



Heat stress and physiological disruption in *Ucides cordatus*: a bioindicator of climate vulnerability in mangrove ecosystems of the Western Atlantic

Esli Emanuel Domingues Mosna ^{IB a,b,d}, Camila Prieto ^{IB a}, Caroline Araújo De Souza ^{IB a,c,d}
and Marcelo Antonio Amaro Pinheiro ^{IB a,b,d}

^aInstituto de Biociências (IB), Campus do Litoral Paulista (CLP), Departamento de Ciências Biológicas e Ambientais (DCBA), UNESP – Universidade Estadual Paulista, São Paulo, Brazil; ^bPrograma de Pós-Graduação em Ecologia, Evolução e Biodiversidade (PPG-EcoEvoBio). UNESP IB/Rio Claro, Rio Claro, Brasil; ^cIFPA – Instituto Federal de Educação, Ciência e Tecnologia, Campus Belém, Belém, Brazil; ^dSão Paulo State University (UNESP), Institute of Biosciences, São Paulo, Brazil

ABSTRACT

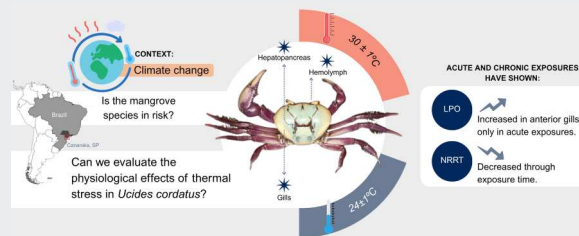
Current projections of climate change indicate potential impacts on coastal ecosystems, including rising seawater temperatures, which may directly affect endemic and ecologically important species such as the mangrove crab *Ucides cordatus*. This study aimed to assess the physiological impacts of climate-induced heat stress in this species, using cytological (NRRT, neutral red retention time) and oxidative stress (LPO, lipid peroxidation) biomarkers across multiple tissues. Crabs were collected from mangroves in Cananéia, SP, Brazil, and subjected to controlled thermal exposure under two regimes: control ($24 \pm 1^\circ\text{C}$) and treatment ($30 \pm 1^\circ\text{C}$), based on IPCC climate projections. Two experimental durations were tested: acute (12–36 hours) and chronic (2–7 days). Crab cells exhibited reduced NRRT in both trials, although no statistically significant differences were observed between thermal groups. Animals exposed to heat stress showed consistently lower NRRT values, suggesting sublethal physiological disruption. Significant differences in LPO levels were detected only during acute exposures and varied by tissue type. These findings provide novel physiological evidence of thermal sensitivity in *U. cordatus*, reinforcing its potential as a bioindicator of ecological vulnerability and its application in integrated monitoring frameworks for mangrove conservation under climate change.

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Introduction

Mangrove forests provide essential ecosystem services in tropical and subtropical coastlines, including shoreline protection against erosion and storms, regulation of water quality, carbon storage, and the provision of nursery habitats that sustain coastal fisheries and the livelihoods of coastal communities (Khan et al. 2025). Despite this relevance, mangroves are highly sensitive to climate-driven changes in temperature, precipitation, and sea-level rise, with projections indicating substantial structural and functional shifts under warming scenarios (Friess et al. 2022; IPCC 2023). These climate pressures interact with local anthropogenic stressors – such as inappropriate disposal of toxic compounds, unplanned coastal occupation, and runoff enriched with pesticides and fertilisers – intensifying habitat degradation and affecting resident biota (Peters et al. 1997; Alongi 2015; Arenas-Sánchez et al. 2016; Saher and Siddiqui 2019; Saravanan et al. 2021; Mishra et al. 2023). Climate and human driven disturbances are already altering mangrove coverage,

CONTACT Camila Prieto prieto.martins@unesp.br São Paulo State University (UNESP), Institute of Biosciences, Coastal Campus, Praça Infante Dom Henrique, s/n, Parque Bitaru, São Vicente, São Paulo, 11330-900, Brazil
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species composition, and ecological integrity, with cascading consequences for associated fauna (Saoum and Sarkar 2024; Ur Rahman et al. 2024).

Climate change can induce physiological stress, biodiversity loss, and shifts in primary productivity, affecting entire food webs (Alongi 2022). In South America, water temperatures may rise by up to 2.5 °C by the end of the century, with severe consequences for coastal ecosystems (Magrin et al. 2014). Even under scenarios of global temperature stabilisation, shifts in the distribution and productivity of exploited marine species are expected, underscoring the need for adaptive monitoring and management (Morée et al. 2024). For crustaceans, warming can impair energy metabolism, antioxidant defences, and behavioural performance, as reported for intertidal species exposed to marine heatwaves or fluctuating temperature regimes (Paganini et al. 2014; Madeira et al. 2018). Rising temperatures are also expected to increase the frequency and severity of extreme events such as prolonged droughts, severe floods, and stronger storms, while reducing extreme cold events, potentially facilitating poleward mangrove expansion and regime shifts in tropical–temperate ecotones (Cavanaugh et al. 2019; IPCC 2023). Together, these trends are expected to modify baseline conditions in estuarine landscapes, potentially narrowing physiological safety margins for resident species.

In coastal environments, climate change may alter water salinity (Lee et al. 2011), increase sedimentation in estuaries, affecting deposition and progradation patterns (Ralston et al. 2013), and promote bioinvasions by allochthonous species (Molinos et al. 2015; Beaury et al. 2019). Such abiotic shifts pose particular risks to shallow systems in estuaries and coastal plains (IPCC 2023). Moreover, climate-driven shifts in hydrology and sediment dynamics may change chemical conditions in ways that influence the exposure of organisms to existing environmental pressures, including contaminants, even when these are not the primary focus of investigation (Lacerda et al. 2022). Linking temperature sensitivity across community, population, and individual levels provides a robust framework for predicting species responses to climate stressors (Kang et al. 2024). In this context, quantifying physiological responses to warming provides mechanistic insight into vulnerability under future scenarios.

For local fauna, water temperature rise can disrupt metabolic pathways, gene expression, and reproductive cycles of ectothermic organisms, while changes in salinity and pH may cause chronic stress leading to population decline or adaptation (Trídico et al. 2010; Magrin et al. 2014; Qyli et al. 2020; Booth et al. 2023; Menon et al. 2023). Abrupt salinity shifts can also impair early life stage development and survival in crustaceans, directly affecting recruitment dynamics (Šargač et al. 2021). Because warming often occurs alongside increased environmental variability, early-warming physiological indicators are valuable for detecting sublethal stress before demographic changes become evident. Among Neotropical crustaceans, the mangrove crab *Ucides cordatus* (Linnaeus, 1763) is a robust sentinel species for biomonitoring due to its broad Western Atlantic distribution, ecological relevance, and sensitivity to contamination. Like other semi-terrestrial brachyurans, *U. cordatus* has physiological adaptations for coping with fluctuating environmental conditions, but these tolerances may be exceeded under future warming and altered salinity regimes (Marin and Tiunov 2023). This species has been widely used in ecotoxicological research, showing consistent cytological and genotoxic responses to pollutants, particularly metals, via biomarkers such as neutral red retention time (NRRT) and micronucleus assays, both reliable indicators of environmental stress (Duarte et al. 2017; Pinheiro et al. 2022; Souza et al. 2022; Vogt et al. 2023). Here, we build on this established biomarker framework to examine thermal stress responses under controlled warming.

Aquatic organisms, including decapod crustaceans, are subject to stressors such as UV radiation, contaminants, and thermal variation, all of which can induce oxidative stress (Vinagre et al. 2012). Antioxidant defences vary according to aerobic metabolic rates, as reported for *Chasmagnathus granulatus* (Dana, 1851) (Maciel et al. 2004). Such variation influences reactive oxygen species (ROS) generation, leading to lipid peroxidation (LPO) and protein oxidation, both of which can cause DNA damage (Halliwell and Gutteridge 1999). However, knowledge of defence systems and physiological adaptations in many ecologically or economically important crustaceans, particularly under climate-related thermal stress, remains limited. Experimental studies show that warming, often acting synergistically with acidification, can reduce antioxidant capacity, increase oxidative damage, and compromise physiological performance in marine and estuarine crabs (Paganini et al. 2014; Qyli et al. 2020). Accordingly, oxidative damage endpoints (e.g. LPO) can complement cellular biomarkers by capturing tissue-level impairment under thermal exposure.

In *U. cordatus*, changes in population density and structure are expected as a result of sea-level rise and shifts in arboreal composition, reinforcing its value as a bioindicator of climate change (Nordhaus et al. 2009;

Pinheiro and Almeida 2015; Pinheiro, in press). The species plays a key ecological role in sediment bioturbation (Araújo-Júnior et al. 2016) and leaf litter processing (Christofolletti et al. 2013), combining high tolerance to environmental variability with sensitivity to xenobiotics (Pinheiro et al. 2012, 2013; Nobre et al. 2022). This duality makes it a valuable model organism in ecotoxicology (Dutra et al. 2009; Duarte et al. 2016, 2017; Pinheiro et al. 2022; Souza et al. 2022; Ramos et al. 2024). Field observations and controlled experiments indicate that exposure to elevated water temperatures – whether from climate warming or thermal effluents – can modify reproductive traits in estuarine crabs, increasing the proportion of ovigerous females and egg production but sometimes reducing abundance due to effects on larval and juvenile stages (Madeira et al. 2018; Vogt et al. 2023; Taylor et al. 2025). This reinforces the relevance of mechanistic assessments linking warming to early sublethal biomarkers and oxidative damage in this species.

Considering projected thermal increases in mangrove environments and previous evidence of biomarker responses in *U. cordatus* to environmental stressors, this study experimentally evaluates the physiological effects of thermal stress in this species. Specifically, we assessed potential cell membrane damage through NRRT and oxidative damage in gill and hepatopancreatic tissues via LPO quantification. We hypothesized that heat treatment would result in shorter NRRT and higher LPO values, indicating thermal stress.

Materials and methods

Sixty specimens of *U. cordatus* were collected from mangroves in Cananéia, in August 2014, in the southern region of the State of São Paulo, Brazil, consisting of intact adults (with all appendages present), in the intermoult stage, and with carapace width (CW) ≥ 60 mm (as stipulated in current legislation – IBAMA 2003), obtained by manual capture ('braceamento'; Pinheiro and Fiscarelli 2001). Immediately after capture, the animals were transported in thermal boxes, kept immobilized to minimize handling-induced stress. In the laboratory, the following biometric parameters were recorded: CW (mm), measured using a precision calliper (0.05 mm), and total wet weight (WW, g), recorded on a precision balance (0.01 g). Subsequently, five specimens were assessed for neutral red retention time (NRRT), based on hemolymph sample and lipid peroxidation (LPO) levels, in the gill and hepatopancreatic tissues (see details in the following section), to determine their initial environmental condition. A baseline control group without thermal treatment was used to compare with the experimental group and to assess whether experimental handling induced any physiological stress (Harding et al. 2004).

The remaining animals ($n = 55$) were distributed among 11 rectangular glass aquaria (50 cm length \times 40 cm width \times 40 cm height) containing brackish water (15 ± 1), sourced from the capture site (5 cm water column depth), with each aquarium housing five specimens. Five PVC pipes (15 cm length \times 10 cm diameter) were placed in each aquarium to serve as artificial burrows, thereby reducing potential stress from behavioural interactions and allowing individuals to climb and partially emerge from the water as needed. The crabs were acclimated for seven days in a temperature-controlled room ($24 \pm 1^\circ\text{C}$) with a 12:12 h light:dark photoperiod, fed with leaves of red mangrove *Rhizophora mangle* L., and monitored daily for salinity and pH. The temperature for the control group ($24 \pm 1^\circ\text{C}$) was recorded using a digital maximum–minimum thermometer, regulated by an air-conditioning system connected to a digital thermostat to prevent significant thermal fluctuations. The aquaria were siphoned daily to remove food remains, organic matter, and faeces, thereby preventing deterioration of water quality from nitrogenous waste (including nitrogenous excretion/urine). Replacement water was added only after it reached the same temperature as that in the aquaria. Two exposure tests (acute and chronic) were conducted, as described below. Throughout the experimental period, the crabs were qualitatively monitored for general behavioural changes as an indicator of physiological stress and animal welfare. Observations were conducted at least twice daily by the same observer, focusing on observable behavioural changes, including abnormal locomotion, prolonged immobility, loss of balance, reduced responsiveness to external stimuli, and atypical postures. No formal behavioural score or quantitative ethogram was applied, as behavioural assessment was not a primary objective of the study.

Acute test

Fifteen crabs were used, distributed among three aquaria with the same dimensions and conditions as the previous ones (five specimens each), and placed in a climate chamber set to $30 \pm 1^\circ\text{C}$ (treatment group),

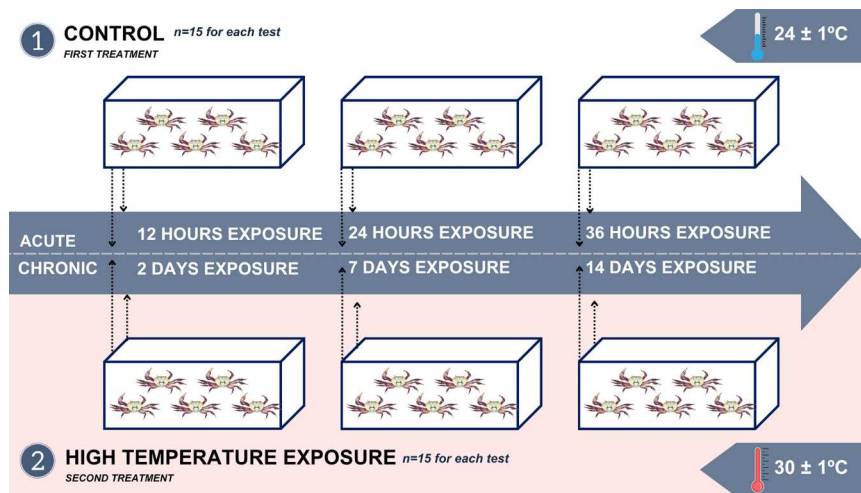


Figure 1. Experimental design used to evaluate thermal stress in the mangrove crab *Ucides cordatus* (Linnaeus, 1763) under two thermal treatments: Control ($24 \pm 1^\circ\text{C}$) and elevated temperature in a climate chamber ($30 \pm 1^\circ\text{C}$). Exposures were classified as acute (12, 24, and 36 h) or chronic (2 and 4 days).

salinity 15 ± 1 , and a 12:12 h photoperiod. Three sampling intervals were established from the start of the experiment (12, 24, and 48 h), with five specimens removed at each interval for haemolymph collection and analysis of two physiological biomarkers: neutral red retention time (NRRT), obtained from haemolymph sampling and processing (see Duarte et al. 2016; Adam et al. 2023), and lipid peroxidation (LPO) in gill tissues (anterior gills, primarily respiratory in function, and posterior gills, mainly osmoregulatory) using the modified FOX protocol, or xylene orange assay, adapted from Hermes-lima et al. (1995) and Jiang et al. (1991, 1992). The same procedure was carried out with fifteen animals from the control group (acclimation conditions), which were kept in aquaria outside the climate chamber (Figure 1).

Chronic test

Similarly, another 15 crabs were placed in the climate chamber under thermal stress (treatment group, $30 \pm 1^\circ\text{C}$), with salinity maintained at 15 ± 1 and a similar photoperiod (12:12 h light: dark). The same procedures as in the acute test were followed, with the application of both protocols (NRRT and LPO), but with sampling at 2, 7, and 14 days post-experiment initiation (Figure 1).

Statistical analyses

Quantitative variables were tested for normality (W, Shapiro–Wilk test) and homogeneity of variances (L, Levene’s test). When the data were not normally distributed ($p < 0.05$), a non-parametric test (KW, Kruskal–Wallis) was applied to compare values across the different heat exposure treatments. When data met the assumptions of normality, an analysis of variance (ANOVA) was performed, with post hoc mean comparisons by Tukey’s test. The means of the primordial values, for both tests, were compared with the respective values of the control group, aiming to verify possible experimental stress. To compare values between the thermal groups (control \times treatment), central tendency tests (Student’s t-test or Wilcoxon–Mann–Whitney test) were applied, depending on normality and homogeneity results (Sokal and Rohlf 2003). Means were compared, and differences were considered significant at the 5% level. All raw experimental data, as well as the step-by-step analysis code, are available in a GitHub repository for reproducibility: <https://github.com/esli-mosna/Thermal-Stress-U.-cordatus.git>.

Results

The crabs analysed had a carapace width (CW) ranging from 62.9 to 87.7 mm (mean \pm standard deviation: 76.3 ± 5.0 mm) and a total wet weight (WW) ranging from 119.25 to 267.46 g (190.10 ± 31.88 g). The

crabs were qualitatively monitored throughout the experimental period to detect conspicuous behavioural alterations potentially associated with increased temperature. No overt behavioural differences were qualitatively detected among climatic treatments in either the acute or chronic tests. A single mortality event was recorded in the control group during chronic exposure.

Table 1. Mean \pm standard deviation (SD) of neutral red retention time (NRRT, min) and lipid peroxidation (LPO, nmol CHP g^{-1}) in *Ucides cordatus* after acute (12, 24, 36 h) and chronic (2, 5, 7 days) exposure under three experimental groups: white (baseline control with no thermal treatment), control (24 ± 1 °C), and elevated temperature treatment (30 ± 1 °C). Where: C = control; T = treatment; \bar{x} = mean; s = standard deviation. Asterisks (*) and bold indicate significant differences ($p < 0.05$) between treatment and control at the same exposure time. Underlined control values indicate significant differences ($p < 0.05$) relative to primordial values, used to verify possible stress effects from experimental handling. Different lowercase letters above bars in the corresponding figures indicate significant differences among exposure times within the same treatment group ($p < 0.05$).

Biomarker	Primordial Values	Thermal Groups	Exposure Tests ($\bar{x} \pm s$)					
			Acute Test			Chronic Test		
			12 h	24 h	36 h	2 days	5 days	7 days
NRRT (min)	120 \pm 38	C	96.0 \pm 25.1	84.0 \pm 13.4	<u>54.0 \pm 22.7</u>	81.0 \pm 29.2	52.5 \pm 28.7	51.0 \pm 13.4
		T	108.0 \pm 16.4	90.0 \pm 21.2	66.0 \pm 22.7	90.0 \pm 24.5	75.0 \pm 17.3	52.5 \pm 8.7
LPO Anterior Gill (nmol CHP/g)	4.4 \pm 0.3	C	4.2 \pm 0.5	4.9 \pm 0.6	4.3 \pm 0.2*	<u>3.3 \pm 0.4</u>	4.2 \pm 0.1	<u>3.4 \pm 0.3</u>
		T	3.9 \pm 0.2	5.0 \pm 0.1	4.9 \pm 0.4*	3.4 \pm 0.2	4.0 \pm 0.2	3.6 \pm 0.3
LPO Posterior Gill (nmol CHP/g)	4.6 \pm 0.2	C	4.4 \pm 0.3*	5.0 \pm 0.5	4.5 \pm 0.1	<u>3.7 \pm 0.3</u>	4.5 \pm 0.1	<u>3.3 \pm 0.3</u>
		T	3.9 \pm 0.1*	4.8 \pm 0.4	4.5 \pm 0.2	3.4 \pm 0.1	4.2 \pm 0.3	3.7 \pm 0.3
LPO Hepatopancreas (nmol CHP/g)	4.8 \pm 0.7	C	4.5 \pm 0.7*	<u>6.3 \pm 0.8</u>	5.4 \pm 0.8	4.5 \pm 0.3	4.8 \pm 0.5	4.1 \pm 0.2
		T	3.7 \pm 0.4*	6.3 \pm 0.4	4.9 \pm 0.7	4.2 \pm 0.5	4.8 \pm 0.4	3.9 \pm 0.3

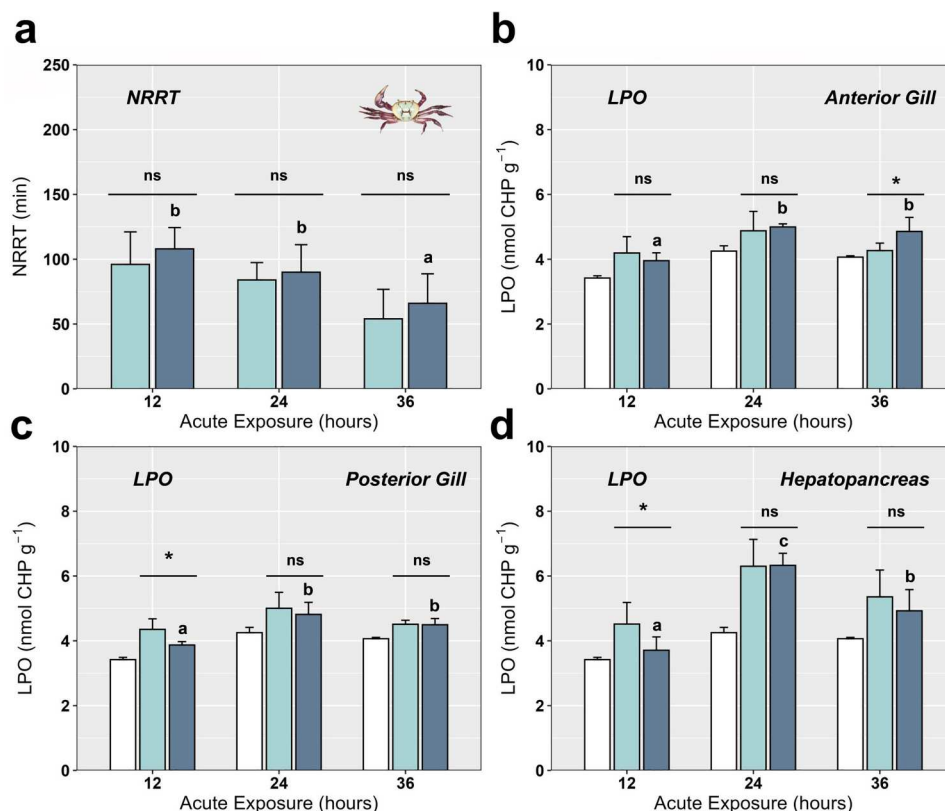


Figure 2. Mean values (bars) \pm standard deviation (vertical lines) for neutral red retention time (NRRT, min) and lipid peroxidation (LPO, nmol CHP g^{-1}) in *Ucides cordatus* after acute exposure (12, 24, and 36 h) under three experimental groups: reagent blank (no biological sample – white bars), control (24 ± 1 °C – light blue), and treatment (30 ± 1 °C – dark blue). Panels indicate: (a) NRRT; (b) LPO in anterior gill; (c) LPO in posterior gill; and (d) LPO in hepatopancreas. Horizontal lines with '*' indicate significant differences ($p < 0.05$) between control and treatment within the same exposure time, whereas 'ns' denotes no significant difference ($p > 0.05$). Different lowercase letters above bars indicate significant differences among exposure times within the treatment group ($p < 0.05$).

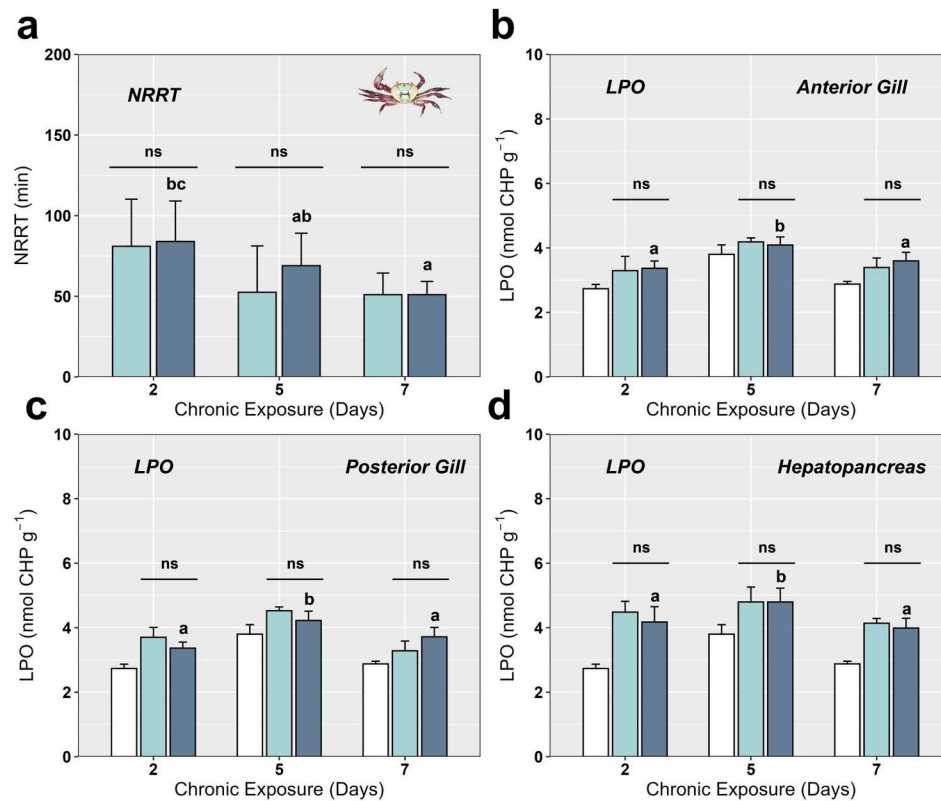


Figure 3. Mean values (bars) \pm standard deviation (vertical lines) for neutral red retention time (NRRT, min) and lipid peroxidation (LPO, nmol CHP g⁻¹) in *Ucides cordatus* after chronic exposure (2, 5, and 7 days) under three experimental groups: reagent blank (no biological sample – white bars), control (24 \pm 1°C – light blue), and treatment (30 \pm 1°C – dark blue). Panels indicate: (a) NRRT; (b) LPO in anterior gill; (c) LPO in posterior gill; and (d) LPO in hepatopancreas. Horizontal lines with ‘*’ indicate significant differences ($p < 0.05$) between control and treatment within the same exposure time, whereas *ns* denotes no significant difference ($p > 0.05$). Different lowercase letters above bars indicate significant differences among exposure times within the treatment group ($p < 0.05$).

The primordial neutral red retention time (NRRT) values differed only in the control group at the 36-hour exposure ($p < 0.05$). For primordial lipid peroxidation (LPO) values, when compared with those from the acute test, a significant difference was found only at the 24-hour exposure in the hepatopancreatic tissue ($p < 0.05$). In the chronic test, statistical differences were observed in the anterior and posterior gill tissues at the 2- and 7-day exposures ($p < 0.01$) (Table 1).

For NRRT, a significant decrease in the mean retention time was observed in both the acute and chronic tests ($p < 0.05$), but no significant differences were found between the control and treatment groups when compared pairwise ($p > 0.05$) (Figures 2a and 3a). In general, crabs kept in the climate chamber at 30 \pm 1°C (treatment) exhibited shorter retention times than those maintained under external conditions at 24 \pm 1°C (control), across all exposure durations, although without statistical significance ($p > 0.05$) (Table 1; Figures 2a and 3a).

For LPO levels, significant differences were observed only in the acute test (Figure 2; Table 1). In this case, LPO levels were significantly higher in the posterior gills at 12 h from the start of the experiment ($t = -3.16$; $p < 0.05$; Figure 2c) and in the hepatopancreas at the same time point ($t = -2.31$; $p < 0.05$; Figure 2d), while in the anterior gills such differences were detected only after 36 h ($t = 2.69$; $p < 0.05$; Figure 2b).

Discussion

This study provides experimental evidence that acute and chronic thermal exposure influences cellular membrane stability and oxidative status in *Ucides cordatus*. As hypothesized, elevated temperature (30 \pm 1°C) altered NRRT and LPO values compared with the control group, although the direction and magnitude of effects varied according to exposure duration and tissue type. These findings reinforce that thermal stress

can induce both immediate and progressive physiological changes in mangrove crabs, with potential implications for their survival and ecological roles in a warming climate.

In coastal ecosystems, projected temperature increases of up to 2.7 °C by the end of the century (IPCC 2023) are often amplified in shallow and estuarine habitats, directly affecting ectothermic organisms such as crabs. Under certain conditions, these changes may compromise physiological homeostasis and trigger oxidative stress (Madeira et al. 2018). In the present study, both acute and chronic exposures of *U. cordatus* produced measurable alterations in LPO and NRRT, indicating physiological responses to thermal variation.

Temperature is a key regulator of metabolism, biochemical reaction rates, and membrane stability in ectotherms (Hochaka and Somero 2002). It can also modulate responses to environmental contaminants (Sokolova and Lannig 2008). Crabs often counteract thermal stress through metabolic adjustments, mobilisation of glycogen reserves, and synthesis of protective proteins such as HSPs (Vogt et al. 2023). The variations observed in our acute and chronic trials likely reflect homeostatic disruption and the activation of compensatory mechanisms (Runcie et al. 2012).

The tolerance of coastal species to multiple stressors is often non-linear, with physiological thresholds beyond which survival and performance can decline sharply (Capparelli et al. 2022). These thresholds may be further reduced when warming acts in synergy with other abiotic factors. In marine and estuarine crabs, simultaneous variation in temperature and pH has been shown to reduce antioxidant capacity and impair metabolic performance (Paganini et al. 2014; Qyli et al. 2020). Warming can also enhance the toxicity of trace metals, thereby intensifying oxidative stress and mortality in benthic invertebrates (Alves et al. 2025), while temperature–salinity interactions may alter larval performance and recruitment success (Geißel et al. 2024).

Thermal sensitivity has also been documented in brachyuran larvae, where developmental performance decreases at elevated temperatures (Gencer 2024). Ontogenetic differences in stress tolerance, as observed in Caribbean king crab larvae and juveniles exposed to combined warming and acidification (Gravinese et al. 2022), highlight the importance of considering life-stage-specific responses. In adults, marine heatwaves can disrupt antioxidant defences, increase lipid peroxidation, and induce heat shock protein synthesis, all of which are reliable biomarkers of acute stress (Madeira et al. 2018; Arrigo et al. 2025).

Oxidative stress results from the accumulation of reactive oxygen species (ROS), which can damage proteins, lipids, and DNA (Lesser 2006). For *U. cordatus*, studies on temperature–physiology interactions are scarce, with most information limited to larval stages (Zarochi et al. 2024). Adults show reduced activity at temperatures below 15 °C (Pineiro and Hattori 2003) and mortality at 10.7 °C (Branco 1993). Larvae display low tolerance to thermal variation, with viability decreasing at 20 °C and survival dropping at higher temperatures (Pineiro et al. 1994; Cottens et al. 2010; Zarochi et al. 2024), potentially limiting dispersal (Anger et al. 1990; Paganini et al. 2014).

The NRRT assay detects lysosomal membrane damage caused by free radical attack (Lowe et al. 1995) and is widely used in ecotoxicology (Hauton et al. 1998; Harding et al. 2004; Martinez-Haro et al. 2016; Soms-Molina et al. 2024). In crabs, reductions in NRRT under warming are often associated with increased LPO and changes in antioxidant enzyme activity (Madeira et al. 2018; Qyli et al. 2020). In our study, mean NRRT declined over time in both exposure regimes, but without significant treatment effects, suggesting that handling-induced stress (Harding et al. 2004) or natural variability may have influenced the results. This reinforces the importance of integrating multiple biomarkers, assessed in different tissues, to detect subtle physiological responses (Pham and Sokolova 2023).

Lipid peroxidation is a widely recognized indicator of oxidative damage to membranes (Maciel et al. 2008; Lushchak 2011). In our experiments, anterior gills showed a significant increase in LPO at elevated temperature, likely reflecting their direct environmental exposure and higher vulnerability to stressors (Zhang et al. 2023). No significant effects were detected in posterior gills or hepatopancreas, possibly due to delayed onset of oxidative damage or more efficient antioxidant defences (Madeira et al. 2018; Zheng et al. 2019; Qyli et al. 2020).

The absence of a consistent LPO pattern across tissues underscores the complexity of thermal physiology in ectotherms (Cheng et al. 2018). Given that decapods are particularly sensitive to temperature fluctuations (Stillman 2003; Zhang et al. 2023), integrated approaches that combine multiple biomarkers, evaluated in different tissues and under varied regimes (Regoli and Giuliani 2014), remain the most effective strategy for early detection of sublethal effects. This is especially important in vulnerable coastal habitats such as mangroves and salt marshes, which are directly affected by sea-level rise and tidal dynamics (Schaeffer-Novelli et al. 2016).

Overall, our findings partially support the initial hypothesis: LPO increased in anterior gills but not consistently across other tissues, and NRRT differences between treatments were not statistically significant. The applied warming scenario did not produce a uniform oxidative stress response but confirmed the tissue-specific nature of thermal effects. Given its ecological relevance, sensitivity to physiological disruption, and measurable biomarker responses (Pinheiro et al. 2012, 2013, 2022; Duarte et al. 2016, 2017), *U. cordatus* remains a robust sentinel species for identifying sublethal stress and a valuable bioindicator of ecosystem vulnerability under climate change. Future studies integrating multi-stressor exposure, life-stage comparisons, and long-term monitoring would further strengthen its application within predictive and adaptive monitoring frameworks.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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ORCID

Esli Emanuel Domingues Mosna  <http://orcid.org/0000-0002-6330-1586>

Camila Prieto  <http://orcid.org/0000-0002-3449-2133>

Caroline Araújo De Souza  <http://orcid.org/0000-0002-3921-0094>

Marcelo Antonio Amaro Pinheiro  <http://orcid.org/0000-0003-0758-5526>

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