sulfoxide (DMSO) (99.9%, Sigma-Aldrich, CAS number: 67–68–5, catalog code: 472301) using a magnetic agitator. The solution was refrigerated (~2ºC) and stored in the dark to prevent degradation. BP-3 was chosen because it has been identified in various studies as the most prevalent and toxic UV filter in freshwater environments (Couselo-Rodríguez et al., 2022; Ramos et al., 2016).

2.3. Experimental design

After acclimation, crayfish were randomly assigned to five experimental treatments (N = 19 per treatment), maintaining a similar size distribution across groups (3.6–5.3 cm): (i) Control (water only) (4 females and 15 males); (ii) Solvent control, DMSO (0.002%) (5 females and 14 males); (iii) BP-3 10 μg/L (4 females and 15 males); (iv) BP-3 100 μg/L (5 females and 14 males); (v) BP-3 1000 μg/L (5 females and 14 males). Each individual was kept in an isolated container (300 mL, with 250 mL of water), ensuring continuous submersion. Exposure lasted 15 days under the same environmental conditions as acclimatization. The selected concentrations followed a logarithmic gradient commonly applied in ecotoxicology to assess sub-lethal effects and support hazard characterization, based on previous work with aquatic organisms (Moreira and Luchiari, 2022). Higher concentrations were also included to simulate potential localized contamination scenarios. Water renewal and feeding were performed every three days to maintain stable exposure conditions. Behavioral assessments were conducted on days 0 (pre-exposure), 7, and 15. At the end of the experiment, all individuals were euthanized, and gills, muscle, and eyes were dissected and stored at −80 °C for biochemical analyses.

Behavioral tests (dispersion and aggression were conducted on days 0 (before exposure), 7, and 15. After completing 15 days, all the individuals were euthanized and dissected to separate the tissues, gills, muscle and eyes. The samples were immediately frozen at −80 °C for subsequent biochemical analysis.

2.4. Behavior assessments

2.4.1. Dispersal test

Dispersal behavior was evaluated following an adapted protocol adapted from (Oliveira et al., 2025b). Each crayfish was placed individually in a metal trough (200 cm long x 25 cm wide) containing approximately 6 cm of water and 1 cm of sediment layer to simulate a natural conditions (see Fig. 1). Crayfish were initially confined 10 cm from the starting point behind a barrier for 30 s (acclimation period). After barrier removal, movement was recorded for 2 min. The total

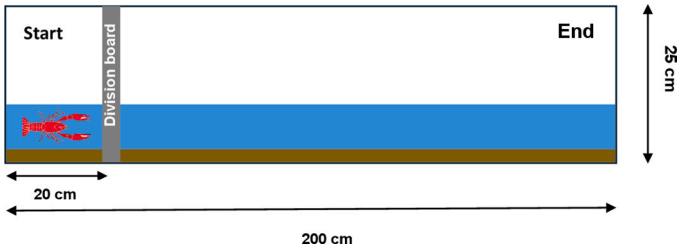

Fig. 1. Schematic view of the experimental layout. The starting point is the point where the crayfish were initially positioned before starting their dispersal. The end corresponds to the maximum length they could disperse. The division board of the room was a translucent plastic plate. The blue color represents the 6 cm of water in the trough, while the brown color represents approximately 1 cm of sediment.

distance traveled, and average velocity were the endpoints evaluated. The average velocity was calculated by dividing the total distance travelled by 120 s. In cases where the crayfish achieved the maximum distance, the time interval used was the amount of time it took them to complete 200 cm.

2.4.2. Mirror aggressiveness test

Immediately after the dispersion test, crayfish were subjected to an aggressiveness assay adapted from Moreira and Luchiari (2022). Each individual was placed 10 cm from a mirror for 2 min to evaluate its response to its reflection. This approach was selected because crayfish exhibit territorial and hierarchical behaviors, and mirror tests have been shown to elicit aggression-related responses (Issa et al., 1999; May and Mercier, 2007). The following behavioral variables were measured: (i) approach latency - the time it takes them to approach the mirror; (ii) time spent facing the mirror - the amount of time they spend in front of the mirror; (iii) number of times facing the mirror - (face to face); (iv) time spent away from the mirror, avoiding it; (vi) number of retreats - interval of time spent avoiding the mirror, assessed as the number of times after facing the mirror that they turned back to face it; and (vii) time spent retreating.

2.5. Biochemical biomarker analysis

2.5.1. Sample preparation

Frozen tissues (gills, eyes, and muscle) were individually homogenized with 1600 μL of 0.1 M potassium phosphate buffer (pH 7.4), on ice, using a sonicator (10% pulse mode, 250 Sonifier, Branson Ultrasonics, Danbury, CT, USA). Extra muscle samples, designated for energy metabolism analysis, were processed similarly but with 1600 μL of ultrapure water instead of buffer.

For lipid peroxidation assessment after homogenization, an aliquot from each replicate (N = 19) of the three tissues was mixed with 8 μL of 4% butylated hydroxytoluene (BHT) in methanol. Additionally, aliquots were taken for protein carbonylation (PC) determination, directly from the homogenate. The remaining homogenates from gills and eyes were subjected to centrifugation at 10,000 × g for 15 min at a temperature of 4 °C. The resulting supernatant was collected for subsequent analyses of catalase (CAT), glutathione-S-transferase (GST), and acetylcholinesterase (AChE) activities, as well as total glutathione content (tGSH).

Muscle homogenates were used to determine AChE activity, lactate dehydrogenase (LDH), electron transport system (ETS) activity, and the levels of proteins, lipids, and sugars. All aliquots were stored at −80 °C until they were analyzed in the days that followed. All biomarker determinations were performed using microassays in 96-well plates and spectrophotometrically measured with the MultiSkan Spectrum microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

2.5.2. Neurophysiological and oxidative stress biomarkers

The protein concentration in the post-mitochondrial supernatant (PMS) was determined using the Bradford method, with bovine γ-globulin as the standard (Bradford, 1976). Acetylcholinesterase (AChE) activity was measured using acetylthiocholine as a substrate, monitoring the increase in absorbance at 412 nm, according to the Ellman method (Ellman et al., 1961) adapted for microplates (Domingues and Gravato, 2018). CAT activity was assessed in the PMS by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm (Clairborne, 1985). The activity of GST was evaluated in the PMS by measuring the conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm (Habig et al., 1974). Total glutathione content (tGSH) was quantified in the PMS at 412 nm using a recycling assay involving GSH and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) in the presence of excess glutathione reductase (GR) (Baker et al., 1990). Lipid peroxidation (LPO) was determined by measuring thiobarbituric acid-reactive substances (TBARS) at 535 nm. Protein carbonylation (PC) was quantified by detecting carbonyl groups using 2,4-dinitrophenylhydrazine (DNPH), with absorbance measured at 450 nm, following the alkaline DNPH method (Mesquita et al., 2014).

2.5.3. Biomarkers of energy metabolism

The total lipid content was quantified by adding chloroform, methanol, and ultrapure water to 300 μL of the homogenate in a 2:2:1 ratio. After centrifugation, sulfuric acid (H_2SO_4) was added to the organic phase of each sample, followed by incubation at 200 °C for 15 min. Absorbance was measured at 375 nm, using tripalmitin as the lipid standard.

For carbohydrate quantification, 300 μL of a second homogenate aliquot was mixed with 15% TCA and incubated at −20 °C for 10 min. After centrifugation at 1000 × g for 10 min at 4 °C, the supernatant was used for analysis by adding 5% phenol and H_2SO_4 to the samples. Absorbance was measured at 492 nm, using glucose as the standard. The resulting pellet was resuspended in 1 M sodium hydroxide (NaOH) and incubated at 60 °C for 30 min, followed by neutralization with 1.67 M hydrochloric acid (HCl). The total protein content was quantified using the Bradford method (Bradford, 1976), with measurements taken at 592 nm and bovine serum albumin as the standard.

The energy equivalents for each biomolecule fraction were calculated using their specific combustion energies: 39,500 mJ/g for lipids, 17,500 mJ/g for glycogen, and 24,000 mJ/g for proteins (Gnaiger, 1983). Electron transport system (ETS) activity was determined using the reduction of iodonitrotetrazolium (INT) as a proxy for the rate of aerobic cellular respiration. The reduction rate of INT to formazan at 490 nm was facilitated by the addition of Triton X-100, a non-ionic detergent. Cellular oxygen consumption rates were calculated based on the stoichiometric relationship, where the reduction of 2 μmol of INT corresponds to the consumption of 1 μmol of oxygen. To express the final ETS activity (Ec) in energy equivalents, the enthalpic equivalent of 480 kJ/mol O_2 was applied, representing the average energy yield from a mixture of lipids, proteins, and carbohydrates (Gnaiger, 1983). The samples intended for LDH activity assessment were subjected to freeze-thaw cycles and subsequently centrifuged at 1000 × g for 10 min at 4 °C. The supernatant obtained after centrifugation was used to determine LDH activity by monitoring the decrease in absorbance at 340 nm, which reflects the oxidation of Reduced Nicotinamide Adenine Dinucleotide (NADH) associated with pyruvate consumption. The method was adapted for microplate analysis, following the procedure described by (Vassault, 1983)

2.6. Statistical analysis

All statistical analyses were conducted using RStudio (version 2024.12.0 Build 467) in the R environment (R Core Team, 2024). The following packages were employed for data handling and analysis: ‘ggplot2’ for data visualization, ‘afex’ for factorial analysis of variance

(ANOVA) and model specification, 'readxl' for importing Excel data files, and 'emmeans' for calculating estimated marginal means and performing post hoc pairwise comparisons. Before performing ANOVA, the assumptions of normality and homoscedasticity were evaluated. Normality of residuals was tested using the Shapiro-Wilk test and examined visually through QQ plots. Homoscedasticity (equality of variances) was assessed via Levene's Test using a function from the car package, alongside diagnostic plots of residuals vs. fitted values.

A Three-way Analysis of Variance (ANOVA) was conducted to evaluate the effects of experimental factors. When significant effects were detected, post hoc pairwise comparisons were conducted using estimated marginal means (emmeans), with p-values adjusted using Tukey's Honestly Significant Difference (HSD) method to control for multiple comparisons among factor levels. Effect sizes, sex and confidence intervals were also inspected when relevant. When sex and size were not significant, they were not included in the analysis. When the concentrations used were not significant, but differences in gender or size were detected, BP-3 concentrations were not included in the analysis. Effect sizes (η^2) were calculated for all ANOVA models. Additionally, post hoc power analyses were conducted for selected effects using Cohen's f derived from η^2.

3. Results

Crayfish mobility was not significantly different for all the factors evaluated (Table 1, $p > 0.05$). Although no statistically significant effects were detected, a small effect size was observed for Treatment ($\eta^2 = 0.03$), while Sex, Size and Day showed negligible effects ($\eta^2 = 0.01$) (Table 1), indicating limited practical influence of these factors (Table S5). The overall mean velocity of crayfish was 0.0093 ± 0.0138 m/s.

Regarding crayfish aggressiveness exposed to BP-3 in response to their mirror image, no significant changes were detected in terms of approach latency ($p > 0.05$, Table 2). When facing the mirror, a significant change was detected in relation to the size of the *P. clarkii*, with bigger crayfish being more prone to face their image (ANOVA: $F = 4.917$, df= 2, $p = 0.0274$, $\eta^2 = 0.02$, Table 2). On the other hand, also the day of observation have effect on the number of retreats (ANOVA: $F = 1.762$, df= 2, $p = 0.0143$, $\eta^2 = 0.03$, Table 2), with the Tukey post hoc tests showed a decrease on the 15th day of exposure when compared to the first day ($p = 0.0124$). As for the duration of retreats or the time individuals spent away from the mirror, no significant differences were found ($p > 0.05$, Table 2). For the remaining variables, effect sizes were generally small ($\eta^2 < 0.02$), indicating limited practical influence of Treatments, Sex, Size, and Day. No significant effect of Size was detected on time spent retreating ($p = 0.0642$), although the observed effect size was small ($\eta^2 = 0.02$) (Table S5).

In the hepatopancreas (Fig. 2), no differences were observed in the activity of CAT, GST, and AChE ($p > 0.05$, Table S1, Fig. 2A, B and D) of animals exposed to BP-3 when compared to control treatment.

Table 1
Analysis of variance (ANOVA) results and effect sizes (η^2) for the velocity of *Procambarus clarkii* following a 15-day exposure to benzophenone-3 (BP-3) at three treatments (10, 100, and 1000 μg/L), plus control and solvent control. The table presents degrees of freedom (df), F-values, p-values, and effect sizes (η^2) for each independent variable: BP-3 concentration ("Concentration"), sex ("Sex"; male or female), body size ("Size"; cephalothorax length), and time ("Day"; assessment days 0, 7, and 15). Effect size (η^2) represents the proportion of total variance explained by each factor, ranging from 0 to 1. Significant effects ($p < 0.05$) are indicated in bold.

	Factor	*df*	*F value*	*p-value*	*η^2*
Velocity	Treatment	4	2.236	0.0654	0.03
	Sex	1	0.594	0.4417	2.05 e-03
	Size	1	3.067	0.0810	0.01
	Day	2	1.612	0.2013	0.01

Table 2
Analysis of variance (ANOVA) results and effect sizes (η^2) for aggressiveness-related behavioral variables of *Procambarus clarkii* following a 15-day exposure to benzophenone-3 (BP-3) at three treatments (10, 100, and 1000 μg/L), plus control and solvent control. The table presents degrees of freedom (df), F-values, p-values, and effect sizes (η^2) for each independent variable: BP-3 concentration ("Concentration"), sex ("Sex"; male or female), body size ("Size"; cephalothorax length), and time ("Day"; assessment days 0, 7, and 15). Behavioral endpoints include approach latency, number and duration of mirror-facing events, time spent away from the mirror, number of retreats, and time spent retreating. Effect size (η^2) represents the proportion of total variance explained by each factor, ranging from 0 to 1, with higher values indicating stronger effects. Significant effects ($p < 0.05$) are indicated in bold.

	Factor	*df*	*F value*	*p-value*	*η^2*
Times away from the mirror	Treatments	4	0.658	0.622	9.37e-03
	Sex	1	2.616	0.107	9.32e-03
	Size	1	0.003	0.956	1.10e-05
	Day	2	0.752	0.473	5.35e-03
Number of retreats	Treatments	4	0.598	0.6641	8.35e-03
	Sex	1	0.000	0.9827	1.64e-06
	Size	1	4.312	0.1855	6.14e-03
	Day	2	1.762	**0.0143**	**0.03**
Facing mirror	Treatments	4	0.487	0.7455	6.86e-03
	Sex	1	1.345	0.2472	4.74e-03
	Size	1	4.917	**0.0274**	**0.02**
	Day	2	0.885	0.4139	6.23e-03
Time spent in front of the mirror	Treatments	4	1.217	0.304	0.02
	Sex	1	0.165	0.685	5.81e-04
	Size	1	2.005	0.158	7.06e-03
	Day	2	1.368	0.256	9.64e-03
Approach latency	Treatments	4	0.0859	0.489	0.01
	Sex	1	0.057	0.812	2.03e-04
	Size	1	0.292	0.590	1.04e-03
	Day	2	1.254	0.287	8.95e-03
Time spent retreating	Treatments	4	0.979	0.4191	0.01
	Sex	1	1.934	0.1654	6.71e-03
	Size	1	2.773	0.0642	0.02
	Day	2	2.894	0.0901	0.01

Regarding oxidative damage biomarkers, no differences were found in LPO and PC ($p > 0.05$, Table S1, Fig. 2E and F).

Regarding tGSH content (Table S1, Fig. 3) (ANOVA: $F=5.557$, df=1, $p = 0.0207$), significant effects were identified by sex, with females showing increased levels compared to males.

In the muscle tissue (Fig. 4), no significant differences were detected at the LPO or PC levels ($p > 0.05$, Table S2, Fig. 4A e B)

In the eyes (Fig. 5), no differences in CAT, GST, AChE activity or tGSH levels were found between treatments ($p > 0.05$, Table S3, Fig. 5A, B, C and D).

However, a significant decrease in GST activity was observed with an increasing individual size (ANOVA: $F=4.217$, df=1, $p = 0.0431$, Table S3, Fig. 6A). Regarding AChE activity, significant effects were also attributed to size, with a decrease in activity as the size of the individuals increased (ANOVA: $F=5.997$, df=1, $p = 0.0164$, Table S3, Fig. 6B).

In muscle tissue, in terms of energy metabolism, (Fig. 7) specifically

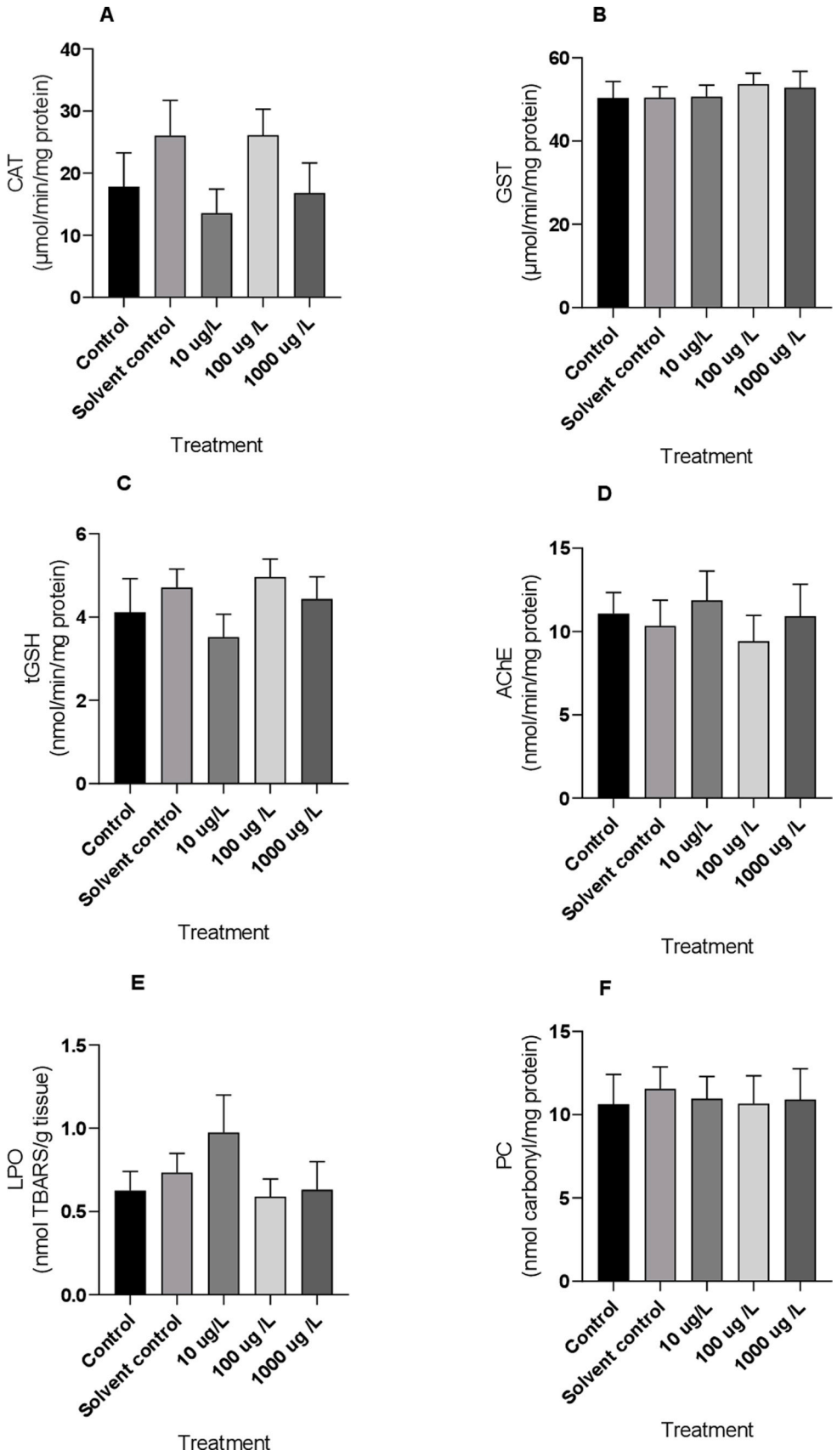

Fig. 2. Biomarkers of oxidative stress in the hepatopancreas of *Procambarus clarkii* exposed for 15 days to different BP-3 treatments (10 µg/L; 100 µg/L; and 1000 µg/L) and controls. Catalase (CAT) activity, glutathione-S-transferase (GST) activity, total glutathione content (tGSH), acetylcholinesterase (AChE) activity, lipid peroxidation (LPO), and protein carbonylation (PC). All bars are presented as mean + SEM.

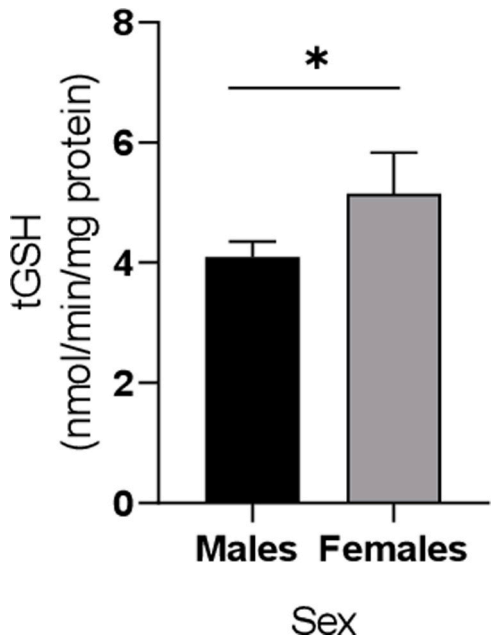

Fig. 3. Total glutathione content (tGSH) in the hepatopancreas of males and females *Procambarus clarkii* exposed for 15 days to different BP-3 treatments (10 μg/L; 100 μg/L; and 1000 μg/L). All values represented in the graph are presented as mean + SEM. * Indicates a significant difference between males and females.

sugar content and aerobic energy production evaluated through ETS activity, no significant changes were recorded ($p > 0.05$, Table S4, Fig. 7A and D). Significant changes in protein content were detected across concentrations ($p = 0.0297$, $F = 2.823$; Table S4; Fig. 7C). However, post hoc (HSD) analyses did not reveal any pairwise differences ($p > 0.05$), despite moderate-to-large effect sizes and moderate statistical power indicated by the power analysis (Table S6).

For lipid content, also significant changes were detected across concentrations ($p = 0.0429$, $F = 2.579$; Table S4; Fig. 7A). However, post hoc (HSD) analyses did not reveal any pairwise differences ($p > 0.05$), despite moderate effect sizes and moderate statistical power indicated by the power analysis (Table S6).

4. Discussion

Dispersion is an ecological process considered key to adaptation to adverse conditions, enabling crayfish to move from an adverse environment to one with favorable environmental conditions (Ramalho and Anastácio, 2015). However, the variation in the distance traveled may depend on multiple factors, including individual characteristics such as personality or housing conditions, as well as environmental factors (Galib et al., 2022; Marques et al., 2015). Moreover, it can also be affected by the perception of environmental threats, whether chemical or physical in nature. Our data shows that exposure to BP-3, did not result in mobility modifications, which suggests a great deal of resilience from this species individuals, which we know to be able to adapt to adverse environments and to hold the intrinsic ability to move even in unfavorable conditions (Moreira and Luchiari, 2022). Evidence of *P. clarkii* resilience by Oliveira et al. (2025b) has also been found when exposed to another drug, fluoxetine, since there were no differences in crayfish dispersal speed with increasing concentrations of the compound, nor related with sex (Oliveira et al., 2025a).

P. clarkii are social animals that exhibit a high level of aggression and tend to establish hierarchies (Issa et al., 1999). In our study, it was possible to observe a decrease in the number of retreats on the 15th day. The fact that there were no differences in the number of retreats depending on BP-3 dosage could mean that in addition to not losing the ability to move, the perception of risk and willingness to fight against an equal size opponent were also not affected. Another study carried out with zebrafish found different results, reporting that when exposed to a higher concentration of BP-3, individuals interacted less within shoals and responded less to their mirror image (Moreira and Luchiari, 2022). While there are still a limited number of studies testing for the effects of BP-3 on fish and other aquatic animals' behavioral response, the absence of significant effects points again towards the greater resilience and behavioural adaptability of the red swamp crayfish. On the other hand, the increase of facing in response to the mirror image as the size of the individuals increases has already been reported by Issa et al. (1999), who showed that larger individuals tend to be bolder, challenging their opponents, while smaller crayfish tend to retreat and avoid fights. Yet, similar patterns have been reported in other crayfish species (May and

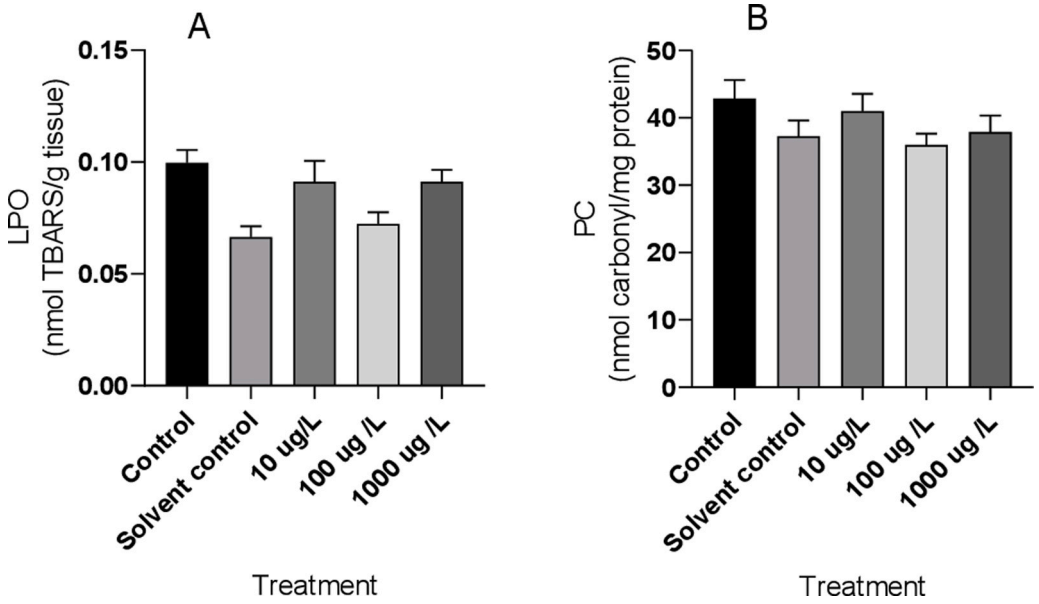

Fig. 4. Biomarkers of oxidative stress in the muscle of *Procambarus clarkii* exposed for 15 days to different BP-3 treatments (10 μg/L; 100 μg/L; and 1000 μg/L) and controls. Lipid peroxidation (LPO), and protein carbonylation (PC). All values represented in the graphs are presented as mean ± SEM.

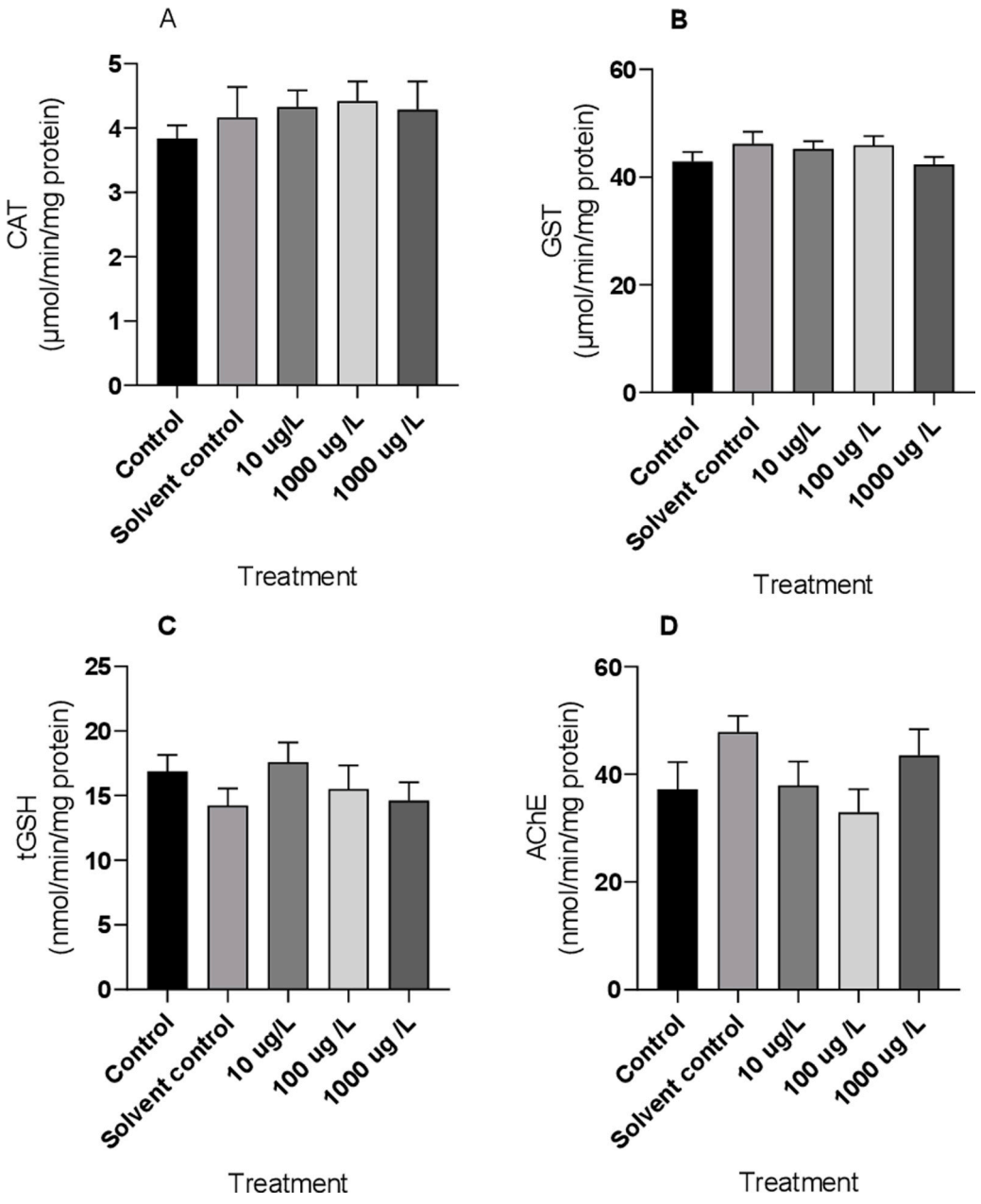

Fig. 5. Biomarkers of oxidative stress in the eyes of *Procambarus clarkii* exposed for 15 days to different BP-3 treatments (10 µg/L; 100 µg/L; and 1000 µg/L) and controls. Catalase (CAT) activity, glutathione-S-transferase (GST) activity, total glutathione content (tGSH), acetylcholinesterase (AChE) activity. All values represented in the graphs are presented as mean ± SEM.

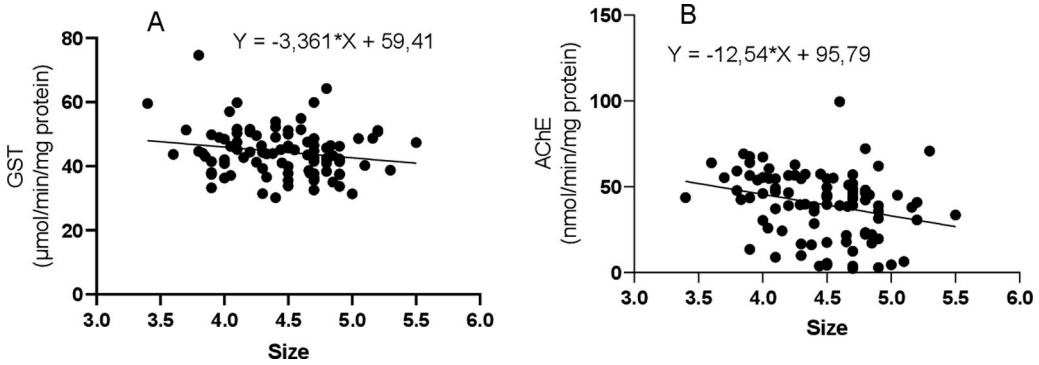

Fig. 6. Biomarkers of oxidative stress in the eyes of *Procambarus clarkii* exposed for 15 days to different BP-3 treatments (10 µg/L; 100 µg/L; and 1000 µg/L) and controls, effects of size. Glutathione-S-transferase (GST) activity, acetylcholinesterase (AChE) activity.

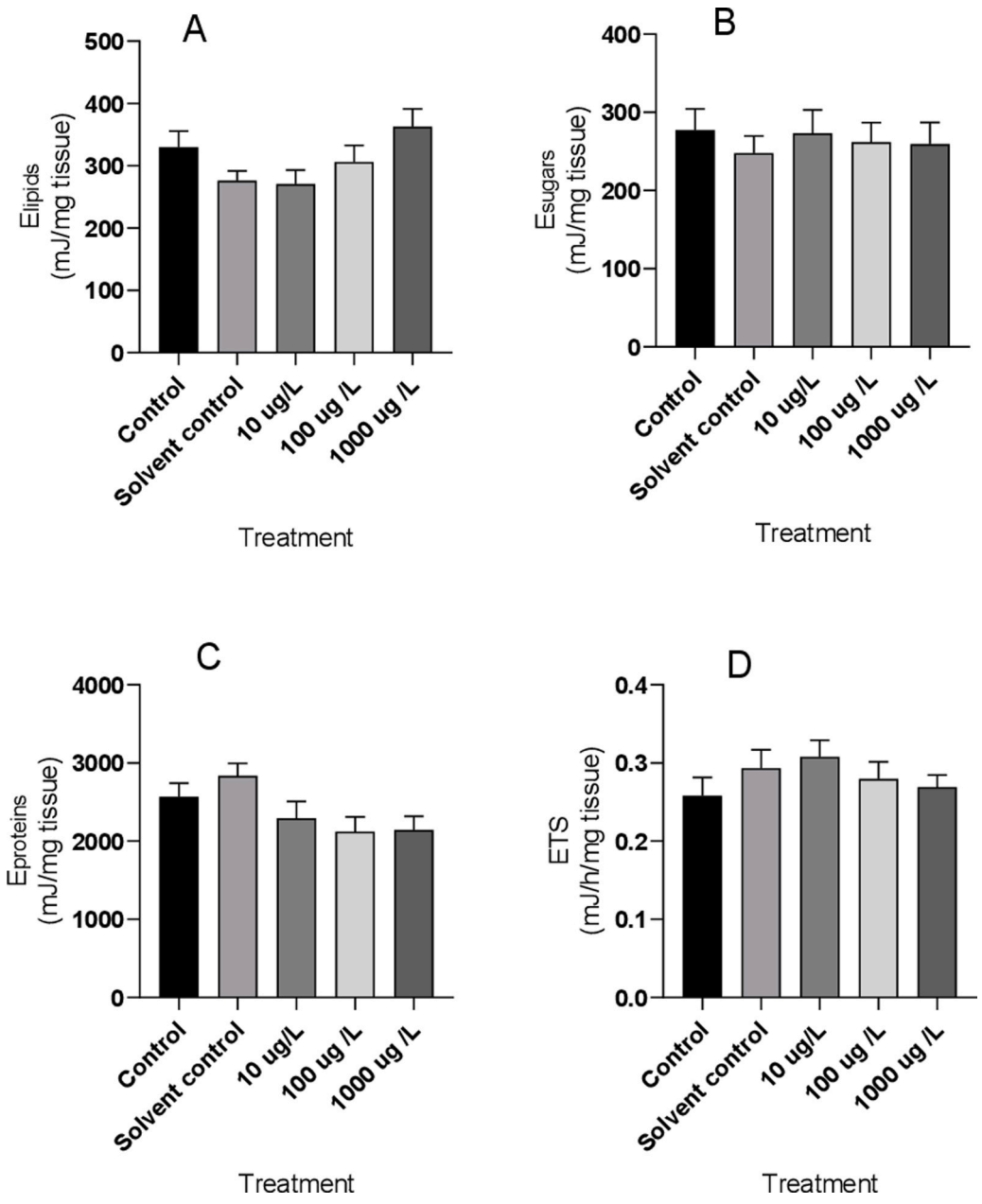

Fig. 7. Biomarkers of energetic metabolism in the muscle of *Procambarus clarkii* exposed for 15 days to different BP-3 treatments (10 μg/L; 100 μg/L; and 1000 μg/L) and controls. Sugar content (E sugars), lipid content (E lipids), protein content (E protein) and electron transport system activity (ETS). All values represented in the graphs are presented as mean ± SEM.

Mercier, 2007). However, crayfish aggressiveness, when assessed solely using a mirror test, has not been validated to the same extent as in fish and may therefore conflate aggression with curiosity or exploratory behaviour

Regarding the analysis of biomarkers, UV filters, including BP-3, are highly lipophilic (Blüthgen et al., 2014), potentially bioaccumulating in various classes of organisms, including crustaceans (Mao et al., 2019). Once present in individuals, BP-3 is capable of inducing toxicity by leading to an excessive production of ROS (reactive oxygen species), activating antioxidant defenses to maintain balance and prevent oxidative damage (Moreira et al., 2023). In the present study, the hepatopancreas (digestive gland) of *P. clarkii* was evaluated as the main metabolic organ, involved in multiple physiological processes such as molting, digestion, storage, and detoxification of various elements (Fernández-Cisnal et al., 2018). As the primary metabolic organ, it would be expected to show heightened antioxidant defenses in comparison to other tissues (Ozden barim oz et al., 2017). However, significant alterations in non-enzymatic defenses were only detected, with an increase in tGSH levels in females compared to males. The lower tGSH levels in males may indicate the consumption of total glutathione in order to neutralize the excess ROS and prevent oxidative damage (Lopes et al., 2023). Despite the increase in total glutathione consumption, antioxidant defenses appear to have been effective, as no oxidative damage was observed at the protein or lipid levels, suggesting that *P. clarkii* was able to cope with the excess ROS resulting from BP-3 exposure. Therefore, the difference between sexes may indicate that antioxidant mechanisms in males are less efficient than in females. This hypothesis has already been reported in *P. clarkii* exposed to hypoxia (Zhu et al., 2023).

In the muscle, no significant differences were found in LPO, suggesting the absence of oxidative damage at the lipid level, even with increasing BP-3 concentrations. The lack of lipid peroxidation in

membrane phospholipids indicates preserved membrane integrity and function, minimizing the risk of cellular or tissue damage (Gago-Tinoco et al., 2014). In these organisms, meat quality is an economically important trait; however, in the absence of oxidative imbalance, this quality is likely to remain unaffected (Cai et al., 2021). In our study, the activity of the CAT enzyme, which acts as the first line of defense against oxidative stress, and the activity of GST (Lopes et al., 2023) were not assessed. However, the absence of lipid damage suggests that antioxidant mechanisms were effective in neutralizing excessive ROS production.

In the eyes, AChE activity, showed a reduction in activity with the increase in individual size. To the best of our knowledge, this is the first study to report this finding for *P. clarkii*; however, previous studies have already reported changes in AChE basal levels with the growth of aquatic crustaceans (Xuereb et al., 2009). In our study, antioxidant defenses were also evaluated in the eyes, and a reduction in GST activity was observed with the increase in individual size. Nevertheless, since the reduction in AChE and GST activities were not associated with compound concentrations, this decrease may alternatively reflect a normal regulatory mechanism related to the growth or age of *P. clarkii*. Knowing these basal levels is highly relevant to accurately interpreting the use of these enzymes as a biomarker of exposure.

In terms of energy metabolism, crustaceans have the ability to regulate their metabolism when exposed to different sources of stress in order to maintain the energy required for their daily activities (Zhu et al., 2023). Here, the energy reserves were measured considering the content of lipids, sugars and proteins in order to analyze the maintenance of the basal state and the adaptive potential of *P. clarkii* to stressful situations. We observed a significant increase in lipid content as the concentration of BP-3 increased. In crustaceans, lipids are an essential source of energy, crucial for maintaining lipid homeostasis. However, when their levels are increased, they can compromise growth and affect the metabolic levels (Li et al., 2022; Pu et al., 2024). The opposite trend was observed for protein content, which decreased with exposure to the evaluated compound, BP-3. In crustaceans exposed to toxic substances, classical studies (Sánchez-Paz et al., 2007) indicate that energy reserves are mobilized in a specific order: glycogen and lipids are utilized first, followed by proteins under conditions of prolonged stress. This suggests that the observed reduction in protein content may reflect both increased degradation and decreased synthesis as a physiological response to toxic exposure. Studies on *P. clarkii* exposed to ammonia have demonstrated oxidative stress and metabolic impairment (Lin et al., 2023) which is consistent with protein mobilization. However, these studies did not directly measure total protein reduction.

Finaly, this study presents some limitations, including the exclusive use of adult crayfish, the application of extreme concentration values that are not frequently observed in natural environments, and the short duration of pollutant exposure; therefore, caution should be exercised when extrapolating these findings to natural conditions.

5. Conclusion remarks

P. clarkii showed a strong capacity to cope with exposure to benzophenone-3 (BP-3), mobilizing proteins and lipids to maintain homeostasis, without exhibiting significant changes in oxidative stress or neurotoxicity biomarkers, while also maintaining their dispersal mobility and aggressiveness. This physiological and behavioral resilience suggests a highly adaptive capacity, which makes sense, considering the species' success as an invasive organism in all sorts of environments, including those more polluted. The findings underscore the need for further ecotoxicological research, particularly involving chronic and multi-stressor exposures, as well as assessments in juvenile stages to determine potential impacts on development, reproduction, and long-term population dynamics. From a fisheries perspective, the persistent entry of BP-3-contaminated crayfish into commercial supply chains may pose risks to food safety and ecosystem health. Regulatory frameworks should consider environmental concentrations of UV filters and their sub-lethal effects on economically important aquatic species, promoting improved wastewater treatment and pollution control policies to safeguard aquatic biodiversity and public health.

CRediT authorship contribution statement

Filipe Banha: Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Cristiana Lopes:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Marta C. Soares:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Andreia Rodrigues:** Writing – review & editing, Methodology, Investigation. **Pedro Anastácio:** Writing – review & editing, Methodology, Conceptualization. **Ana Luchiari:** Writing – review & editing, Methodology. **Daniel Oliveira:** Formal analysis. **Pedro Brandão:** Writing – review & editing, Formal analysis. **Sílvia Pires:** Investigation.

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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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2026.105040.

Data availability

Data will be made available on request.

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