Thermal Biology and Metabolic Scope of Two Species of Juvenile Gastropod Mollusks Inhabiting Kelp Forests

Ana Denise Re-Araujo[1,*], Fernando Díaz[1,§], J. Pablo Sánchez-Ovando[1,§], Fabiola Lafarga-de la Cruz[2], Laura Alvarez-Lee[1], and Luis Enrique Angeles-Gonzalez[1]

[1]*Laboratorio de Ecofisiología de Organismos Acuáticos, Departamento de Biotecnología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Baja California, 22860, México. *Correspondence: E-mail: denisre@cicese.edu.mx (Re-Araujo)*
E-mail: fdiaz@cicese.edu (Díaz); sanchezjp@cicese.edu.mx (Sánchez-Ovando); ibeth@cicese.mx (Álvarez-Lee); luis.angeles0612@gmail.com (Angeles-Gonzalez)

[2]*Laboratorio de Genética Acuícola, Departamento de Acuicultura, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Baja California, 22860, México. E-mail: flafarga@cicese.mx (Lafarga-de la Cruz)*

[§]*FD and JPSO contributed equally to this work.*

Ana Denise Re-Araujo: https://orcid.org/0000-0002-9639-9866

Fernando Díaz: https://orcid.org/0000-0003-1614-6795

J. Pablo Sánchez-Ovando: https://orcid.org/0000-0001-6238-8934)

Fabiola Lafarga-de la Cruz: https://orcid.org/0000-0002-5101-9364

Laura Alvarez-Lee: https://orcid.org/0000-0002-5266-1623

Luis Enrique Angeles-Gonzalez: https://orcid.org/0000-0003-3537-4574

Temperature is one of the main abiotic factors that influence the growth, reproduction, food availability, distribution, and survival of many species of marine ectotherms. *Megastraea undosa* and *Megathura crenulata* are gastropod mollusks with economic, commercial, and biomedical importance that are part of the kelp forest community in the temperate waters of the northern Pacific of Mexico. As

knowledge about the thermal biology of these two species is scarce, this study aimed to determine the effect of different acclimation temperatures on the thermal biology and metabolic scope of these two species. 120 *M. undosa* juveniles were collected from the wild and acclimated to four temperatures (13, 16, 19, and 22°C ± 1°C), while 90 *M. crenulata* juveniles were cultured in an aquaculture facility (Stellar Biotechnologies, Inc) and acclimated to three temperatures (17, 20, and 23°C ± 1°C). Subsequently, experiments were performed to determine the thermal tolerance, thermal window, thermal safety margin, future thermal safety margin, and thermal metabolic scope of these species. The thermal tolerances of both species were relatively similar (*M. undosa* juveniles: CTmax = 25.3, 21.0, 25.4 and 27.4°C, CTmin = 6.0, 9.2, 16.3 and 17.3°C; *M. crenulata* juveniles: CTmax = 27.7, 28.1 and 28.8°C, CTmin = 11.2, 12.1 and 14.7°C). Both species had a small thermal window area (*M. undosa*: 77.2°C^2; *M. crenulata*: 65.25°C^2). The values of the thermal safety margin and the future thermal safety margin of both species were lower during the summer than winter. The optimal temperature proxies of *M. undosa* and *M. crenulata* juveniles were 16 and 20°C, respectively. This study shows that the two species of marine gastropods could be moderately affected by ocean warming, being forced to modify their current distribution patterns. The data on thermal limits and optimal temperatures can be used to initiate and maximize the cultivation of both species, which would also help mitigate the impact of overfishing on natural populations.

Key words: Aquaculture, Climate change, *Megastraea undosa*, *Megathura crenulata*, Ocean warming.

Citation: Re-Araujo AD, Díaz F, Sánchez-Ovando JP, Lafarga-de la Cruz F, Alvarez-Lee L, Angeles-Gonzalez LE. 2025. Thermal biology and metabolic scope of two species of juvenile gastropod mollusks inhabiting kelp forests. Zool Stud **64:**06.

BACKGROUND

Temperature is one of the main abiotic factors that influence the growth, reproduction, food availability, and distribution of aquatic individuals (Pörtner 2002; Sanford 2002; Pörtner et al. 2008;

IPCC 2022). In marine ectotherms, this variable exerts its effects at different levels of organization; for example, molecular, biochemical, physiological, and behavioral (Stillman and Somero 2000; Mora and Ospína 2001; Folguera et al. 2011; Nguyen et al. 2011). Since the Industrial Revolution, anthropogenic emissions of CO_2 into the atmosphere have been increasing, causing the average global temperature to increase by 0.74°C since 1760. By the end of this century, the average sea surface temperature is expected to increase by 2–4°C (Lüthi et al. 2008; Meinshausen et al. 2011; IPCC 2022).

Mollusks of the Class Gastropoda are among the most important in the marine environment because they represent approximately 80% of all mollusk species. Many species of marine gastropods are distributed along the northern Pacific coasts of Mexico, playing significant roles in ecosystems and have economic importance due to their use in aquaculture for human consumption or extraction of substances for biomedical use (Harris and Markl 1999; DOF [Official Gazette of the Federation] 2018). Two such species are *Megastraea undosa* (W. Wood, 1828) and *Megathura crenulata* (G. B. Sowerby I, 1825), which inhabit kelp forests (Cox and Murray 2006; Torres-Moye et al. 2013).

Megastraea undosa, commonly known as the wavy turban snail, is a generalist herbivorous gastropod distributed on rocky and sandy substrates associated with algal beds at depths of up to 80 m, and ranging from Point Conception, Santa Barbara, California, USA to Asuncion Island, Baja California, Mexico (McLean 1978; Gotshall 1994; Taniguchi and Rogers-Bennett 2001). It is a slow-growing species that can live up to 12 years (Gluyas-Millán et al. 1999). Currently, *M. undosa* supports an economically important fishery off the west coast of Baja California Sur, Mexico. In 1980, commercial exploitation of this species formally began, with a production of 75.7 tons obtained with relatively little effort (DOF [Official Gazette of the Federation] 2018). In 1989, fishing efforts increased, generating a maximum production of 353.6 tons of fresh tripe with operculum. However, between 2000 and 2013, the average production on the western coast of Baja California Sur remained at 200 tons, with a notable decrease (<150 tons) in 2015 and 2016. In the state of Baja California, the capture of this species is lower than in Baja California Sur. In 2009, the highest catch peak was reached at approximately 70 tons, but by 2014, the fishing of this species fell significantly, and by 2016, the catch in tons was almost zero (DOF [Official Gazette of the Federation] 2018). Furthermore, on the western coast of Baja California Sur, this species has a typical density of 2.2-2.4 No./m^2 and

individuals of different sizes can be found (minimum: 7-28 mm, maximum: 144-180 mm), with a dominance of small sizes in the southernmost fishing site (Hernández-Padilla et al. 2021).

Megathura crenulata, commonly known as the giant keyhole limpet, is an omnivorous gastropod with an herbivorous tendency, feeding on macroalgae (i.e., giant kelp *Macrocystis pyrifera* and stipitate kelp *Eisenia arborea*), as well as associated invertebrates (i.e., tunicates, bryozoans and hydrozoans, Oakes et al. 2004; Martin et al. 2011; Mazariegos-Villarreal et al. 2013). It belongs to the family Fissurellidae J. Fleming, 1822 and is the only identified species of its genus to date (Martin et al. 2011; Cunha et al. 2019). This species is endemic to rocky intertidal and subtidal areas (to 25 m deep) along the coast of California, from Monterey Bay, USA to Asuncion Island, Baja California, Mexico. This species experiences temperatures ranging from 16 to 20°C along its distribution (Smith and Carlton 1975; Morris et al. 1980). In addition, the hemolymph of *M. crenulata* contains a metalloprotein utilized for biomedical applications (Harris and Markl 1999). *Megathura crenulata* can be maintained in captivity without negatively affecting the relative levels of keyhole limpet hemocyanin (KLH) isoforms, demonstrating that a reliable long-term supply of KLH can be provided through aquaculture. The commercial use of *M. crenulata* has not yet been established in the Baja California Peninsula; however, cultivation of this species could provide a sustainable source for extracting KLH (Oakes et al. 2004). The average size of this species is 120 mm and minimum and maximum size is 10-40 mm and 187-220 mm, respectively (Kushner et al. 2013; Aguilar-Mora 2012; Reed 2020). Recently, Bonett-Calzada et al. (2024) published the complete genome of *M. crenulata*.

Despite the economic and biomedical importance of these two species of marine gastropods and the effects of imminent climate change, few studies have focused on understanding the effect of temperature on their thermal biology (Díaz et al. 2011, 2015). Therefore, this study aimed to determine the effect of different acclimation temperatures on the thermal biology and metabolic scope of juveniles of *M. undosa* and *M. crenulata* distributed in the temperate waters of the northern Pacific of Mexico.

MATERIALS AND METHODS

Collection of specimens, maintenance, and acclimation

120 *Megastraea undosa* juveniles were collected from Campo Kennedy, Ensenada, Baja California, Mexico (31°42'08.6"N, 116°41'07.9"W) at a depth of 10 m. The wavy turban snails ranged from 30.6-114.6 g wet weight and the typical size was 3.5-6.5 cm (4.97 ± 0.69, mean ± SD). Likewise, 90 *Megathura crenulata* juveniles were F1 from wild caught animals cultured in an aquaculture facility (Stellar Biotechnologies, Inc). The limpets were in a range of 4.2–17.6 g wet weight and the typical size was 3.7–5.0 cm (4.35 ± 0.31). The juveniles of both species were transferred to the Wet Laboratory of the Department of Marine Biotechnology at CICESE (Centro de Investigacion Cientifica y de Educacion Superior de Ensenada, Baja California, Mexico), where they remained to monitor their survival for 24 h. Initially, juveniles of both species were placed separately in two reservoirs with a capacity of 2000 L. The physical-chemical parameters of the seawater in the reservoirs were constant (temperature: 18 ± 1°C, dissolved oxygen: above 6 ± 0.5 mg L^{-1}, salinity: 35‰, pH: 8.1, and photoperiod: 12–12 hours light/dark).

Acclimation temperature experiments were carried out separately for the *Megastraea undosa* and *Megathura crenulata* juveniles. The *M. undosa* juveniles were acclimated to four temperatures (13, 16, 19, and 22°C ± 1°C), whereas the *M. crenulata* juveniles were acclimated to three temperatures (17, 20, and 23°C ± 1°C). The acclimation temperatures selected for both species reflect the temperature range of the natural habitat where these two gastropod species are distributed, as both species can be found in both low intertidal and subtidal areas (Morris et al. 1980; Taniguchi and Rogers-Bennett 2001).

Low and high temperatures were monitored and controlled with chillers (LN-2600, China) and heaters (H₂Pro, USA), respectively. For the acclimation of the *M. undosa* and *M. crenulata* juveniles, four and three 2000 L reservoirs with seawater were used, respectively (one reservoir for each acclimation temperature), with 30 juveniles in each reservoir. Subsequently, juveniles of both species were acclimated for 30 days. This acclimation period was used to ensure that the thermal life history that the juveniles previously experience was eliminated, thus ensuring that the responses observed during the experiments were due to the acclimation temperatures and not the thermal life history of the organisms. After the acclimation period, it was observed that the juveniles were healthy because the

food provided was consumed entirely and the juveniles adhered strongly to the walls of the tanks, and none died.

During the acclimation period (30 days), the juveniles were fed *ad libitum* with the macroalgae, *Macrocystis pyrifera* (Linnaeus) C. Agardh, 1820. The reservoirs were siphoned and washed weekly to reduce the accumulation of fecal matter and associated bacteria.

Thermal tolerance (maximum and minimum critical temperatures) and thermal window

To determine thermal tolerance (maximum and minimum critical temperature), the loss of locomotor stability and attachment capacity (detachment from the acrylic plates) of the juveniles were monitored when they were exposed to temperatures higher or lower than their acclimation temperatures, according to the tenacity methodology described by Morley et al. (2011) and Díaz et al. (2015). This methodology evaluates the ability of mollusks to remain attached to a substrate until a stressor such as maximum or minimum temperatures which causes the loss of this attachment capacity. To achieve this, 10 *M. undosa* juveniles and 8 *M. crenulata* juveniles from each acclimation temperature were not fed for 24 h prior to the trial, and then a monofilament fishing line was attached to the shell of individuals using cyanoacrylate adhesive (CorAffix®). In both species, individuals were placed on acrylic plates (30 × 25 cm) in 40 L aquaria with aeration and left undisturbed overnight. Subsequently, a piece of lead (representing an extra weight of 50% of the weight of each individual) was placed at the other end of the monofilament as a counterweight and the temperature was increased or decreased to determine the endpoint of maximum critical temperatures (CTmax) and minimum critical temperatures (CTmin), respectively.

To determine CTmax, a 1000 W thermostatically controlled heater and an air stone were placed in each aquarium. The temperature was raised from the acclimation temperatures at a rate of 1°C every 30 min to allow the core temperature of the individuals to equilibrate with the surrounding water temperature. This rate approximates the natural daily temperature increases in the habitat of both species (Cox 1974; Tomanek and Somero 1999; Stenseng et al. 2005). When the individuals were observed to detach from the acrylic plates (*i.e.*, loss of attachment capacity), the temperature stopped increasing and the temperature value that corresponded to the CTmax was recorded. To determine

CTmin, the same methodology was followed as for CTmax, and the criterion to determine the endpoint of CTmin was the same as that used for CTmax, but with decreasing temperatures. A chiller (LN-2600, China) was used to reduce the temperature at a rate of 1°C every 30 min. Immediately after the individuals were detached from the acrylic plates after having reached CTmax and CTmin, they were transferred to their respective 2000 L reservoirs to recover at either of the original acclimation temperatures.

As controls to determine the effect of the extra weight of the lead piece (which represented an extra weight of 50% of the weight of the juveniles) on the juveniles loss of attachment to the acrylic plate, 10 *Megastraea undosa* and *Megathura crenulata* juveniles were placed in aquaria and allowed to acclimate to 13, 16, 19, and 22.0°C ± 1°C and 17, 20 and 23°C ± 1°C, respectively. All were monitored to measure the time at which they detached from the plates. The experiment was terminated after six hours because the individuals never detached from the plates. This control demonstrated that at the acclimation temperatures the individuals did not detach because they were healthy, and not stressed and subsequent loss of attachment was due to the individuals having already reached the maximum or minimum critical temperature and was not due to the extra weight of the lead piece.

The thermal window area expressed in °C^2 was obtained from the CTmax and CTmin data according to Bennett and Beitinger (1997).

Thermal safety margin and future thermal safety margin

One approach to determining how vulnerable ectothermic animals are to environmental warming involves comparing the upper thermal safety margin (TSM) (Deutsch et al. 2008; Huey et al. 2012). The thermal safety margin (TSM = CTmax – maximum habitat temperature) for *Megastraea undosa* and *Megathura crenulata* and the future thermal safety margin (FTSM = CTmax – (maximum habitat temperature + 3°C)) were estimated (Vinagre et al. 2019). We downloaded the seasonal surface temperature data (day data: spring, summer, autumn and winter) from Aqua MODIS Level 3. This dataset was obtained from NASA's OceanColor Web (https://oceancolor.gsfc.nasa.gov/), which provides information on oceanic temperature changes at a spatial resolution of approximately 4 x 4 km. The data covers the period from 2016 to 2022. We restricted the layers of surface temperature to a

maximum of 5 km distance, meaning that any pixel value beyond that distance was not considered. Additionally, we cropped the environmental layers based on the latitudinal distribution of each species. Finally, we calculated the average value of the maximum habitat temperature for the distribution area of each species during two seasons, summer and winter.

Thermal metabolic scope

The thermal metabolic scope (TMS) was determined with the novel method of Paschke et al. (2018). The highest point of the TMS represents the metabolic optimum that individuals reach when acclimated to a specific temperature, when individuals can have a greater amount of energy that they can use to maximize their different physiological requirements (i.e., growth, reproduction, etc.). Therefore, TMS can be used as a proxy for optimal temperature (Paschke et al. 2018; Díaz et al. 2021).

To determine the TMS, it was first necessary to calculate the temperature that induced the maximum metabolic rate (TIMRmax), known as high metabolic rate (HMR) and the temperature that induced the minimum metabolic rate (TIMRmin), known as low metabolic rate (LMR). TIMRmax and TIMRmin were determined by adjusting the average of the CTmax to 90% and CTmin to 110%, respectively, determined for each acclimation temperature (Paschke et al. 2018). Subsequently, for each acclimation temperature of each species, a 40 L aquarium was used as an intermittent respirometry system. Respirometric chambers of 1000 mL (with valves that allowed control of the flow of seawater from the aquarium into the chambers) were placed inside each aquarium (Σn *M. undosa* = 4 temperatures x 7 individuals = 28; *M. crenulata* = 3 temperatures x 8 individuals = 32). In each aquarium, there was an extra respirometric chamber, which did not have an individual inside and was used as a control to measure microbial oxygen consumption in the seawater to allow the necessary corrections. Oxygen consumption in each chamber was measured with mini-sensors (one per chamber) (Loligo Systems, Copenhagen, Denmark) connected to a PC-controlled fiber optic trace oxygen transmitter (OXY-10 trace transmitter, PreSens Precision Sensing GmbH, Regensburg, Germany). The seawater temperature in the aquariums and inside the chambers was previously calculated as TIMRmax and TIMRmin for each acclimation temperature. Once the individuals were inside the chambers, the seawater valve was kept open until an oxygenation value close to saturation was reached. This value corresponded to the

first reading of dissolved oxygen, and subsequently the chambers were closed for 5 min, taking data every 30 seconds. The oxygen consumption rate (OCR) was calculated with equation 1. Finally, the TMS expressed in mg O_2 h^{-1} kg^{-1} w.w., was plotted and calculated as the difference between HMR and LMR, also as mg O_2 h^{-1} kg^{-1} w.w.

(Equation 1) $OCR = (C_1 - C_2)\ V\ /\ (W * T)$

where $C_1 - C_2$ was the difference in the initial concentration minus the final concentration of dissolved oxygen (mg O_2 L^{-1}); V was the volume (in liters) of the chamber where the measurements were made; W was the weight of the organism in kg; and T was the time in hours during which oxygen consumption was measured (Cerezo-Valverde et al. 2006).

Statistical analysis

Fixed factor one-way analysis of variance (ANOVA) was applied to determine whether acclimation temperatures had a significant effect on the thermal tolerance (CTmax and CTmin values), high and low metabolic rate, and thermal metabolic scope of each species separately. However, when these data did not meet the assumption of normality (Shapiro-Wilk: $P < 0.05$) the Kruskal-Wallis one-way non-parametric analysis was used. When data did not meet both the assumptions of normality and homoscedasticity (Levene: $P < 0.05$), before applying the ANOVA or the Kruskal-Wallis (K-W) analysis, the data were transformed with the square root function (Zar 2010). When these statistical tests detected significant differences ($P < 0.05$), multiple comparison tests were used to determine statistical differences between acclimation temperatures. Sigma Plot v.15 software was used to perform all statistical tests and graphics.

RESULTS

The thermal tolerance of juvenile individuals of both species studied was significantly affected by acclimation temperatures (Table 1). The thermal tolerances of both species were relatively similar. In *Megastraea undosa* juveniles, CTmax values increased from 25.3 to 27.4°C as the acclimation temperature increased from 13 to 22°C, but at the acclimation temperature of 16°C the CTmax value decreased. CTmin values increased from 6.0 to 17.3°C as the acclimation temperature increased from 13 to 22°C.The thermal window area of *M. undosa* juveniles was 77.2°C^2 (Fig. 1A). For *Megathura crenulata* juveniles, CTmax and CTmin values increased from 27.7 to 28.8°C and 11.2 to 14.7°C as the acclimation temperature increased from 17 to 23°C, respectively. The thermal window area of this species was 65.25°C^2 (Fig. 1B).

Table 1. Statistical analysis to investigate thermal tolerance (CTmax and CTmin) for juveniles of *Megastraea undosa* ($n = 10$) acclimated at 13, 16, 19 and 22°C and *Megathura crenulata* ($n = 8$) acclimated at 17, 20 and 23°C

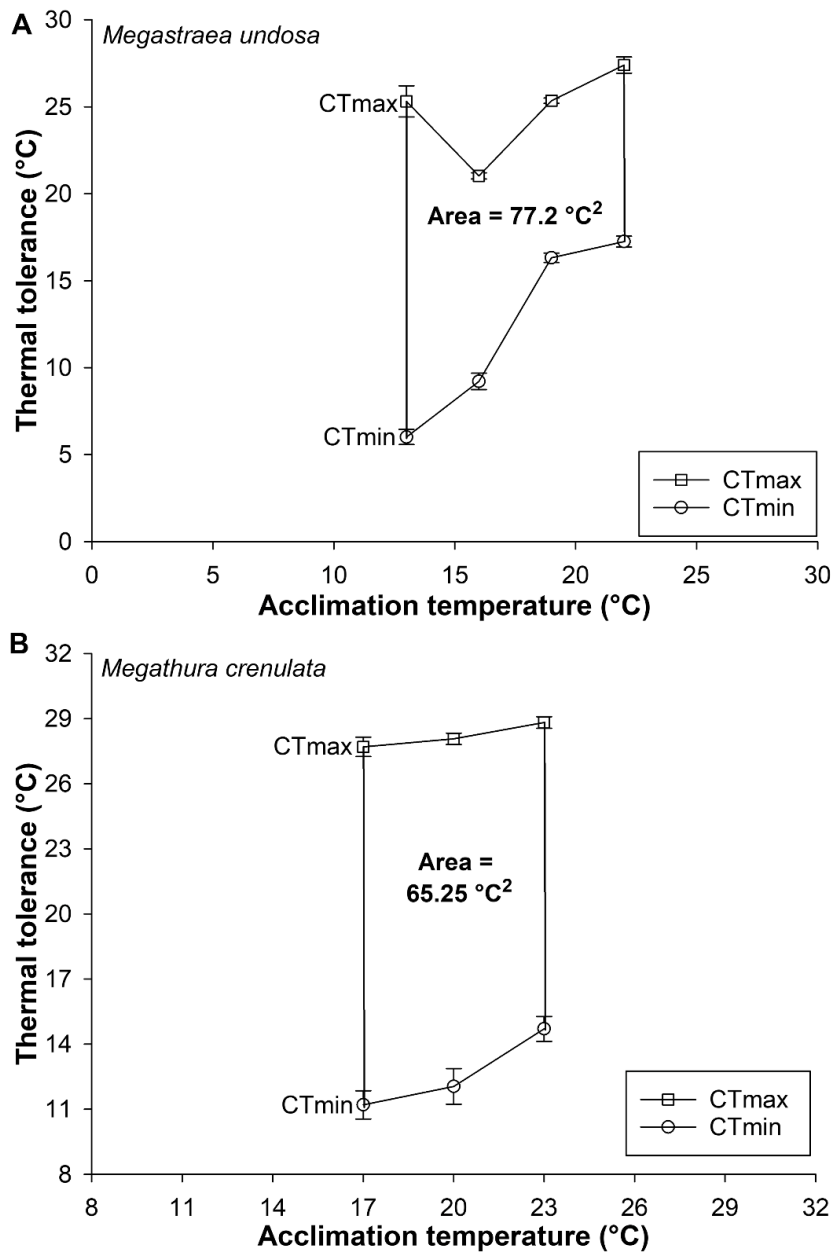| Gastropod species	Thermal tolerance	Statistical analysis
Megastraea undosa	CTmax	K-W: H $_{(3, 40)}$ = 24.8, $P < 0.001$ Dunn's test: 13 > 16°C ($P < 0.05$) 16 < 19°C ($P < 0.05$) 16 < 22°C ($P < 0.001$)
	CTmin	ANOVA: F $_{(3, 36)}$ = 203.8, $P < 0.001$ Tukey's test: $P < 0.001$ in all temperature comparisons, except for 19 = 22°C ($P > 0.05$)
Megathura crenulata	CTmax	ANOVA: F $_{(2, 21)}$ = 2.9, $P > 0.05$
	CTmin	K-W: H $_{(2, 24)}$ = 9.7, $P < 0.05$ Dunn's test: 17 < 23°C ($P < 0.05$)

Fig. 1. A) Thermal window of *Megastraea undosa* juveniles acclimated to 13, 16, 19 and 22°C (mean ± standard error (*SE*), n = 10). B) Thermal window of *Megathura crenulata* juveniles acclimated to 17, 20 and 23°C (mean ± *SE*, n = 8).

For *M. undosa*, the average value of maximum habitat temperature was 20.38 and 15.09°C for summer and winter, respectively. For *M. crenulata*, these values were 19.75°C for summer and 14.67°C in winter. In both species, present-day thermal safety margins (TSM) were positive (*M. undosa*: 10.21°C in winter and 7.02°C for the summer; *M. crenulata*: 13.03°C in winter and 9.05°C for the summer). However, the future thermal safety margin (FTSM) values were lower than those

obtained for the present-day thermal safety margins and were lower during summer than during winter (*M. undosa*: 7.21°C in winter and 4.02°C for the summer; *M. crenulata*: 10.03°C in winter and 6.05°C for the summer).

In *M. undosa* juveniles, acclimation temperatures had a statistically significant effect on high metabolic rate (HMR) or low metabolic rate (LMR) (Table 2). The HMR of this species increased significantly at the acclimation temperature of 16°C and decreased significantly at 13, 19 and 22°C (Fig. 2A). The LMR increased significantly at 16°C and LMR values were similar at 13, 19 and 22°C (Fig. 2A). The highest value of the thermal metabolic scope (TMS) was obtained at 16°C (Fig. 3A), indicating that at this temperature the highest metabolic performance was achieved, and this temperature is a proxy of the optimal temperature where *M. undosa* juveniles can obtain more energy for growth, reproduction, or other physiological requirements. In addition, acclimation temperatures only affected the HMR of *M. crenulata* juveniles (Table 2). The HMR increased significantly at the acclimation temperature of 20°C and decreased significantly at 17 and 23°C (Fig. 2B). The values of the LMR were similar at 17, 20 and 23°C (Fig. 2B). The highest value of TMS was obtained at 20°C (Fig. 3B) and was used as the proxy of the optimal temperature.

Table 2. Statistical analysis to investigate high metabolic rate (HMR) and low metabolic rate (LMR) for juveniles of *Megastraea undosa* ($n = 7$) acclimated at 13, 16, 19 and 22°C and *Megathura crenulata* ($n = 8$) acclimated at 17, 20 and 23°C

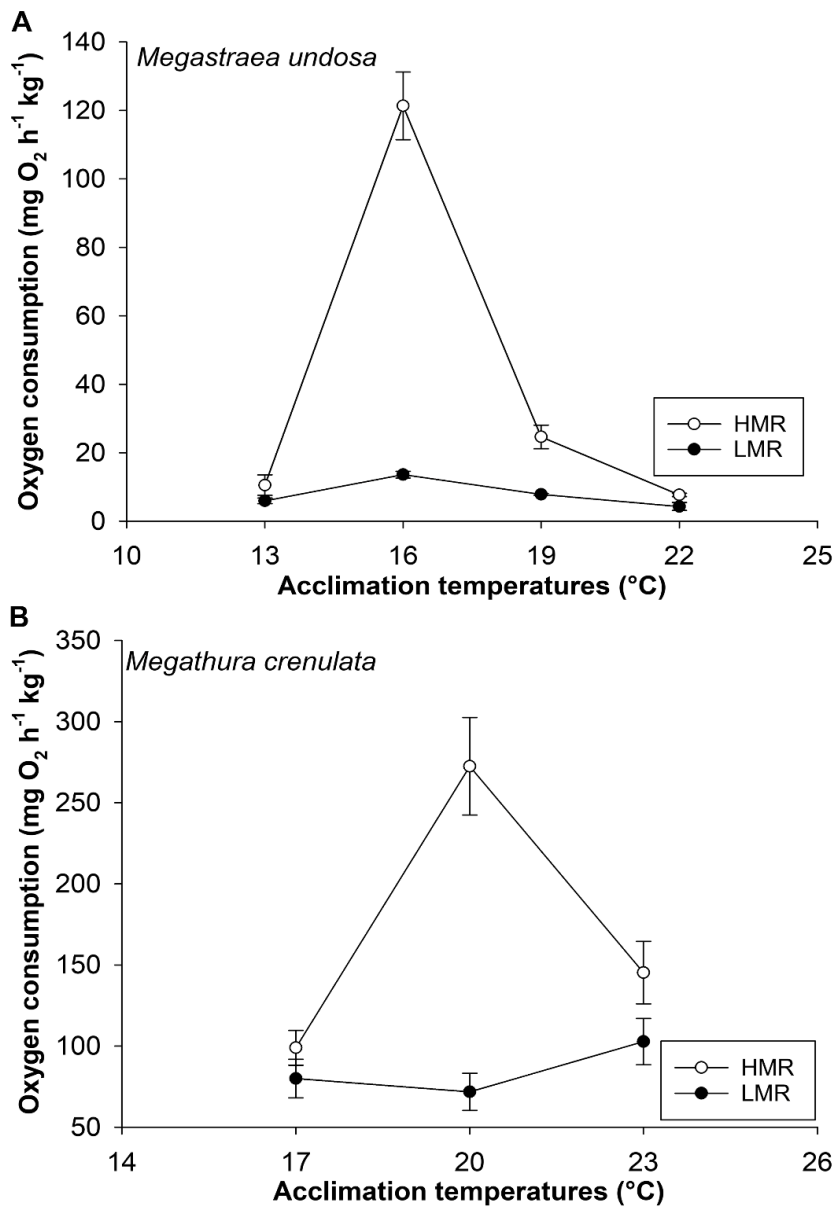| Gastropod species	Metabolic rates	Statistical analysis
Megastraea undosa	HMR	K-W: H $_{(3, 28)}$ = 20.5, $P < 0.001$ Dunn's test: 13 < 16°C ($P < 0.001$) 22 < 16°C ($P < 0.001$)
	LMR	K-W: H $_{(3, 28)}$ = 18.1, $P < 0.001$ Dunn's test: 13 < 16°C ($P < 0.05$) 22°C < 16°C ($P < 0.001$)
Megathura crenulata	HMR	ANOVA: F $_{(2, 21)}$ = 17.4, $P < 0.001$ Tukey's test: 17 < 20°C ($P < 0.001$) 23°C < 20°C ($P < 0.001$)
	LMR	ANOVA: F $_{(2, 21)}$ = 1.6, $P > 0.05$

Fig. 2. High metabolic rate (HMR), low metabolic rate (LMR) of *Megastraea undosa* and *Megathura crenulata* juveniles acclimated to 13, 16, 19 and 22°C and 17, 20 and 23°C, respectively. A) HMR and LMR of *M. undosa* juveniles (mean ± standard error (*SE*), n = 7). B) HMR and LMR of *M. crenulata* juveniles (mean ± *SE*, n = 8).
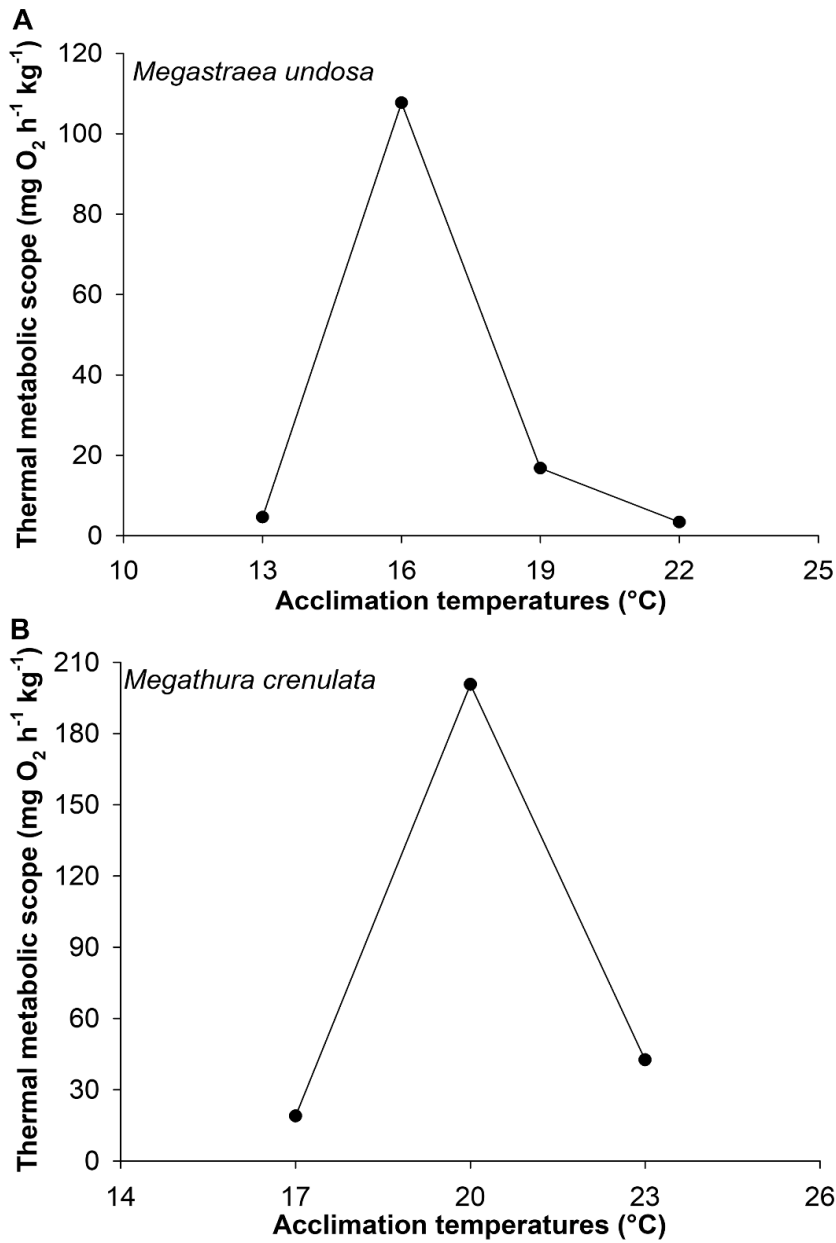
A

B

Fig. 3. Thermal metabolic scope (TMS) of *Megastraea undosa* and *Megathura crenulata* juveniles acclimated to 13, 16, 19 and 22°C and 17, 20 and 23°C, respectively. A) TMS of *M. undosa* juveniles. B) TMS of *M. crenulata* juveniles.

DISCUSSION

Despite the economic, commercial, and biomedical importance of *Megastraea undosa* and *Megathura crenulata*, studies on the thermal biology of these two gastropod mollusk species are limited (Díaz et

al. 2011 2015). These types of studies are not only important for understanding the possible effects of ocean warming on these two species of marine gastropods (IPCC 2022), but they are also informative for the aquaculture of both species because they provide data on thermal limits and optimal temperatures at which juveniles can be cultured to obtain larger and better-quality individuals for human consumption. Strengthening and encouraging the cultivation of these two species would help mitigate the impact of overfishing on natural populations.

The thermal tolerances of both species were relatively similar and can be explained by the fact that they share similar ecological niches and distribution (Morris et al. 1980; Taniguchi and Rogers-Bennett 2001). *Megastraea undosa* juveniles and adults tend to prefer deeper and therefore colder waters (10.9–18.2 m) as a strategy to escape from their predators (Cox and Murray 2006; Hernández-Padilla et al. 2021). *Megathura crenulata* also prefers cold subtidal waters (up to 25 m deep, Smith and Carlton 1975; Morris et al. 1980), but juveniles commonly prefer living in intertidal zones where they can easily find refuge between rocks. The thermal window area for both juvenile gastropods (*M. undosa*: 77.2°C^2; *M. crenulata*: 65.25°C^2) was smaller than those reported by Manríquez et al. (2020) for juveniles of another gastropod species, the muricid *Concholepas concholepas* (Bruguière, 1789). Díaz et al. (2021) indicated that the thermal window of adults of the gastropod *Kelletia kelletii* (Forbes, 1852), a species that has the same distribution area as *M. undosa* and *M. crenulata*, was 163.5°C^2 in a range of acclimation temperatures from 13-22°C. All the above suggests that *M. undosa* and *M. crenulata* are stenothermic species, that is, both species are adapted to live within a narrow temperature range. Somero (2010) and Peck et al. (2014) reported that tropical species as well as those living in very stable thermal environments, live close to their thermal limits and have reduced acclimatory capacities to environmental change. As a result, populations of *M. undosa* and *M. crenulata* that inhabit northern or southern regions with relatively stable environments are likely to be affected by ocean warming compared to those populations that inhabit temperate, subtidal waters with moderately variable environments.

TSM and FTSM are two indices that help compare the vulnerability of marine ectotherms to ocean warming. These indices and CTmax values can be useful for understanding the thermal ecology and adaptive capacity of coastal species as well as for predicting future distribution changes and/or local extinctions (Vinagre et al. 2019). Evidence has shown that laboratory studies can be a good proxy

of species distribution, aggregations and potential vulnerability to temperature (Payne et al. 2021; Angeles-Gonzalez et al. 2023). In the present study, positive values of the present-day thermal safety margin and future thermal safety margins were obtained, but these values were lower during summer than winter. FTSM values were smaller than those obtained for the present-day thermal safety margin. These results indicated that *M. undosa* and *M. crenulata* could probably survive future ocean warming; however, both species would be mostly affected during the summer by the increase in temperature, forcing them to modify their current distribution patterns, both latitudinal and bathymetric, as already documented for many marine species around the world (Mueter and Litzow 2008; Sunday et al. 2015; Kleisner et al. 2016; Assan et al. 2020; Spies et al. 2020). Recent studies have indicated that sites near ocean upwelling areas, as well "cold spots" located in the western Baja California Peninsula and along the western Pacific coast of USA, respectively, could probably be used as thermal refuges by many marine species, including *M. undosa* and *M. crenulata* (Helmuth et al. 2006; Angeles-Gonzalez et al. 2023 2024).

Historically, overfishing of *M. undosa* and *M. crenulata* has led to a decline in their populations in the study region. Likewise, extreme events, such as marine heat waves (*e.g.*, "The Blob"), have been linked to declines in populations of both species (DOF [Official Gazette of the Federation] 2018; Viglione 2021). Another factor that has contributed to the decline in the populations of both species has been the loss of their habitat (giant kelp *Macrocystis pyrifera*), also caused by the increase in temperature in the Northeast Pacific Ocean (Edwards and Estes 2006; Wernberg et al. 2018; Cavanaugh et al. 2019; Arafeh-Dalmau et al. 2021). Without a doubt, overfishing, marine heat waves and habitat loss have negatively affected the populations of *M. undosa* and *M. crenulata*. If these phenomena continue to occur, it is highly likely that a local extinction of both species will occur.

Thermal metabolic scope (TMS) is useful for calculating a proxy for optimal temperature; it is also a good indicator of stress when TMS values approach zero (Paschke et al. 2018). In this study, *M. undosa* individuals acclimated at 13 and 22°C had TMS values close to zero, indicating that at these temperatures juvenile individuals of this species are possibly under stress. Under the ocean warming scenario, these species might prefer to migrate to areas with lower temperatures; however, the stress caused by such conditions could result in negative physiological effects (i.e., production of organisms with smaller sizes, low reproductive success, etc.).

CONCLUSIONS

The juveniles of the two species of marine gastropods are likely to be moderately affected by future ocean warming, being forced to modify their current distribution patterns; however, marine heat waves could accelerate this migration process or even lead to their local extinction. Data generated on thermal limits and optimal temperatures can be used to initiate and maximize the cultivation of these two species of economic, commercial, and biomedical importance. Such information can be used to help people who are still dedicated to fishing for these species to begin cultivating them, obtaining larger and better-quality individuals for human consumption, which would also help mitigate the impact of overfishing on natural populations.

Authors' contributions: AD. Re-Araujo, F. Díaz and JP. Sánchez-Ovando contributed to study design, performed the laboratory work, collected the data, analyzed the information, and wrote the original draft; F. Lafarga-de la Cruz provided the *M. crenulata* specimens and collected the data; L. Álvarez-Lee performed the laboratory work and collected the data; LE. Angeles-Gonzalez obtained the averages of sea temperatures in the region where the two species are distributed and reviewed and edited the draft.

Competing interests: The authors declare that they have no competing interests.

Availability of data and materials: The data generated and analyzed during the current study are available from the corresponding author.

Consent for publication: All the authors consent to the publication of this manuscript.

Ethics approval consent to participate: This research followed the guidelines specified by the research permits from CICESE (permit number CBE/PRES-O/001).

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