



Physiological response of *Palaemon elegans* to multi-anthropogenic stressors: assessing the impact of marine heatwaves and UV filters contained in sunscreens

Alexandro Autiero^{a,c,*} , Ico Martínez^a, Sarah Montesdeoca-Esponda^b, Catarina Vinagre^c, May Gómez^a, Alberto Navarro^a, Alicia Herrera^a

^a Marine Ecophysiology Group (EOMAR). IU-ECOQUA, Universidad de Las Palmas de Gran Canaria, Canary Islands, Spain

^b Instituto de Estudios Ambientales y Recursos Naturales, Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain

^c CCMAR – Centre of Marine Sciences, University of Algarve, Faro, Portugal

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ABSTRACT

Sunscreens UV filters have been identified as emerging pollutants, representing a toxic threat to aquatic environments. In addition to that, regions with intense sunscreen usage are usually exposed to marine heat waves.

This study shows the combined effects of high-water temperatures associated with sunscreen exposure in *Palaemon elegans* (Rathke, 1836). A full factorial experiment tested two temperature conditions (20 and 32 °C) and two sunscreens (one eco-friendly and the other non-eco-friendly) over 12 h. Shrimp were exposed to both stressors and sampled after 30 min (T1), 6 h (T6) and 12 h (T12). At each sampling point, metabolic biomarkers (cytochrome *c* oxidase, electron transport system) and oxidative stress biomarkers (glutathione-S-transferase, superoxide dismutase, lipid peroxidation) were analysed in the muscle and hepatopancreas. In the muscle, metabolic biomarkers showed that at T12, ETS activity was upregulated, showing a high metabolic demand at elevated temperatures, 32 °C. Meanwhile, COX activity was downregulated, suggesting possible mitochondrial dysfunction due to the increased accumulation of reactive oxygen species (ROS), further enhanced by exposure to chemicals present in the non-eco-friendly sunscreen. LPO activity indicated the presence of oxidative stress in organisms exposed to high temperatures, 32 °C, in combination with the non-eco-friendly sunscreen. In contrast, oxidative stress biomarkers such as GST and SOD showed that these antioxidant defences function effectively at 20 °C, but their efficacy fails at 32 °C, probably due to significant ROS accumulation associated with elevated temperatures and chemical pollutants. UV filters accumulation over time and temperature was analysed using UHPLC. Results show that the concentration (µg/g) of UV filters contained in the eco-friendly and non-eco-friendly sunscreens increased over time under higher temperature (32 °C). This indicates that marine heat waves can enhance the uptake of certain chemicals over just 12 h of exposure.

1. Introduction

Worldwide marine and coastal ecosystems are under increasing pressure from multiple human-induced stressors. Climate-driven atmospheric anomalies, related to a significant warming of the biosphere, which has led to widespread negative impacts on ecosystems and the sustainability of natural resources (Cardinale et al., 2012), are one of these. Marine heat waves (MHWs) are periods of anomalously warm water lasting five or more consecutive days (Hobday et al., 2016) that can indirectly amplify the toxicity of anthropogenic stressors (de

Luzinais et al., 2024), and chemical pollutants (Cadena-Aizaga et al., 2022a). Furthermore, given the elevated abundance of marine litter such as microplastics (Herrera et al., 2019), MHWs might further exacerbate the effects of these pollutants. These events pose considerable threats to marine ecosystems (Cavole et al., 2016; Oliver et al., 2017) as projections indicate that heat waves are expected to occur more frequently and with greater intensity (Wedler et al., 2023). Regional case studies have shown that MHWs can significantly alter ecosystems, resulting in widespread mortality, shifts in species distribution, and changes to community structure (Smale et al., 2019). Therefore, it is

* Corresponding author. Marine Ecophysiology Group (EOMAR). IU-ECOQUA, Universidad de Las Palmas de Gran Canaria, Canary Islands, Spain
E-mail addresses: alexandro.autiero101@alu.ulpgc.es (A. Autiero), alicia.herrera@ulpgc.es (A. Herrera).

crucial to investigate the complexity of MHWs in-depth and understand their impact on marine ecosystems.

Beyond the thermal stress, marine organisms are increasingly exposed to new chemical pollutants, such as ultraviolet (UV) filters contained in sunscreens, which accumulate in coastal waters (Cadena-Aizaga et al., 2022a). The rapid expansion of tourism, particularly in subtropical regions, combined with an increased awareness of the harmful effects of prolonged overexposure to UV sun radiation (Thallinger et al., 2023) has increased the use of sunscreen (Lapides et al., 2023) also in regions like the Canary Islands (Spain). In fact, to mitigate the risks of sunburn, photoaging, and skin cancer, the use of sunscreens has widely increased (Henderson et al., 2022; Sander et al., 2020; Silva et al., 2018). While sunscreens play a crucial role in protecting human health, they have become a source of marine pollution (Tovar-Sánchez et al., 2013). UV filters, the active components in sunscreens, are now recognised as emerging pollutants due to their persistence and accumulation in coastal waters (Chatzigianni et al., 2022). UV filters such as Benzophenone-3 (BP3), octinoxate, and others are known to leach into marine environments mainly through recreational activities (Fig. 1) and wastewater discharges (Montesdeoca-Esponda et al., 2021). Also, the accelerated growth of coastal tourism, coupled with the widespread application of sunscreen, constitutes an important pathway contributing to the introduction of UV filters, as reported by Sánchez-Quiles & Tovar-Sánchez (2015). Significant concentrations of these compounds have been detected in coastal areas (Sánchez Rodríguez et al., 2015; Sharifan et al., 2016), especially in primary marine consumers (Isabel Cadena-Aizaga et al., 2022). Consequently, they pose a toxic threat to marine life (Bachelot et al., 2012) as they are reported to be endocrine disruptors both in mammals (Ma et al., 2023; Schlumpf et al., 2008) and fish (Kinnberg et al., 2015). Furthermore, studies have proven that they are neurotoxic (Araújo et al., 2018) and lead to oxidative stress in different marine species (Nataraj et al., 2020).

Regarding the impacts on marine life, enzymatic biomarkers have emerged as critical tools for early detection of environmental stressors (Samanta et al., 2018; Sanchez and Porcher, 2009). They are widely used as proxies of stress response in marine organisms (Valavanidis et al., 2006). Biomarkers might be very species-specific, differing

according to organisms and type of stressor (Madeira et al., 2013), complicating the identification of consistent patterns. That is why, generally, scientists remain divided on their use, due to their varying predictive capacities: while high-level biomarkers (considering their genetic expression) are more ecologically relevant, they are slow to respond and difficult to detect, low-level biomarkers (enzymatic biomarkers) offer early warnings about stressors, but they are limited in predicting broader biological effects, responding differently according to the stressors (Armon and Hänninen, 2015; Lam, 2009). Biomarkers have been employed to elucidate and assess the effects of MHWs on aquatic organisms showing that the oxidative stress response increases with temperature but also that it is a species-specific reaction (Madeira et al., 2015, 2016; Madeira et al., 2012; Vinagre et al., 2012, 2014, 2018).

To date, little scientific research has investigated the combined effects of MHWs and sunscreens in crustaceans. While considerable research exists on each factor singularly, the lack of information on the possible interaction of both stressors is still poorly explored. Due to the increasing occurrence and duration of MHWs and the widespread presence of UV filters in coastal waters, it is fundamental to fill this gap in research and assess the potential threat to marine organisms.

This study aimed to understand the physiological stress response of *Palaemon elegans* (Rathke, 1836) when exposed simultaneously to a MHW and sunscreens. Two different temperatures (20 and 32 °C) and two different sunscreens (one eco-friendly, the other non-eco-friendly) were tested during a 12-h full factorial experiment. Metabolic biomarkers such as Cytochrome c oxidase (COX) and Electron Transport System (ETS), and oxidative stress biomarkers, such as Glutathione-S-Transferase (GST), Superoxide Dismutase (SOD), Lipid peroxidation (LPO), were analysed to evaluate the shrimp's response.

P. elegans is a common rockpool dweller where it is likely to be exposed to higher concentrations of sunscreen than organisms that live in open coastal areas. Particularly, rocky shores that harbour bathing pools, which could be highly frequented during high-season peaks, can become ecological traps during ebb tides associated with MHWs (Vinagre et al., 2018). Given that, the oxidative stress response to temperature has already been deeply studied in this species, therefore, it was

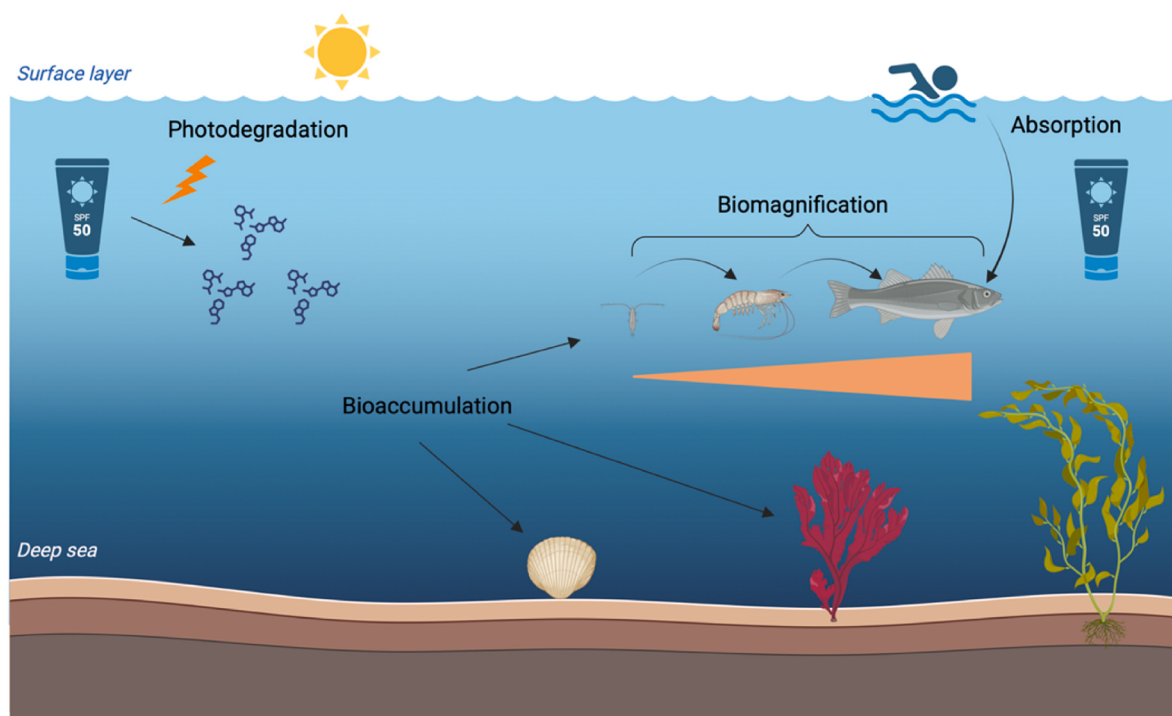


Fig. 1. Source and fate of UV filters in marine environments.

considered a good model to understand the combined effects of temperature and sunscreen exposure.

2. Materials and methods

2.1. Animal collection and husbandry

Shrimps were collected by a hand net during low tide from rocky pools located along the north and east coasts of Gran Canaria (Canary Islands, Spain), in Fig. 2.

They were transported to the laboratory in tanks at a controlled temperature. Shrimps were acclimatised and kept at a controlled room temperature of 20 °C, in direct contact with natural light and fed three times a week *ad libitum* with cultured *Artemia franciscana*. Tank parameters (temperature, dissolved oxygen, ammonia, nitrites and nitrates) were measured twice a week.

2.2. Experimental preparation and set-up

A 12-tank aerated aquarium system was set up and filled with filtered seawater. Each tank was filled with thirty randomly selected organisms. Tanks corresponding to the MHW treatment had a thermostat and were previously warmed up to 32 °C, to mimic heatwave conditions and to bring the organisms close to their species-specific CTMax (Madeira et al., 2012; Vinagre et al., 2016). Both tanks corresponding to the normal

temperature of 20 °C and to the simulated MHW were kept in a thermo-constant room. Their temperature was kept constant throughout the whole experiment (12 hours).

Before the start of the experiment, sunscreen solutions were prepared for each treatment. A concentration of 60 mg L⁻¹ (Sendra et al., 2017; Sureda et al., 2018; Yang et al., 2024) was chosen to simulate upper-bound, short-term scenarios in near-shore environments, where recreational activities can be intense and where as much as 2.68 g of sunscreen may be released into the sea per person during each beach session (Milinkovitch et al., 2024). The concentration used reflects a possible worst-case scenario during stagnant conditions, occurring in popular rock pools, in high peak season periods. The main ingredients (i. e., UV filters), their sun protection factor (SPF), and the format (i. e., application type) of the sunscreen composition are listed in Annex 1 (Table S1).

To simulate the introduction of sunscreens into the environment, they were thoroughly homogenized in filtered seawater. Dilution was achieved in the aqueous medium through continuous agitation (Araújo et al., 2020) with a magnetic stirrer for 50 min. During the process, beakers were covered with aluminium foil to avoid sunscreen degradation (Romanhole et al., 2016) and contamination due to sprinkling. Shrimps were starved for 24 h before the start of the experiment. Once all the solutions were introduced into the tanks, the initial sampling, considered immediate response, (T1) was conducted after 30 min. Subsequent samplings were carried out at 6-h intervals, with the second

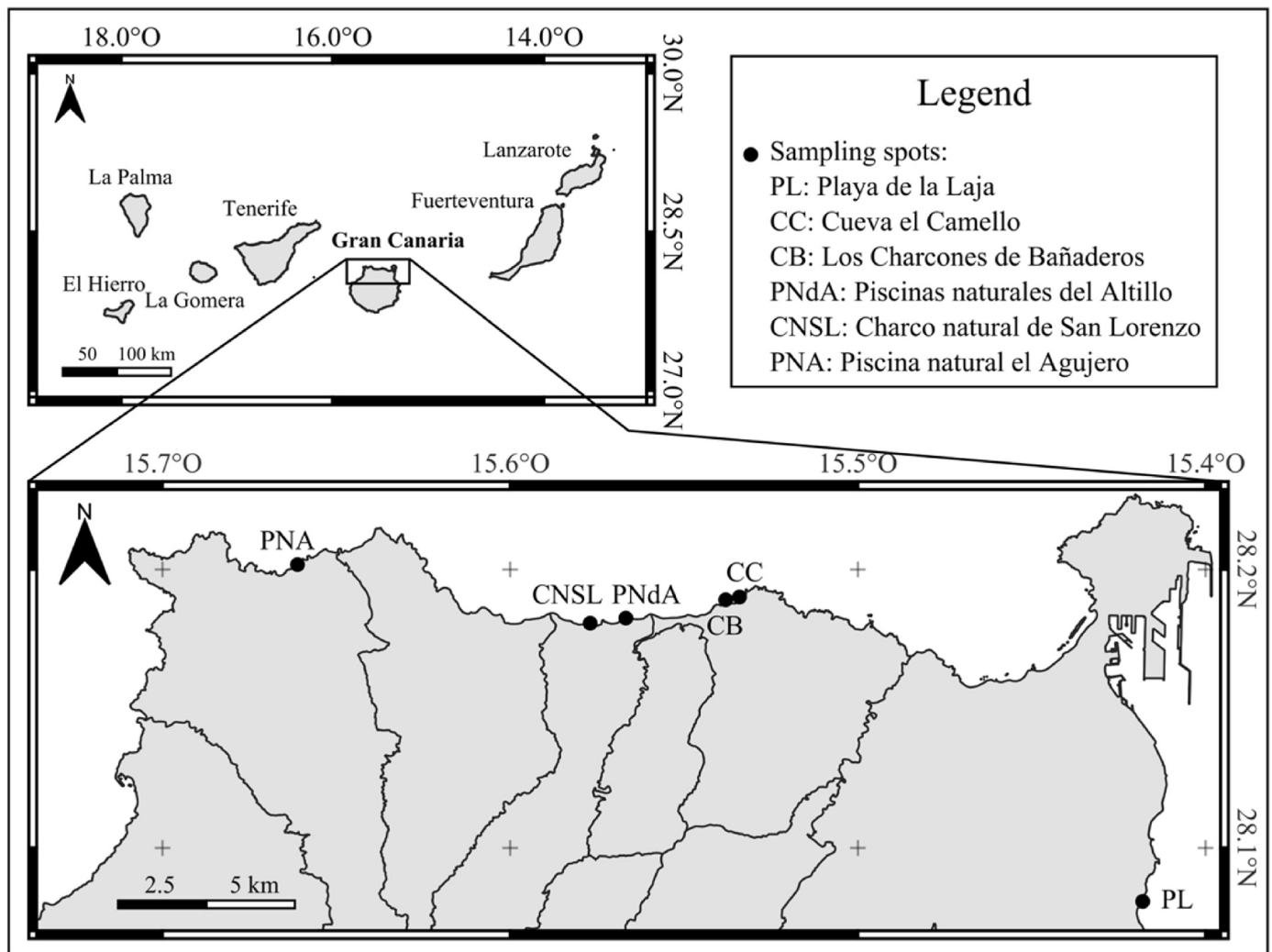


Fig. 2. Location of the sampling spots in the north, and northeast of Gran Canaria where specimens of *P. elegans* were collected.

sampling (T6), intermediate response, occurring 6 h after T1, and the third sampling (T12), prolonged exposure, 6 h after T6.

Eight shrimps from each of the 12 tanks were sampled at every sampling time. Five individuals were used for biomarker analysis and three for liquid chromatography analysis. A hand net exclusively reserved for each sunscreen treatment was used to collect shrimps. Once collected, they were sedated in ice (Saborowski et al., 2022). Full body length was measured and weighed. Muscle tissue and hepatopancreas from each individual were extracted on a Petri dish placed on ice, stored in vials and immediately frozen in dry ice. Muscles and hepatopancreas were then stored at -80°C until the analysis. Three different workstations were designated to prevent contamination among the treatments.

2.2.1. Pre-treatment and extraction procedure of UV filters from samples of *P. elegans*

Once the shrimps were sedated, their body length was measured and weighed. Afterwards, they were washed with distilled water, stored in aluminium boxes, covered with aluminium foil, and frozen at -80°C until further analysis. Before the extraction procedure, the samples were freeze-dried for 24 h in small glass vials. A porcelain mortar with a pestle was then used to homogenise and grind the samples into powder.

To extract the target compounds, a microwave-assisted extraction (MAE) method from Guazé et al. (pending publication) was adopted. 50 mg of the dried powder was weighed with a precision balance and placed into microwave digestion vessels. The microwave oven used for extraction was a TITANMPS with 16 vessels (230 V, 50–60 Hz, 40 bar), purchased from PerkinElmer (Madrid, Spain). 7 mL of Hexane (HEX) was used as an extractant solvent. MAE equipment was then used to heat solvents at 68°C in contact with samples for 15 min. After allowing samples to reach room temperature, the solvent was evaporated with a nitrogen stream to reconstitute in a solvent compatible with the detection system. This step enables sample concentration by using a lower volume (1 mL of MeOH). Then, samples were sonicated for 30 min and filtered through a $0.2\ \mu\text{m}$ syringe into chromatographic glass vials.

The accumulation of the studied UV filters – Butyl methoxydibenzoylmethane (BMDBM), BP3, 2-Ethylhexyl Salicylate (EHS) and Octocrylene (OC) were analysed using ultra-high-performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS). The detailed chromatographic separation and determination conditions for each UV filter, together with specific detection limits, are included in detail in supplement materials. Information on sunscreens and their composition is also described in Table S2 of Annex 1.

2.3. Oxidative stress biomarkers analysis

Five shrimps in each treatment were singularly homogenized for 2 min in a Potter-Elvehjem type Teflon glass tissue grinder rotating at 2600 rpm in 1 mL of 0.1 M sodium potassium phosphate buffer (0.1 M Na_2HPO_4 , 0.1 mM KH_2PO_4 , 75 μM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 1.5 % Polyvinylpyrrolidone and 0.2 % Triton X-100) at pH 8.5 (Owens and King, 1975). Samples were kept at $0-4^{\circ}\text{C}$ in an ice bath throughout the process. Crude homogenates were centrifuged for 10 min at 4000 rpm, 0°C , and the supernatant fluid was used for measurements. All biomarker analyses were conducted using a FLUOstar Omega microplate reader from BMG Labtech. The enzymatic activities (ETS, COX, SOD, GST, LPO) were measured at their corresponding temperature of 20 and 32°C . Detailed analysis protocols for each biomarker and liquid chromatography analysis can be found in Annex 1.

2.4. Statistical analysis

All statistical analyses were performed using RStudio (version 2024.12.1 + 563). UV filter accumulation over time was analysed using linear regression models. For each UV filter (BMDBM, BP3, Octocrylene and EHS), concentration ($\mu\text{g/g}$) was regressed against time to evaluate the rate of accumulation at the different time points (T1, T6, T12).

Separate models were fitted for each temperature condition (20 and 32°C) to assess temperature-specific effects on accumulation. The assumptions of linear regression were checked for each fitted model. The normality of residuals was evaluated with the Shapiro-Wilk test and homogeneity of variance was assessed by examining residual vs. fitted values plots with the Breusch-Pagan test. Differences were considered significant if $p < 0.05$. Mann-Whitney-Wilcoxon test was employed to analyze the different biomarkers (COX, ETS, GST, SOD, LPO) in the muscle and the hepatopancreas between two independent temperature groups (20 and 32°C) within the same treatment (control, eco-friendly and non-eco-friendly), since both the normality and homoscedasticity of data were not met ($p > 0.05$). Differences were considered significant if, $p < 0.05$. Graphics were performed with RStudio using the package ggplot2.

3. Results

3.1. UV filter accumulation

The accumulation of the UV filters ($\mu\text{g/g}$) in *P. elegans* contained in the two different sunscreens (eco-friendly and non-eco-friendly) at 20 and 32°C at short (T1), intermediate (T6) and prolonged (T12) exposure is shown in Fig. 3. Linear regression analysis revealed marginally significant ($p = 0.05$) time and temperature-dependent accumulation for Butyl Methoxy Dibenzoyl Methane (BMDBM) and accumulation for Octocrylene (OC). Respectively found in the eco-friendly and non-eco-friendly sunscreens and at elevated temperatures (32°C). No statistically significant ($p > 0.05$) time and temperature-dependent accumulation ($\mu\text{g/g}$) was found in any of the other UV filters contained in sunscreens and at any low temperatures (20°C).

Under simulated heat-wave conditions (32°C), BMDBM had a time-dependent increase in concentration. Minimal levels were observed after 1 hour, intermediate accumulation after 6 hours and maximal accumulation after 12 hours of exposure. At 32°C , BMDBM increased over the total exposure time, with a final concentration level higher than that measured at the beginning. Data indicate a marginally significant linear accumulation ($p = 0.05$) during the exposure under warm conditions, with 65.3 % of the variability explained by the time trend at 32°C . Likewise, OC exhibited a marginally significant linear accumulation ($p = 0.05$) over time at 32°C , indicating a measurable uptake of this compound over the exposure period, rising progressively across the same time intervals. This results in a greater accumulation by 12 hours compared to the initial level. The slope suggests that higher temperatures accelerated the accumulation rate of OC, with 65.6 % of the variability explained by the time at 32°C .

Regarding the rest of the UV filters, EHS contained in the non-eco-friendly sunscreen shows at T1 a higher concentration at both 20 and 32°C , it decreases at the intermediate and increases again at prolonged exposure. Although it does not show a significant time-dependent accumulation at 32°C , 70.6 % of the variability of EHS is explained by time at 32°C . Regarding BMDBM contained in the non-eco-friendly sunscreen, it showed a low concentration in *P. elegans*, compared to the eco-friendly sunscreen, at T1. Its concentration peaked in the intermediate exposure (T6), and then decreased, showing no clear patterns of accumulation ($p > 0.05$). BP3, present only in the non-eco-friendly sunscreen, also showed no clear accumulation pattern ($p > 0.05$), reaching a plateau at T6 for both temperatures. The potential ecological impact of these compounds along the trophic chain and under changing environmental conditions warrants further and more detailed investigation.

3.2. Muscle biomarker analysis

COX activity (nmol/min/mg protein) shown in Fig. 4, exhibits statistically significant changes in short and prolonged exposure to both contaminants and heat stress. In contrast, COX activity does not show

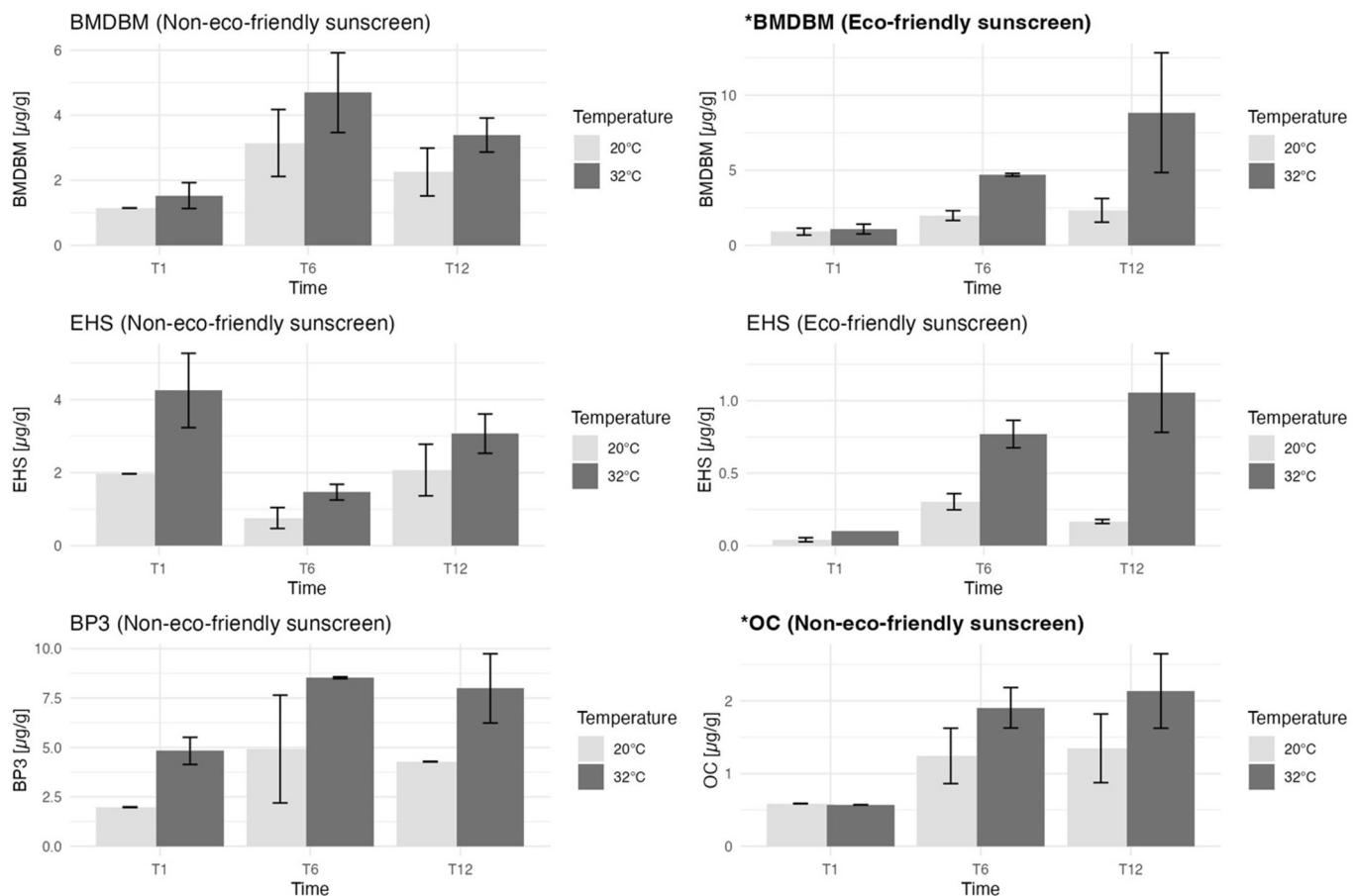


Fig. 3. Represents the accumulation (mean $\pm$ SD, represented by bars) of UV filters ($\mu\text{g/g}$) detected in *P. elegans* using UHPLC from the non-eco-friendly and eco-friendly sunscreens at the three sampling times (T1, T6, T12) under two temperature conditions (20 and 32 °C). Each panel shows a different UV filter: BMDBM and EHS (present in both sunscreens), BP3 (present only in the non-eco-friendly sunscreen) and OC (present only in the non-eco-friendly sunscreen). Marked with an asterisk * and in bold, the UV filter that shows a marginally statistically significant positive linear relationship ($p = 0.05$) between the concentration ($\mu\text{g/g}$) and the exposure time (T1, T6, T12), at 32 °C.

any significant changes ($p > 0.05$) in the intermediate exposure. At the short exposure time (Fig. 4, a), the non-eco-friendly treatment shows a statistically significant difference ($p < 0.05$) between the two temperature conditions, with lower values at 32 °C. A clear decline in COX activity was observed in the prolonged exposure (Fig. 4, c) at 32 °C for all treatments, with the most pronounced decrease in the non-eco-friendly sunscreen group ($p < 0.05$).

As shown in Fig. 5, ETS activity ($\mu\text{l O}_2/\text{min}/\text{mg}$ protein) was significantly elevated ($p < 0.05$) after short exposure at 32 °C in the absence of sunscreens, compared with the 20 °C condition. At intermediate exposure durations, no significant differences were detected among temperature groups ($p > 0.05$), suggesting a possible metabolic adaptation. However, following prolonged exposure (12 h, c), all treatments exhibited a significant increase in ETS activity at 32 °C ($p < 0.05$), with the largest elevation observed in the control group, indicating that elevated temperature may enhance ETS activity, particularly after extended exposure times at 32 °C.

Fig. 6 shows that GST activity (nmol/min/mg protein), was significantly elevated ($p < 0.05$) after prolonged exposure (12 h, c) at 20 °C. In contrast, at short (30 min, a) and intermediate (6 h, b) exposure durations, no significant differences were detected among temperature groups ($p > 0.05$).

Fig. 7 shows that SOD activity (nmol/mg protein), was significantly lower ($p < 0.05$) after prolonged exposure (12 h, c) at 32 °C. In contrast, at short (30 min, a) and intermediate (6 h, b) exposure durations, no

significant differences were detected among temperature groups ($p > 0.05$).

As shown in Fig. 8, LPO activity (nmol TBARS/mg protein) was significantly higher ($p < 0.05$) after short exposure (30 min, a) at 32 °C in the control treatment, compared with the 20 °C condition. At intermediate exposure durations (6 h, b), LPO activity was significantly lower at 32 °C in the control treatment compared to the 20 °C condition. Finally, a significant ($p < 0.05$) increase LPO activity was observed at 32 °C in the non-eco-friendly sunscreen treatment after the prolonged exposure (12 h, c).

3.3. Hepatopancreas analysis

Analysis of the hepatopancreas of *P. elegans* revealed no significant differences ($p > 0.05$) for the biomarkers examined across the different treatments, control, eco-friendly sunscreen and non-eco-friendly sunscreen, and within the temperature conditions (20 and 32 °C) at all sampling times (T1, T6 and T12).

4. Discussion

This study shows the combined effects of MHWs and UV filters contained in an eco-friendly and non-eco-friendly sunscreen in *P. elegans* and highlights physiological ROS production and metabolic adaptations triggered by the multi-stressor exposure. Elevated temperatures and

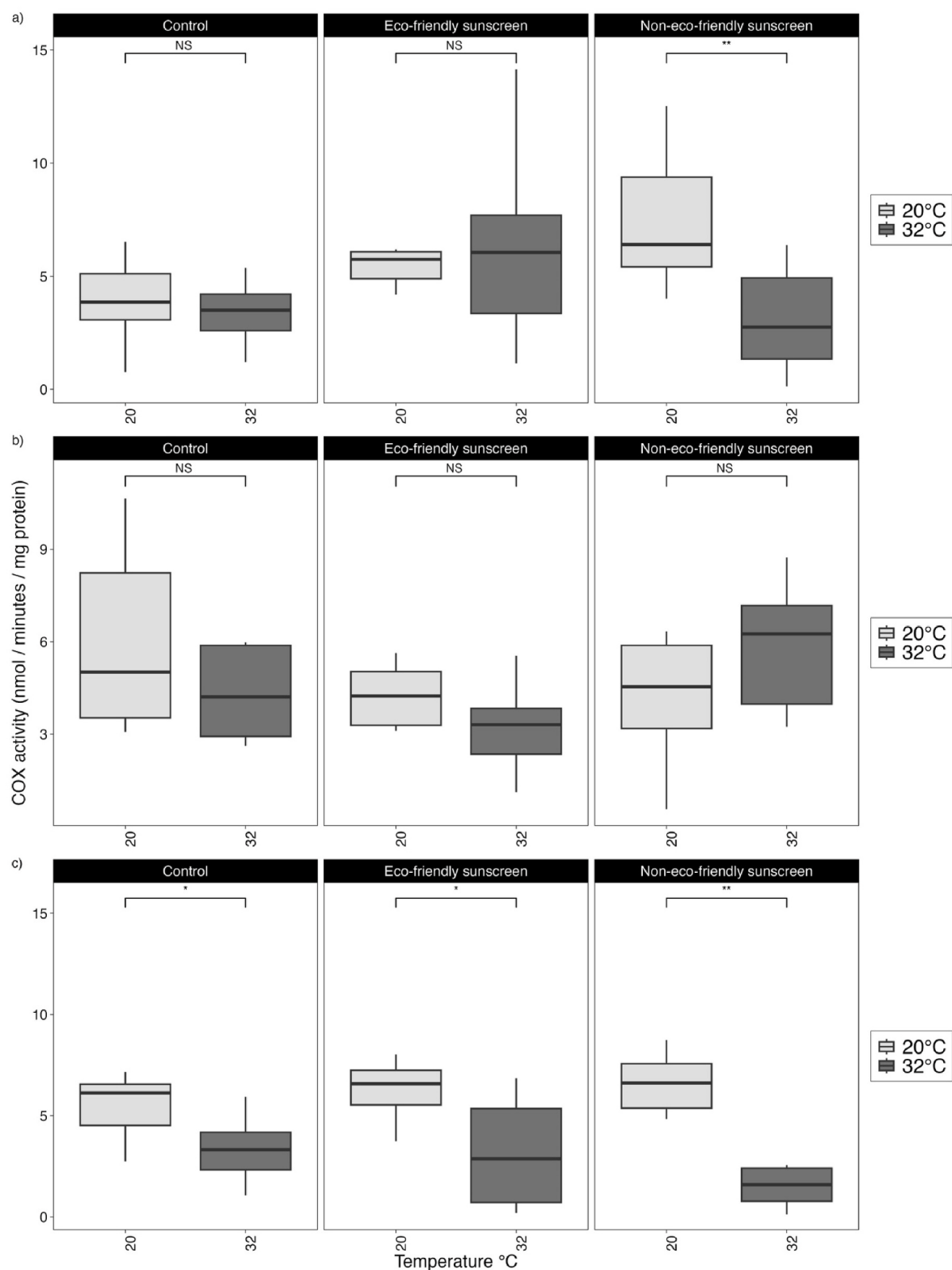


Fig. 4. COX activity (nmol/min/mg protein) at T1 (a), T6 (b) and T12 (c) sorted by treatment condition (control, eco-friendly and non-eco-friendly sunscreen) and compared across two temperature exposures (20 and 32 °C). The boxplots illustrate that at T1, there is a significant difference ($p < 0.05$) in COX levels between groups exposed to 20 and 32 °C under the non-eco-friendly sunscreen and at T12 in all treatments between groups at 20 and 32 °C ($p < 0.05$).

exposure times were identified as the dominant stressors. UV filters further intensify the physiological responses and accumulate over 12 h in the organisms. This reflects changes in the susceptibility to both thermal stress and chemical pollutants.

4.1. Ecological implications of UV filters bioaccumulation

Crustaceans, such as *P. elegans*, commonly found in nearshore waters, have been indicated as reliable bioindicators of anthropogenic

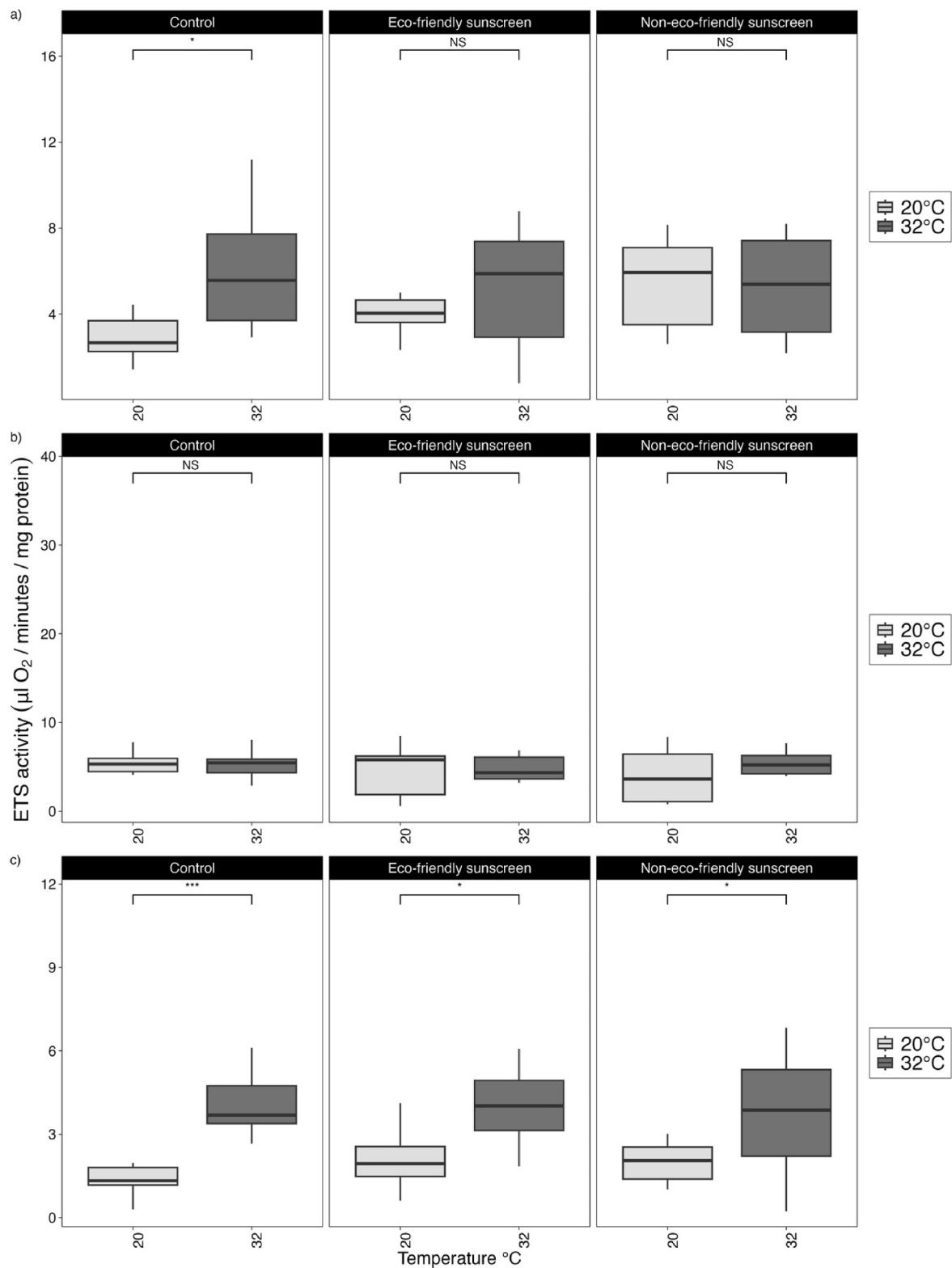


Fig. 5. ETS activity (μl O₂/min/mg protein), at T1 (a), T6 (b) and T12 (c) sorted by treatment condition (control, eco-friendly and non-eco-friendly sunscreen) and compared across two temperature exposures (20 and 32 °C). The boxplots illustrate that at T1, there is a significant difference ($p < 0.05$) in ETS levels between groups exposed to 20 and 32 °C in the control group and at T12 in all treatments between groups at 20 and 32 °C ($p < 0.05$).

pollutants due to their sensitivity to chemical substances (Key and Fulton, 2002), as they are frequently exposed to anthropogenic contaminants (Cadena-Aizaga et al., 2022b; Pisani et al., 2022). BMDBM, present in the eco-friendly sunscreen and OC, present in the

non-eco-friendly sunscreen, showed, respectively, a marginally significant accumulation ($p = 0.05$) in *P. elegans*, suggesting that MHWs might intensify the bioaccumulation of these UV filters. One potential explanation may be related to the fact that the metabolic rate of crustaceans

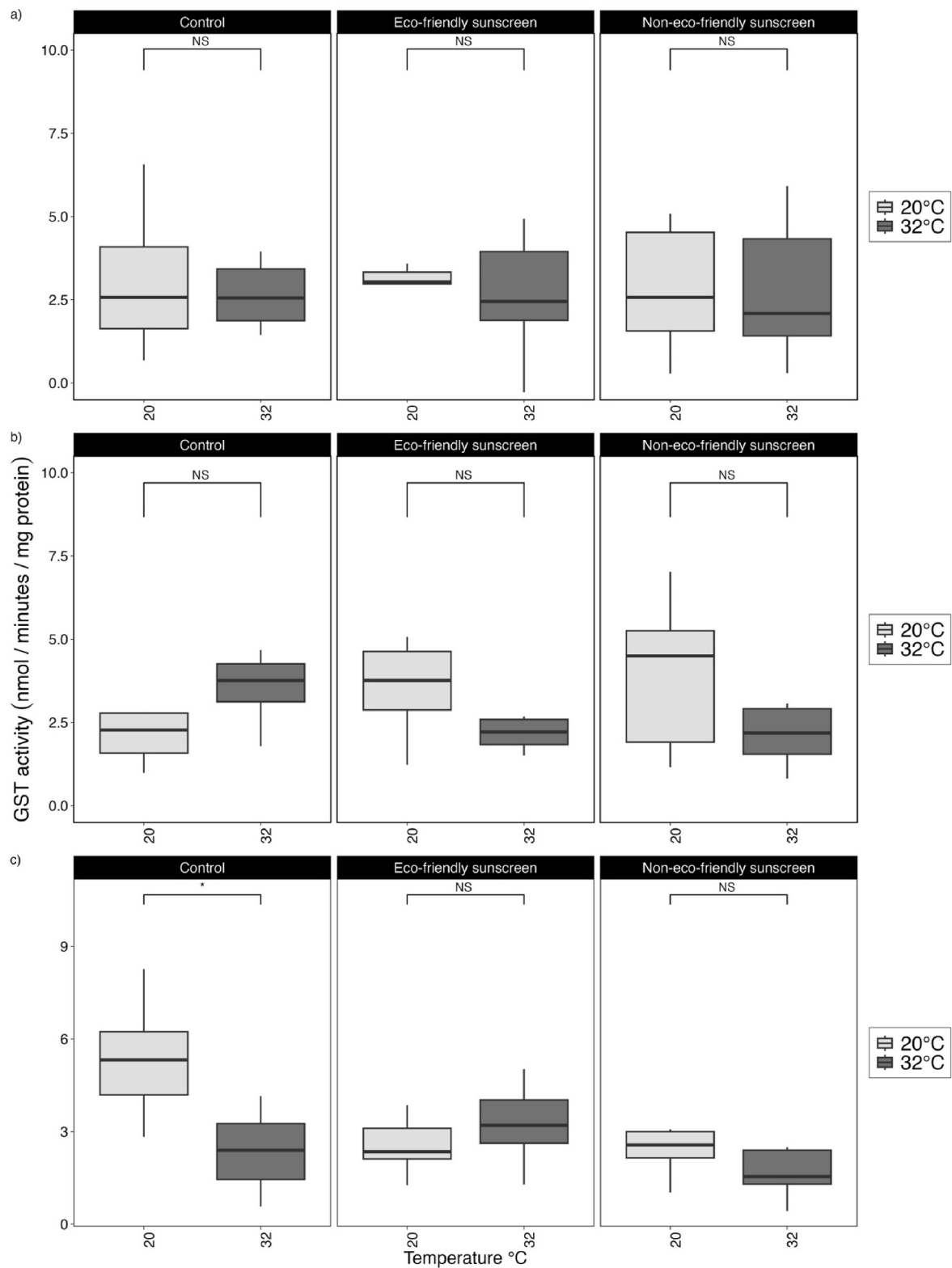


Fig. 6. GST activity (nmol/min/mg protein), at T1 (a), T6 (b) and T12 (c) sorted by treatment condition (control, eco-friendly and non-eco-friendly sunscreen) and compared across two temperature exposures (20 and 32 °C). At T12, the boxplots reveal a statistically significant decrease in GST activity in the control group at 32 °C relative to 20 °C ($p < 0.05$), whereas no significant temperature-related differences were observed at T1 or T6 ($p > 0.05$).

increases at higher temperatures (González et al., 2010), allowing them to cope with environmental and thermal fluctuations (Denisse Re et al., 2006). The associated rise in oxygen consumption may facilitate the accumulation of UV filters, particularly at 32 °C. Elevated levels of UV

filters in crustaceans have been linked to adverse physiological effects, including the alteration of endocrine-related genes (Rodríguez, 2024). Additionally, due to the accumulation over time of these compounds, *P. elegans* might be the vector for biomagnification to higher trophic

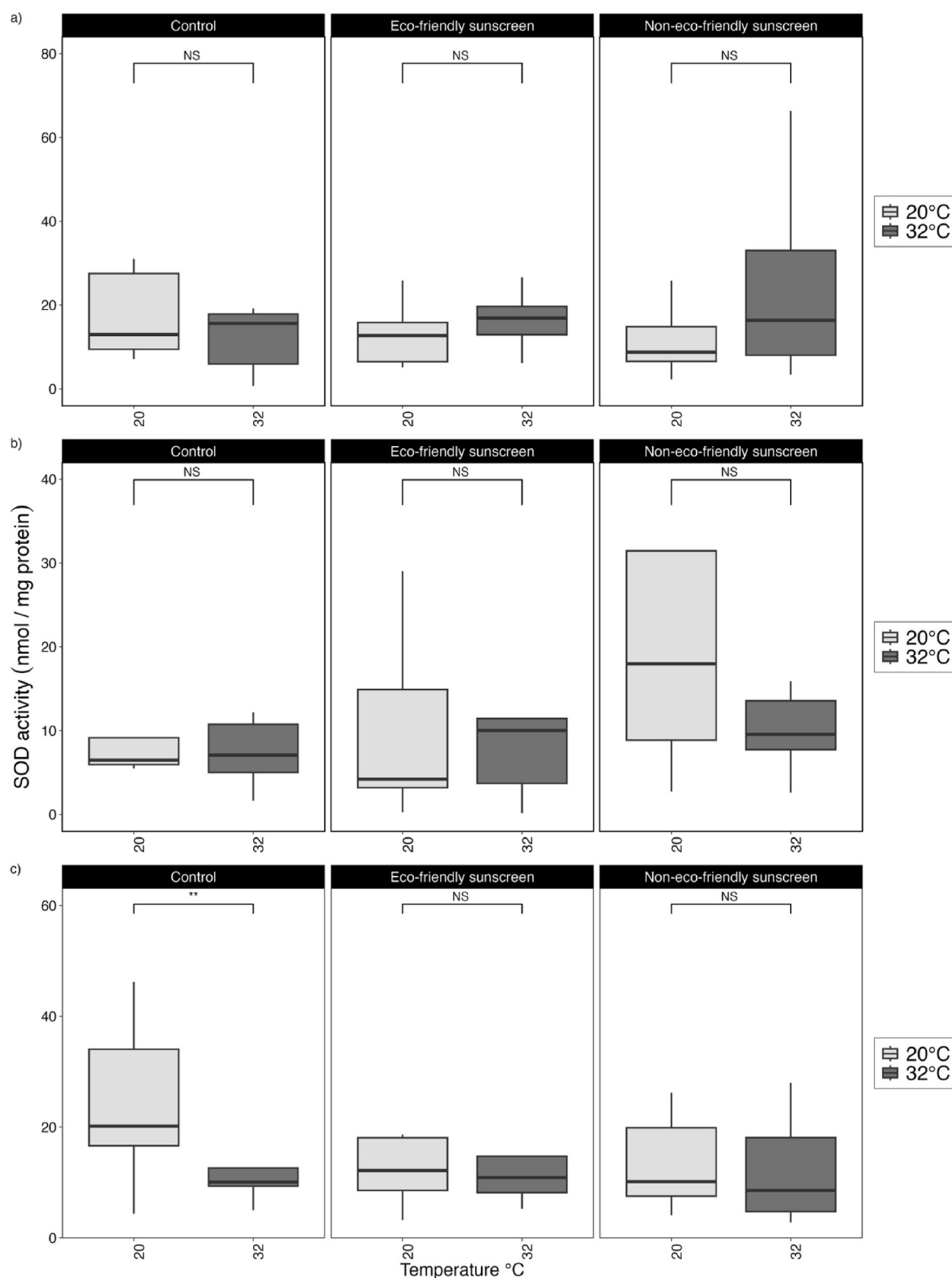


Fig. 7. SOD activity (nmol/mg protein) at T1 (a), T6 (b) and T12 (c) sorted by treatment condition (control, eco-friendly and non-eco-friendly sunscreen) and compared across two temperature exposures (20 and 32 °C). At T12, the boxplots reveal a statistically significant decrease in SOD activity in the control group at 32 °C relative to 20 °C ($p < 0.05$), whereas no significant temperature-related differences were observed at T1 or T6.

levels of UV filters, with critical ecological implications. The projected increase, together with an unregulated use of sunscreens in coastal areas, could worsen this scenario and further intensify this issue in coastal marine ecosystems.

4.2. Metabolic stress response

COX represents a transmembrane molecule found in the mitochondria of eukaryotes, which plays a fundamental role in producing energy

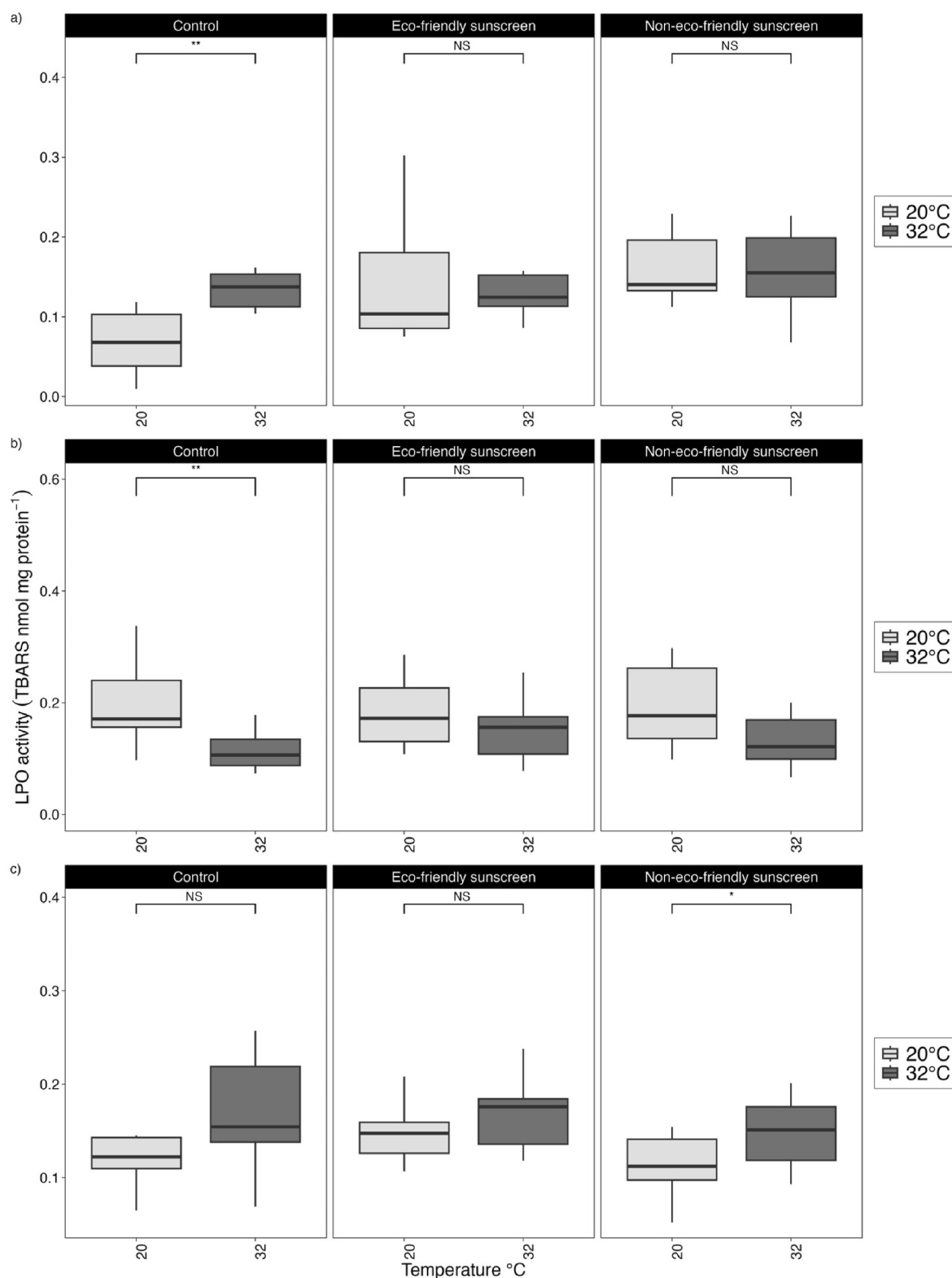


Fig. 8. LPO activity (nmol TBARS/mg protein) at T1 (a), T6 (b) and T12 (c) sorted by treatment condition (control, eco-friendly and non-eco-friendly sunscreen) and compared across two temperature exposures (20 and 32 °C). At T1, the boxplots reveal a statistically significant increase in LPO activity in the control group at 32 °C relative to 20 °C ($p < 0.05$), and at T12 a statistically significant increase in LPO activity in the non-eco-friendly group at 32 °C ($p < 0.05$).

via ATP generation through the electron transport system (Antonini et al., 1970; Wikström and Sharma, 2018), being a fundamental regulator enzyme in the electron transport chain (ETC) (Srinivasan and Avadhani, 2012). The observed statistically significant decrease of COX

activity at short exposure (T1) for the non-eco-friendly treatment at 32 °C, and at prolonged exposure ($p < 0.05$) (T12), for all treatments at 32 °C, suggests a potential mitochondrial dysfunction, possibly due to excessive ROS accumulation in the ETC, affecting ATP generation. In

fact, previous studies indicate that COX dysfunction is highly related to increased mitochondrial reactive oxygen species promoting oxidative stress (Fornuskova et al., 2010; Galati et al., 2009). Under normal conditions, COX uses oxygen, allowing the electron flow and ATP production. MHW in a rocky tidal pool might lead to low oxygen concentrations (Roman and Pierson, 2022), accumulating electron-rich intermediates (NADH, FADH₂) in the ETC, with increased oxidative damage (Srinivasan and Avadhani, 2012). COX downregulation at a short exposure (T1) only in the non-eco-friendly treatment at 32 °C indicates that sudden heat stress associated with the specific treatment causes disruptions in the energy metabolism of *P. elegans*. An intermediate exposure suggests that the physiological adjustments employed conferred thermal acclimation. However, after prolonged exposure (12 h), the downregulation of COX activity observed for all treatments at 32 °C highlights that temperature is a dominant factor driving mitochondrial impairment. This suggests that extended thermal stress might overwhelm compensatory mechanisms, as shown also in other studies (Su et al., 2020), leading to disruptions in the electron transport chain and ATP synthesis reduction.

At 32 °C and at short exposure (T1) in the control and at prolonged exposure (T12), all treatments showed an upregulation of the ETS activity, indicating an increased cellular metabolic demand under the acute thermal stress. This higher activity in ETS suggests that *P. elegans* allocated more energy towards essential metabolic mitochondrial processes at higher temperature, in contrast to individuals kept at a lower temperature (20 °C). ETS is a metabolic biomarker that reflects the potential respiration rate of cells (Herrera et al., 2014). Accordingly, an increase in thermal stress implies that more of the energy budget of the organism is being used to meet immediate metabolic demands, leaving less energy available for other processes like somatic growth and reproduction (Simčić et al., 2014), increasing ETS activity (Lemos, 2021). Therefore, the significant increase ($p < 0.05$) in ETS at 32 °C (T1 and T12) indicates that the shrimp were actively adjusting their metabolism to cope with the heat-induced stress, presumably by accelerating ATP production to satisfy the higher energy requirements for cellular homeostasis.

These findings are consistent with previous studies and reinforce the role of ETS as a reliable indicator of metabolic response to thermal stress. Studies on *Dicentrarchus labrax* have demonstrated that ETS activity serves as an accurate proxy for estimating metabolic rate under elevated temperatures (Madeira et al., 2016; Vinagre et al., 2014). In those experiments, fish exposed to thermal stress showed increased ETS activity related to higher oxygen consumption, confirming that ETS measurements closely show organism metabolism. Similarly, it was observed that ectothermic aquatic organisms inhabiting warmer waters exhibit elevated metabolic rates as a compensatory response to heat stress (Simčić et al., 2015). The increased ETS activity in our study can be viewed as part of a generalized physiological strategy among ectotherms to meet the increased energy demand imposed by high temperatures. This higher metabolic activity implies that, on the one hand, *P. elegans* shows an adaptive resilience, upregulating its energy-producing pathways to cope with thermal stress. On the other hand, the physiological cost of that is related to a lack of energy resources that may no longer be available for growth, reproduction or other functions. This trade-off underscores the complex nature of metabolic adaptation under multi-stressor conditions. In this context, the enhanced ETS activity highlights how *P. elegans* copes within a multi-stressor environment, close to a real-case scenario. Overall, the increase in ETS activity at 32 °C (at T1 and T12) shows the capacity of the organisms to adjust their metabolism to stressful environments, reinforcing the value of ETS as an informative biomarker of thermal stress response in marine ectotherms.

4.3. Oxidative stress response

In this study, oxidative stress biomarkers indicate a compromised

antioxidant system under the influence of high temperature and chemical stressors. While normal physiological activities produce reactive molecules and free radicals that can damage various cellular structures (Félix et al., 2020), oxidative stress occurs when ROS production exceeds their removal (Aranda-Rivera et al., 2022). Nonetheless, cellular defenses play a crucial role in detoxifying these molecules, thereby mitigating their harmful effects (Birben et al., 2012).

Our findings underscore the importance of cautious interpretation of oxidative stress biomarkers under multiple stressors. The decrease in GST activity after prolonged exposure at 32 °C only in the control, despite the expectation of the opposite, highlights a potential threshold effect. In fact, once the organism is pushed past stress threshold, antioxidant enzymes like GST may no longer respond, and their activity may decline due to enzyme inactivation or depletion of substrates (like glutathione). Furthermore, GST might not be involved in the detoxification of the compounds present in the sunscreens in *P. elegans*. This might be why no significant difference ($p > 0.05$) was found in the eco-friendly and non-eco-friendly sunscreens. Similar results were observed in other shrimp species exposed to chemical pollutants, where significant oxidative damage was observed without a corresponding measurable increase in GST (Dorts et al., 2009). This biomarker plays a fundamental role in detoxifying and preventing lipid peroxidation (Sureda et al., 2018) and is widely used for evaluating how organisms respond to stressors like pollution and temperature fluctuations (Cheung et al., 2001; Gowland et al., 2002). However, it also frequently provides values that are difficult to interpret (Cunha et al., 2005) and that lead to inconsistent results (Domingues et al., 2010). In our study, the absence of a clear GST response to sunscreen chemicals does not mean these substances had no effect. Their impact may manifest in other ways or become evident beyond the 12-h window or at different concentrations. Our results imply that GST might remain unchanged or become suppressed under extreme or multi-stressor environments. Thus, elevated temperature stress appears to have a dominant effect that can impair the enzyme response and suppress the expected induction. Finally, the additional chemicals did not produce an additive effect on GST, highlighting the complexity of the antioxidant network.

After a prolonged exposure (12 h) SOD activity in the control treatment showed statistically significant differences ($p < 0.05$), with higher enzyme levels at 20 °C compared to 32 °C. Superoxide dismutase, a cytosolic enzyme widely used to indicate cellular stress resulting from environmental contaminants (Serafim et al., 2012), catalyses the conversion of superoxide radicals to hydrogen peroxide (Downs et al., 2001). Verlecar et al. (2007) found higher SOD activity at 32 °C than 26 °C in the digestive gland and gills of *Perna viridis*. Comparable patterns of SOD variability were reported in gastropods where an increased temperature exposure resulted in higher SOD activity (De Oliveira and Greco, 2015). Our findings indicate that in *P. elegans*, prolonged exposures to high temperatures (32 °C) may exceed the organism's thermal tolerance of SOD activity, which was higher at 20 °C than at 32 °C and only in the control group. Similar results were obtained in a study conducted by Vinagre et al. (2014) where SOD activity, for the same species, decreased from a 20–32 °C thermal exposure. The prolonged exposure might have compromised this antioxidant defence system, leading to a reduced capacity of SOD to detoxify superoxide radicals into hydrogen peroxide (Ilijin et al., 2021). Furthermore, the exposure to different sunscreens appears to exacerbate this effect, resulting in no differences ($p > 0.05$) among the treatments (eco-friendly and non-eco-friendly) at 20 and 32 °C.

Our data show that after a short exposure (30 min) high temperature (32 °C) appeared to induce higher LPO levels only in the control, compared to the lower temperature ($p < 0.05$). This indicates that short-term exposure to thermal stress is enough to trigger an increase in oxidative damage in *P. elegans*. In contrast, the addition of sunscreen did not produce a clear difference in LPO between temperatures, suggesting that the chemical stressors' effects on LPO might require a longer exposure time. In the same treatment, by the intermediate exposure (6

h), LPO levels were lower at 32 °C ($p < 0.05$), suggesting a transient acclimatisation or mitigation of lipid peroxidation. However, the causes of this drop are not fully clear and could also be influenced by experimental variability. After a prolonged exposure (12 h), a clear synergistic effect of thermal and chemical stress is evident. In fact, LPO levels were significantly higher at 32 °C than at 20 °C in the presence of the non-eco-friendly sunscreen ($p < 0.05$), suggesting that the combined effect of the stressors enhanced higher lipid peroxidation, whereas at lower temperatures, the increase was lower. This rise in LPO under the combined stressors indicates substantial oxidative damage to cell membranes by 12 h. Such damage is consistent with the mechanism of LPO, where accumulated ROS and peroxides can disrupt membrane lipids, leading to loss of membrane fluidity and impaired cell function (Ferreira et al., 2015; Lesser, 2006). The non-eco-friendly sunscreen likely contains UV filters that trigger additional ROS production that is further increased by the high-temperature exposure. These findings align with patterns observed in other marine organisms facing thermal stress. Similar studies on *Dicentrarchus labrax* showed a significant increase in LPO activity once temperature exceeded the species-specific thermal optimum (Madeira et al., 2016; Vinagre et al., 2012). In our study, *P. elegans* was exposed to a simulated MHW of 32 °C, whereas Madeira et al. (2012, 2012) showed that the CTMax value of the shrimp was 34.08 °C. Elevated temperature in combination with sunscreen pollution, can synergistically exacerbate lipid peroxidation and cellular damage and may lower the CTMax of *P. elegans* but the exact outcomes and thresholds are species-dependent. Further studies should compare different species and include longer exposure durations at different concentrations to identify any adaptive response that might mitigate LPO under multi-stressor conditions.

4.4. Insight from hepatopancreas analysis

The hepatopancreas, as the primary organ for digestion and xenobiotic detoxification in aquatic invertebrates (Bianchini and Monserrat, 2007), is extremely sensitive to environmental fluctuations (Webb, 2011). In this study, hepatopancreas biomarker levels did not differ significantly ($p > 0.05$) among treatments or temperature conditions at any of the sampling times (T1, T6, T12). This lack of significant response may be due to methodological limitations, particularly the relatively small sample size and the pooling of tissue from only a small number of shrimp per treatment, which reduced the statistical power to detect subtle changes. Furthermore, the high variability in hepatopancreas responses may reflect the organ's rapid dynamic reaction to stress, possibly indicating that at the sunscreen concentration and temperature tested in this study, the antioxidant defence mechanisms would be enough to remove ROS in this organ (Lavarías and García, 2015) or that physiological priorities had shifted toward other homeostatic pathways under the combined stressors. These findings underscore the challenges of establishing clear oxidative stress patterns in the hepatopancreas and highlight the need for future studies under a multi-stressor environment.

5. Conclusions

This study demonstrates that the physiological responses of *P. elegans* to multi-stressors are both species-specific and stressor-dependent. The simulated MHWs (32 °C) were identified as the primary driver of metabolic and oxidative stress, with non-eco-friendly UV filters intensifying the observed impacts.

Key findings.

1. Higher temperatures (32 °C) likely enhanced the absorption of UV filters contained in the eco-friendly and non-eco-friendly sunscreens, with an increased accumulation in *P. elegans* during 12-h exposure. Given the important role of *P. elegans* in the food web, these chemical compounds may be further biomagnified to larger predators and

become a threat to a wider range of species, including different trophic levels.

2. Regarding metabolic biomarkers, at a prolonged exposure (12 h) ETS activity at 32 °C across all treatments indicates an increased metabolic demand related to thermal stress. This upregulation suggests an adaptive response to cope with the heat stress and to meet immediate metabolic demands. COX activity instead was downregulated, at 32 °C, indicating that temperature is the dominant factor (over 12 h) driving mitochondrial impairment.
3. The antioxidant defences activity (GST and SOD) were not sufficient to counteract ROS production at 32 °C, indicating that the antioxidant system might be overwhelmed by the prolonged heat stress and the sunscreens. LPO levels provided evidence of enhanced ROS production at 32 °C in the non-eco-friendly sunscreen, indicating that higher temperatures, in combination with the non-eco-friendly sunscreen might act synergistically and increase cellular membrane damage.
4. The hepatopancreas data did not show clear patterns due to the small sample size, highlighting the need for improved methodologies.

These findings highlight the importance of considering a multi-stressor environment in coastal ecosystems when assessing the impacts of climate change and anthropogenic pollutants on marine invertebrates. Specifically, in areas like the Canary Islands, where mild temperatures make it a major tourist destination, increasing the pressure on the coastal ecosystems. Regulatory measures to limit or modify the composition of sunscreens are crucial for preserving biodiversity and ecosystem health. The results shown in this study also highlight the interaction between MHW and UV filters, with temperature emerging as the dominant stressor influencing physiological pathways. This justifies the need for further investigation of their combined impact.

CRedit authorship contribution statement

Alexandro Autiero: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ico Martínez:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Sarah Montesdeoca-Esponda:** Writing – review & editing, Methodology, Investigation, Data curation. **Catarina Vinagre:** Writing – review & editing, Supervision. **May Gómez:** Writing – review & editing. **Alberto Navarro:** Writing – review & editing, Methodology, Investigation. **Alicia Herrera:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2025.107226>.

Data availability

Data will be made available on request.

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