

Thermal Physiological Performance and Thermal Metabolic Scope of the Whelk *Kelletia kelletii* (Forbes, 1850) (Gastropoda: Neptuneidae) Acclimated to Different Temperatures

Fernando Díaz^{1,*§}, Ana Denise Re-Araujo^{1,§}, Eugenio Carpizo-Ituarte², Zaul Garcia-Esquivel², Ernesto Larios-Soriano¹, Leonel Perez-Carrasco¹, and Ernesto Lerma¹

¹Laboratorio de Ecofisiología de Organismos Acuáticos. Departamento de Biotecnología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE). Carretera Ensenada-Tijuana # 3918 Ensenada, Baja California, Mexico.

*Correspondence: E-mail: fdiaz@cicese.mx (Díaz).

E-mail: denisre@cicese.mx (Re-Araujo); efnesto.larios.soriano@uabc.edu.mx (Larios-Soriano); lperez@cicese.mx (Perez-Carrasco); jlerma@cicese.mx (Lerma)

²Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California. (UABC). Carretera Ensenada-Tijuana # 3917 Ensenada, Baja California. E-mail: ecarpizo@uabc.edu.mx (Carpizo-Ituarte); sgarcia@uabc.edu.mx (Garcia-Esquivel)

[§]FD and ADRA contributed equally to this work.

Received 30 April 2020 / Accepted 21 May 2021 / Published 30 July 2021

Communicated by Benny K.K. Chan

Investigations of thermal limits are crucial to understanding climate change ecology because it illuminates how climate will shape future species distributions. This work determined the preferred temperature, critical threshold limits represented by the Critical Thermal Maximum (CTMax) and (CTMin), thermal window, oxygen consumption rate and thermal metabolic scope of *Kelletia kelletii* acclimated to 13, 16.0, 19.0 and 22.0 ± 1°C to determine if this species is sensitive to global warming. The preferred temperature (PT) of *Kelletia*'s whelk was determined using the acute method. The acclimation temperature significantly affected the thermal preference of the marine snail ($P < 0.05$) and increased from 13.2 to 24.2°C as the acclimation temperature increased from 13.0 to 22.0°C. The PT was 13.4°C. The acclimation temperature did not significantly affect the CTMax ($P > 0.05$), obtaining a range of 29.2 to 30.2°C. The CTMin had an interval of 9.2°C, at acclimation temperatures of 13 to 16°C, and increased significantly ($P < 0.05$) at 12.3°C in the acclimation interval of 19–22°C. The thermal window for the different acclimation temperatures was 163.5°C². The oxygen consumption rate of the snails increased significantly ($P < 0.05$) when the acclimation temperature increased from 13.0 to 22.0°C, peaking at 63.6 mg of O₂ kg⁻¹ h⁻¹ w.w. in snails maintained at the highest acclimation temperature. The thermal metabolic scope increased significantly ($P < 0.05$) when the acclimation temperature was 13.0°C, with values of 68.7 mg O₂ h⁻¹ kg⁻¹ w.w., then decreased significantly ($P < 0.05$) to 27.9 mg O₂ h⁻¹ kg⁻¹ w.w. at 32°C. Therefore, the thermal aerobic scope was highest at the temperatures that *K. kelletii* preferred. These results may partially explain their pattern of distribution on the Baja California coast.

Key words: Preferred temperature, Critical thermal limits, Thermal windows, Metabolic response.

BACKGROUND

In aquatic environments, temperature is a constantly changing factor that can significantly alter the metabolism, growth, and reproduction of many organisms (Kordas and Harley 2016). Temperature is, therefore, a key component and resource along with water velocity, depth, substrate and food availability in determining the suitability of habitats to certain species and predicting potential impacts related to habitat changes (Pörtner and Peck 2010). Consequently, species-specific temperature preference (T_{pref}) and temperature maxima inform temperature limits, making them useful proxies for quantifying the optimal temperature zone for growth and respiratory metabolism (Salas-Garza et al. 2014).

Sedentary and sluggish species that have little capacity for large-scale movement typically do not redistribute themselves in response to changes in their environment. For these species, short-term fluctuations in water temperature may be ecologically important, and long-term increases in temperature may be especially concerning (Rangel and Johnson 2018). In the intertidal shores, species-specific temperatures can be determined from bio-mimetic loggers, which contain a small thermal sensor, embedded with resin inside shells of specific intertidal species (Chan et al. 2016; Wang et al. 2020).

Thermal windows represent the minimum and maximum temperatures that an organism can successfully tolerate, and thus they provide a general insight into the thermal niche of organisms calculated from the CTMax and CTMin of animals previously acclimated to different temperatures (Fry 1947). The thermal window is considered ecologically relevant because it provides an indicator of how organisms tolerate temperature changes. This area, reported as $^{\circ}\text{C}^2$, can be used as a comparative index of thermal tolerance among species (Eme and Bennet 2009). This thermal window is a useful criterion for understanding not only thermal behavior but also how different species are adapted to live in specific environments successfully (Pörtner and Farrell 2008; Pörtner 2010; Noyola et al. 2015; Flynn and Todgham 2018).

One approach to determining how vulnerable ectothermic animals are to environmental warming involves comparing the upper thermal safety margin (TSM), *i.e.*, the difference between the upper thermal performance limit (heat tolerance) and the environmental temperature (Deutsch et al. 2008; Huey et al. 2012). The CTMax and TSM values will be useful references for future studies on the thermal ecology and adaptation capacity of coastal species, as well as a possible basis for explaining future distributional shifts

and/or local extinctions (Vinagre et al. 2019).

The aerobic power budget, often measured as aerobic scope (AS), is the surplus proportion of the energy flux (and the corresponding amount of metabolic power used to support this flux) that is left after the basal maintenance costs of an organism are met. It reflects the energy that an individual can invest in somatic and gamete production, and other fitness related functions (Guderley and Pörtner 2010; Sokolova et al. 2012). In practice, AS is calculated as the difference between the maximum metabolic rate (MMR) and the standard metabolic rate (SMR), the latter being a widely used indicator of the minimal rate of energy required to maintain life (Paschke et al. 2018). In sedentary animals or in those that do not display athletic swimming behavior, the most frequently used method is manual chasing, which involves touching, pinching, or using air exposure to provoke maximum activity in the specimen (Ern et al. 2014 2015; Norin et al. 2014). This method induces a high metabolic rate, but it is difficult to ensure that all experimental animals receive a chase stimulus of a similar magnitude.

Paschke et al. (2018) proposed a new conceptual approach: measuring weight-specific oxygen consumption when it is stimulated to provoke high metabolic rates (HMR), *i.e.*, when ectotherms are exposed to a temperature high enough to invoke their maximum metabolic rate for 5 min (temperature that induced maximum metabolic rate: TIMR max). According to our trials with invertebrate species (Rodríguez-Fuentes et al. 2017), TIMR max in most of the organisms was seen at temperatures 5–10% below CTMax. Similarly, a minimum metabolic rate can be obtained when the activity is depressed for 5 min by exposure to a temperature low enough to provoke a forced low metabolic rate (LMR) (temperature that induced resting metabolic rate: TIMR min; TIMR min is set to temperatures 5–10% above CTMin). The 5 min duration is long enough to provoke the activation of respiratory metabolism, but short enough to keep animals from experiencing the harmful effects of heat exposure (Paschke et al. 2018).

The Kellet's whelk, *Kelletia kelletii*, is a large predatory buccinid gastropod commonly found in subtidal kelp forest, rocky reefs and cobble-sands interfaces. Juveniles and adults occupy a vertical distribution of 2 to 70 m deep from Isla Asuncion, Baja California, Mexico to Monterey, CA, USA (Rosenthal 1970; Romero et al. 2012). Surveys conducted at the northern range edge of the southern neogastropod *Kelletia kelletii* in the northeast Pacific demonstrate that the boundary shifted north approximately 400 km from the late 1970s/early 1980s to the 2000s (Herrlinger 1981; Zacherl et al. 2003). It is important to study the

thermal tolerance of *Kelletia*, as such data can be used to predict any further geographical range shift of the species (e.g., Sanda et al. 2019).

Thus, from a thermal point of view, understanding how climate change will affect species requires analyses of the thermal physiological traits in the laboratory (Bennett et al. 2018). This information could help uncover information to reduce the uncertainty around interpreting future impacts of global warming in the species studied; this is a pressing issue because climate change represents one of the greatest challenges that mankind faces (Vinagre et al. 2019; Angeles-Gonzalez et al. 2020).

The present study aimed to predict the potential consequences of increasing sea temperature on the Kellet's whelk, *Kelletia kelletii*, as well as determine whether this species is sensitive to global warming and determine its preferred temperature, thermal tolerance, thermal window width, thermal safety margin, and thermal metabolic scope.

MATERIALS AND METHODS

Collection and Maintenance

Kelletia kelletii adults (weighing 39.3–79.1 g) were collected by deep sea hook diving at a depth of 5–10 meters in the area of Tres Hermanas in Punta Banda, Baja California, México (31°42' Lat. N; 116°45' Long. W.). Organisms ($n = 120$) were kept for seven days under laboratory conditions in four 2,000 L reservoirs at 35‰ salinity and $16 \pm 1^\circ\text{C}$ in a flow-through water system (these were the same conditions measured at capture). The photoperiod was maintained in 12–12 h light-dark cycles with 30 min transition periods between them. Kellet's whelk was fed with the mussel *Mytilus* sp. every three days during the acclimation period. Water levels in the reservoirs were kept high enough to prevent emersion. The reservoirs were washed weekly to reduce the accumulation of fecal matter and associated bacteria.

Preferred Temperature

To determine the preferred temperature, we used 84 whelks previously acclimated for 4–6 weeks in 2000 L reservoirs at temperatures of 13, 16, 19 and $22 \pm 1^\circ\text{C}$ according to the methods described by Salas-Garza et al. (2014). Forty-eight hours before beginning each experiment, the organisms were tagged with a plastic mark. The preferred temperature experiments were performed in a trough with a volume of 150 L, as described by Díaz et al. (2006). A Neslab

thermoregulator model HX 150 (Newington, NH) was connected to the right end of the trough to cool the water to 8°C ; a 1000 W heater was placed at the left end of the trough, which was connected to a temperature-controlled heater to heat the water to 30°C , generating a stable temperature gradient ($y = 6.20 + 1.60x$, $r^2 = 0.98$, where x = segments of gradient, and y = temperature of the gradient segments). An aeration system consisting of a high efficiency diffuser hose was placed along the gradient to maintain dissolved oxygen concentrations of 6.6–8.5 mg L⁻¹ and to eliminate vertical thermal stratification from the water column. The water column depth in the trough was 9 cm, and to maintain high water quality, it was refilled at a rate of 12.0–14.0 L h⁻¹.

The acute method, described by Reynolds and Casterlin (1979), was used to determine the preferred temperature. Three organisms, randomly chosen and within the same weight range, were introduced to the gradient at the virtual segment where the water temperature was the same as the acclimation temperature. The location of the organisms and the temperature where the whelk was located in the gradient were recorded using a digital infrared thermometer (Steren HER-425) every ten minutes for 120 min. The final preferred temperature was determined to be the point at which the preferred temperature of the organisms intersected with the acclimation temperature, with the equality line. For each experimental condition, seven repetitions were done (N total = 21). The whelk was not fed for the 24 h before testing, according to recommendations by Nelson et al. (1985) and Beamish and Trippel (1990).

A control group of 15 individuals was placed in the gradient system (in groups of three and previously acclimated to different temperatures), with the heater and cooler turned off (resulting in a constant temperature of 20°C along the gradient, which was the temperature of the water entering the system). This control experiment allowed us to determine that the final location was indeed due to temperature selection and not a preference for any particular site inside the tube.

Critical Thermal Maximum

To determine the critical thermal maximum (CTMax), 14 Kellet's whelks were acclimated in four 500 L reservoirs at 13.0, 16.0, 19.0 and $22.0 \pm 1^\circ\text{C}$ (N total = 56) and a salinity of 35‰ for four weeks. The whelks were not fed for the 24 hr leading up to the trial, and a loop of monofilament was attached to their shell's surface using epoxy clay. *Kelletia kelletii* adults were then placed on acrylic plates (30 × 25 cm) in 40 L aquaria with aeration and left undisturbed overnight.

To determine the temperature at which the organism detaches from the plates (according to Morley et al. (2011)), a mono filament fishing line was hooked onto the loop attached to the whelk shell, and a 90 g mass was placed at the other end of the line. Each aquarium contained a thermostatically controlled 1000 W heater and an air stone. The temperature was raised from the acclimation temperatures of 13.0, 16.0, 19.0 and 22.0 \pm 1°C at a rate of 1°C every 15 minutes to allow the core of temperature to equilibrate to the surrounding water temperature. This rate approximates natural daily temperature increases in the intertidal habitat (Cox 1974; Tomanek and Somero 1999; Díaz et al. 2000 2006 2011; Stenseng et al. 2005; Salas-Garza et al. 2014).

Critical Thermal Minima

The critical thermal minimum (CTMin) was assessed from 14 Kellet's whelk from each experimental condition using the methodology described in Noyola et al. (2015). A stainless steel coil was connected to a chiller (PolyScience IP-35) inside the 40 L aquarium to allow the temperature to decline at a rate of 1°C every 15 min. To determine the temperature at which the organism detaches from the plates (according to Morley et al. (2011)), a mono filament fishing line was hooked onto the loop attached to the whelk shell, and then a 90 g mass was placed at the other end of the line. Criteria for determining the end point of the CTMin were the same as those used for CTMax.

CTMax and CTMin were determined between 9:00 and 14:00 h; visual monitoring was performed and recorded to define the time and temperature at which *K. kelletii* detached. Immediately after detaching, each Kellet's whelk was transferred into 2000 L tanks for 96 h to recover at its acclimation temperature (organisms were used only once). The data obtained from whelks that did not fully recover after reacclimating were discarded. The thermal window area was obtained with the CTMax and CTMin data following Bennett and Beitinger (1997) and expressed in °C².

As controls to determine the effect of 90 g mass on the lost attachment of plates, 10 organisms were placed and allowed to acclimate at 13.0, 16.0, 19.0 and 22.0°C \pm 1°C (N = 30). All were monitored to measure the time at which they detached from the plates. The experiment was terminated after six hours because the whelks never detached from the plates.

The thermal safety margin (TSM = CTMax–Maximum Habitat Temperature) was estimated for Kellet's whelk. The future thermal safety margin was also estimated (CTMax – (Maximum Habitat Temperature + 3°C)) according to Vinagre et al. (2019).

Oxygen Consumption Rate

The oxygen consumption rate (OCR) was measured in a semi-open respirometer system, as described by Díaz et al. (2007), consisting of 10 chambers of 1000 ml each. Kellet's whelks were acclimated for 30 days at 13, 16.0, 19.0 and 22.0°C \pm 1°C. Eighteen whelks from each acclimation temperature were individually introduced into the respiratory chamber, 24 h before initiating measurements to minimize the effect of stress due to manipulation. All measurements were performed between 9:00 and 13:00 h. To minimize the possible influence of body weight on oxygen consumption, a narrow weight range (mean \pm SD wet weight, 58.5 \pm 4.1 g) of Kellet's whelks was used.

The water flow in the chambers remained open for two hours before being shut off. Initial and final oxygen concentrations were measured with dipping probe oxygen mini-sensors (Loligo Systems, Copenhagen, Denmark) connected to a PC-controlled fiber optic trace oxygen transmitter (OXY-10 trace transmitter, PreSens Precision Sensing GmbH, Regensburg, Germany).

Subsequently, the chambers remained closed for 1 h to avoid a diminution of the dissolved oxygen of 25 to 30%, since this constitutes a stress factor according to Stern et al. (1984). Before re-establishing the water flow, one water sample was taken to measure the final concentration of dissolved oxygen.

The oxygen consumption rate (OCR) was calculated according to equation 1 (Cerezo Valverde et al. 2006; Zheng et al. 2008).

$$\text{OCR} = (C_1 - C_0) V / (W \times T) \quad (\text{Equation 1})$$

where C_1 is the change in the oxygen content in the respirometric chambers before and after the test, C_0 is the change in the oxygen content in the blank (control), V is the volume of the chamber; W is the weight of whelk in kg wet biomass and T is the time duration in hours.

The 10th chamber was used as a control to measure oxygen consumption by the microorganisms present in the water, and the necessary corrections were made. Two repetitions were carried out for each test. The results of the oxygen consumption rate are given in mg O₂ h⁻¹ kg⁻¹ wet weight (w.w.).

Thermal Metabolic Scope determined with a novel methodology

To obtain thermal metabolic scope values, 80 organisms weighing 59.0 \pm 3.7 g were placed in a closed

recirculation system and divided into four groups. Every group was acclimated to 13.0, 16.0 19.0 and 22°C for 21 days. To determine the thermal metabolic scope, we used the TIMR (Temperature Induced Metabolic Rate).

The methodology uses the determination of CTMax to 50%, which is a range of temperatures where the high activity is enhanced as the extreme temperature. Based on this, we proposed measuring the weight of specific oxygen consumption when the organism is stimulated to invoke high metabolic rates (HMR) when exposed to 90% of the CTMax (TIMRMax). Similarly, a minimum metabolic rate can be obtained from the CTMin when the activity is depressed by exposing the organism to 110% of the CTMin (TIMRMin), reaching a metabolic condition in which the low metabolic rate (LMR) is forced. Considering that activity may be a good indicator of the metabolic performance of ectotherms, it is possible to assume that the thermal metabolic scope (TMS) induced by temperature can be obtained as HMR–LMR, where TMS reflects the high and low activity under extreme thermal conditions.

For each acclimation temperature, 10 animals were used to calculate TIMRMax and 10 for TIMRMin, according to the novel methodology proposed by Paschke et al. (2018). To determine TIMRMax, organisms were placed individually in a 60 L aquarium equipped with a 1000 W heater and constant aeration in their respective acclimation temperature. For organisms that remained in these conditions for 30 min, the temperature was increased 1°C min⁻¹ every 15 min until reaching 90% of its CTMax; they were then immediately transferred into an intermittent respirometric system (Díaz et al. 2007) with a temperature similar to that of the CTMax (90%). Oxygen consumption was measured every 30 seconds for 5 minutes with an oximeter (Oxy-10 mini, Presens) equipped with an optical fiber sensor. This corresponded to the temperature that induces a high metabolic rate (HMR).

To determine TIMRMin, marine snails were exposed to a temperature reduction at a rate of 1°C min⁻¹, similar to that described to obtain CTMin. When organisms reached a CTMin of 110%, they were immediately transferred to an intermittent respirometric system (Díaz et al. 2007) with a temperature similar to that of CTMin (110%). Oxygen consumption was measured every 30 seconds for 5 minutes with an oximeter (Oxy-10mini, PreSens) equipped with an optical fiber sensor. This corresponds to the low metabolic rate (LMR).

The thermal metabolic scope TMS was calculated according to novel methodology as the difference between the HMR (high metabolic rate) and LMR (low metabolic rate) (both as mg O₂ h⁻¹ kg⁻¹ w.w.).

Statistical Analysis

Normality (Shapiro-Wilk $P > 0.05$) tests and homoscedasticity (Levene $P > 0.05$) applied to each data group failed. The preferred temperature, CTMin, CTMax, Oxygen Consumption Rate and TIMR were analyzed by the Kruskal-Wallis nonparametric test followed by a Dunn's multiple comparisons *post-hoc* test. Sigma Plot (V 12.5) software was used for all tests and graphics.

RESULTS

When the water temperature was kept constant (control treatment), adult *Kellet's* whelks were found to be distributed freely along the gradient (400 cm) after 2 hours, indicating that they showed no preference for any particular segment. Acclimation temperature significantly affected the thermal preference of *Kellet's* whelk ($P < 0.05$), and thermal preference increased from 13.2 to 24.2°C as the acclimation temperature increased from 13.0 to 22.0°C. The preferred temperature obtained was 13.4°C (Fig. 1). Acclimation temperature did not significantly affect the critical thermal maximum ($P > 0.05$) from 29.2 to 30.2°C. The critical thermal minimum had an interval of 9.2°C in acclimation temperatures from 13 to 16°C, and increased significantly ($P < 0.05$) to 12.3°C when the acclimation interval was 19–22°C (Fig. 2). The thermal window width delimited by the values of CTMax and CTMin for the different acclimation temperatures was 163.5°C² (Fig. 2).

The value obtained for the thermal safety margin (TSM) for *K. kelletii* had an interval for temperatures experimented in its habitat during the winter of 14.5 to 18.7°C for the summer. Future safety margins were also positive, ranging from during winter 11.5 to 15.7°C for the summer.

The oxygen consumption rate of the *Kellet's* whelk (OCR) increased significantly ($P < 0.05$) as acclimation temperature incremented from 13.0°C to 22.0°C. The *Kellet's* whelk peaked at 63.6 O₂ kg⁻¹ h⁻¹ w.w. in a higher acclimation temperature (Fig. 3).

The Low Metabolic Rate increased significantly in the acclimation interval from 13.0 to 22.0°C with values of 18.5 to 19.3 mg O₂ h⁻¹ kg⁻¹ w.w., and remained constant from 16.0 to 19.0°C. High Metabolic Rate increased significantly ($P < 0.05$) in acclimation temperatures from 13.0°C with values of 68.7 mg O₂ h⁻¹ kg⁻¹ w.w., followed by a significant decrease ($P < 0.05$) to 27.9 mg O₂ h⁻¹ kg⁻¹ w.w. at 32°C (Fig. 4). The Thermal Metabolic Scope increased significantly ($P <$

0.05) to $50.2 \text{ mg O}_2 \text{ h}^{-1} \text{ kg}^{-1}$ w.w. when the acclimation temperature was 13.0°C , followed by a significant decrease at 32°C (Fig. 5).

DISCUSSION

Marine organisms have a variety of temperature-preference relationships that can be categorized into

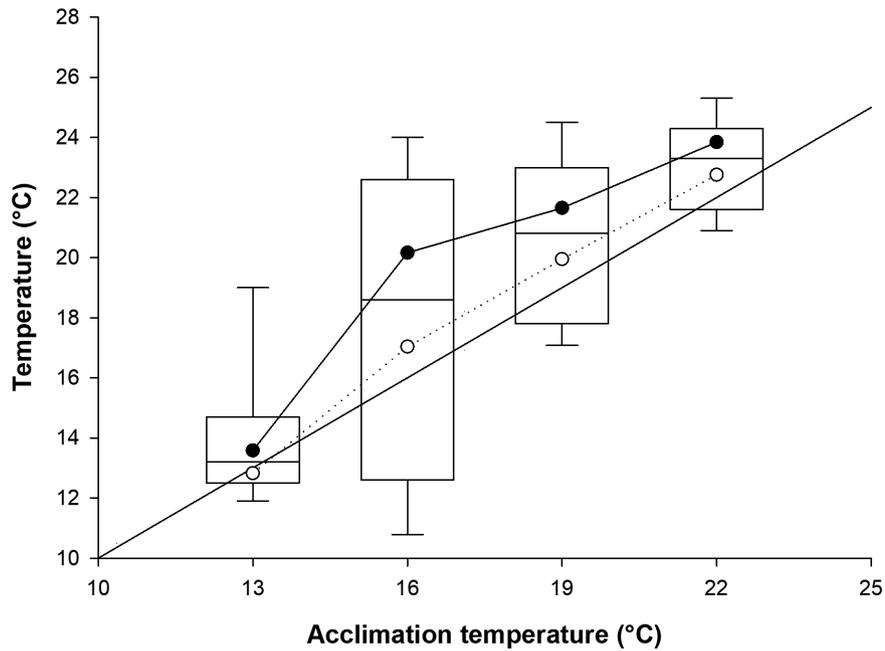


Fig. 1. Preferred temperature of *Keltia keltii* adults acclimated to different temperatures. The zone, bordered by circles, represents the 95% confidence interval of the median. The bars include 50% of the organism's distribution. The 45° construction continuous line represents the point where preferred and acclimation temperatures are equal.

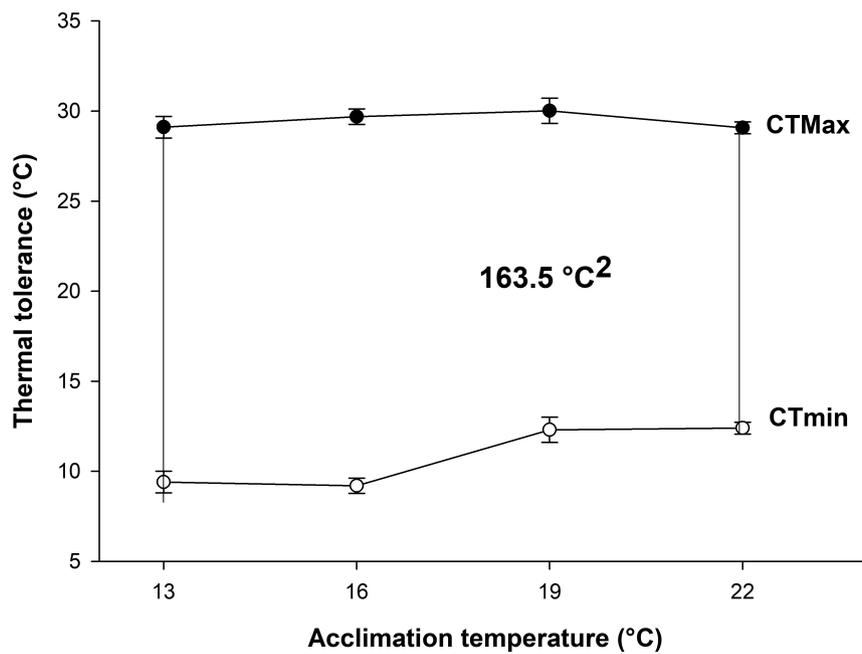


Fig. 2. Thermotolerance represented by critical thermal minima (CTMin) and critical thermal maxima (CTMax) of *Keltia keltii* adults acclimated to different temperatures. The zone bordered by circles represents the 95% confidence interval of the median. The bars include 50% of the distribution and the vertical lines represent the quartiles.

three classes based on whether they have a positive, independent or negative function of the acclimation temperature (Johnson and Kelsch 1998). The temperature-preference relationships obtained for Kellet's whelk were a positive function over the range of acclimation temperatures from 13 to 22°C. Similar

results were obtained for fishes crabs, and black sea urchins (Cherry et al. 1977; Kelsch 1996; Padilla-Ramirez et al. 2015; Cumillaf et al. 2016; Díaz et al. 2017). According to Zacherl et al. (2013), Kellet's whelk that inhabit Baja California are exposed to seasonal temperatures during the winter of 14.5 to

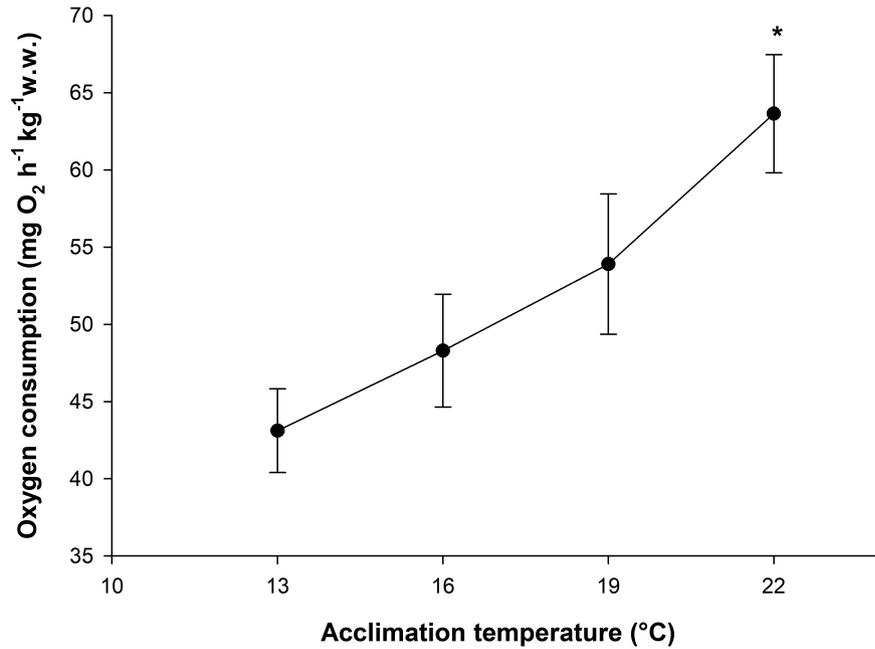


Fig. 3. Oxygen consumption rate of *Kelletia kelletii* acclimated to different temperatures.

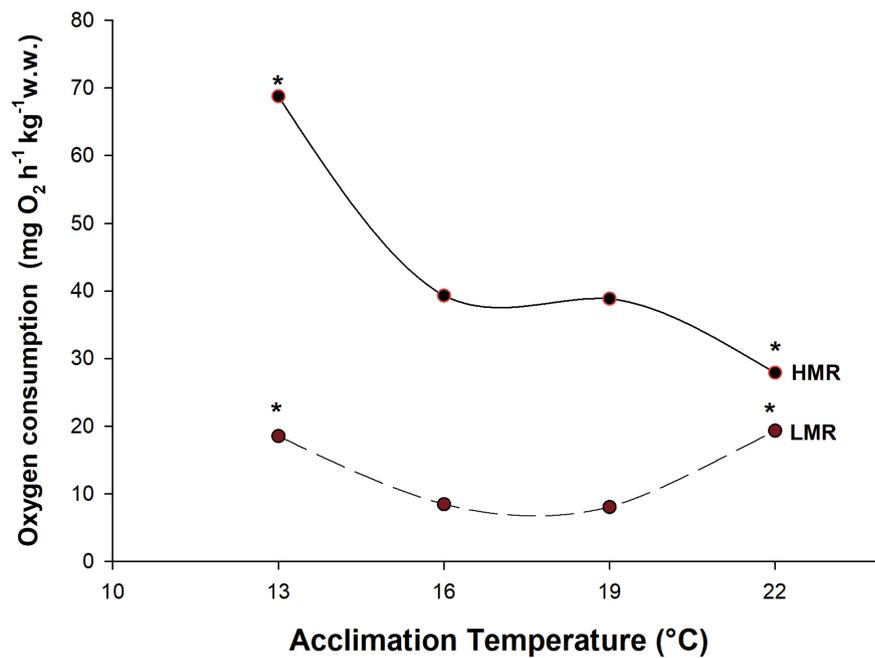


Fig. 4. High metabolic rate (HMR, closed red circles) and low metabolic rate (LMR, closed black circles) of *Kelletia kelletii* obtained with the temperature-induced metabolic rate (TIMR) method, acclimated to different temperatures. The asterisk above the points indicates significant difference ($P < 0.05$).

18.7°C for the summer. This response is typical of species experiencing thermal cycles of relatively high amplitude and duration in their habitats (Johnson and Kelsch 1998). This seems to be an adaptative response to the changing temperatures that Kellet's whelk experience in their natural habitat.

Kellet's whelk's final preferred temperature was 13.4°C, representing the temperature that the species is adapted to. At coldwater upwelling sites in north-central Baja California, subtidal seawater temperatures can typically be as cold as 10 to 14°C north of Point Conception. Yet, these sites have some of the highest densities of *K. kelletii*, and recruitment is consistently strong. Zacherl et al. (2013) found that *Kelletia kelletii* adults mate and produce viable eggs capsules at these temperatures, and the hatched larvae can survive and grow in this temperature range. According to Rosenthal (1970), incubation time seems to depend on water temperature. In the laboratory, the hatching period for *Kelletia kelletii* was 30 to 34 days for capsule developing in water temperatures between 13.5–17.5°C. This shows that physiological processes are optimal within the preferred temperature range. Díaz-Herrera et al. (1996) found a similar relationship in *Bulla gouldiana*. As shown by Nichelman (1983), organisms living within the final thermal preference or thermal optimal temperature range are subject to minimal heat stress; their physiological functions are optimized, resulting in maximum growth.

Thermal tolerance has evolved to allow animals to live under a wide range of conditions, from stable

and benign to extreme and sometimes highly variable. CTMax and CTMin determinations suggested that marine snails are subjected to a continuous and constant linear increase and decreased in temperature until an endpoint is reached. According to Cowles and Bogert (1944), the CTMax and CTMin endpoint reflects a temperature at which locomotory movements become disorganized and the Kellet's whelk loses its ability to hold onto acrylic plates. Smith (1991) and Morley et al. (2011) mentioned that the attachment capacity of marine gastropods can be measured as the sum of two main components: the proteinaceous or mucopolysaccharide secretions, and the muscular activity that maintains the suction force from the foot and its shape. It has also been shown that increases in temperature affects the attachment capacity of different species, such as sea urchins, abalones, sea cucumbers, wavy turban snail, California sea hare and giant keyhole limpet. This decreased attachment capacity could lead the organism to detach and ultimately die; thermal attachment is, therefore, a snail's response that reflects the effect of temperature in organisms.

When Kellet's whelks went through the process of reaching the endpoint of CTMax, they had several particular responses, especially snails that started to lift their shells and to expose their mantle (this has also been observed in other abalone species—*i.e.*, the wavy turban snail and keyhole limpet (Hines et al. 1980; Hahn 1989; Hecht 1994; Gilroy and Edwards 1998; Díaz et al. 2000 2006 2011 2015). Hines et al. (1980) suggested that these responses, from an adaptive point of view, are

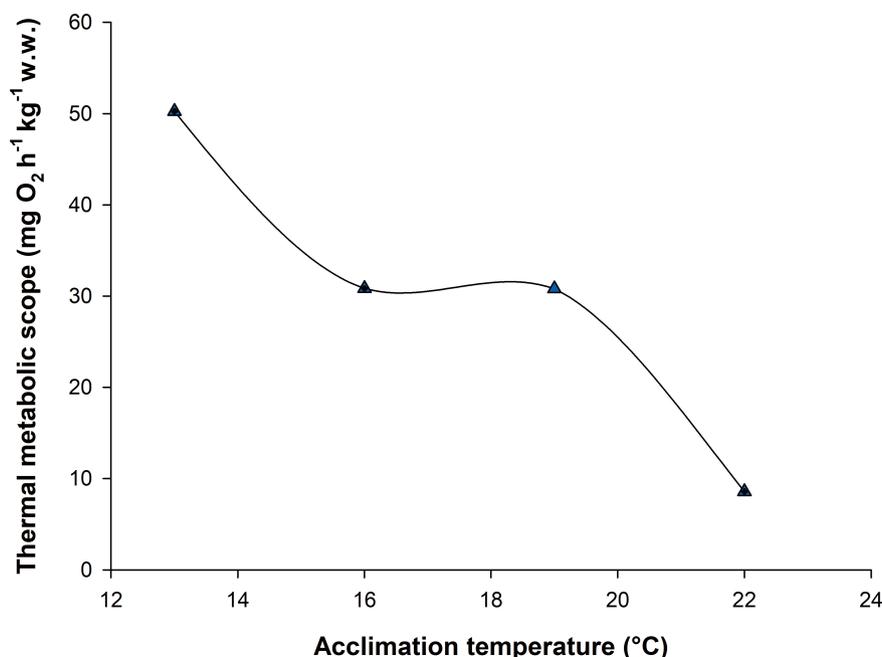


Fig. 5. Thermal metabolic scope (TMS) of *Kelletia kelletii* acclimated to different temperatures.

used as an evaporative cooling system, although this could also increase desiccation and leave marine snails more vulnerable to predation or disease.

Living close to thermotolerance thresholds is also characteristic of temperate invertebrates, particularly those inhabiting intertidal areas. Many marine snails, particularly those living at intertidal environmental template areas, already live on the edge of their thermotolerance limits and may not survive even small changes in temperature, as showed in Kellet's whelks (Somero 2009; Zacherl et al. 2013; Peck et al. 2014).

This study obtained a wide thermal window of the 163.5°C² for Kellet's whelks, which was lower than the window area of 210°C² reported in juveniles of *Octopus maya* (Noyola et al. 2013) and 202°C² in the fighting conch *Strombus pugilis* (Noyola et al. 2015). Overall, the thermal window area provides a convenient and useful comparative index of eurythermality in mollusc species that have shown an intermediate degree of eurythermality, such as octopus, fighting conch, and Kellet's whelk. Understanding the thermal window of a species and how additional environmental factors reduce or enlarge that niche is a fundamental prerequisite to determining that species' habitat requirements and how its productivity and distribution may be altered (Pörtner and Peck 2010). According to Fry (1947), Rezende et al. (2014) and Karelitz et al. (2017), the thermal window represents the minimum and maximum temperatures that organisms can successfully tolerate and provides a comprehensive insight into the thermal niches occupied according to species distribution. It can be seen that an increase in temperature due to global warming will reduce the thermal window of the Kellet's whelk, resulting in a reduction in the population and distribution range of the species since its performance is negatively affected and the species cannot survive unless the temperature only increases for a limited period of time.

Vinagre et al. (2019) mentioned that for different species of gastropods that live in temperate climates had a thermal safety margin of 9.48 to 16.65°C. Lugo et al. (2016) reported that species that inhabit the Gulf of California e.g., as *C. perplexus* and *C. ximenes* had greater high thermal safety margin, which is typical of species that are distributed throughout tropical climates, whereas those that inhabit the Pacific coast, like *C. californicus*, had a lower thermal safety margin. In the present study we obtained a temperature interval of 14.5 to 18.3°C in Kellet's whelk, which is distributed throughout temperate climates on rocky reefs from Baja California Mexico to Monterey Bay, California, USA (Zacherl et al. 2013). Deutsch et al. (2008) and Vinagre et al. (2019) mentioned that the thermal safety margin represents the average environmental warming that an

ectotherm can tolerate before its performance drops to a fatal level. According to Madeira et al. (2012), temperate species like Kellet's whelk may be more vulnerable to warming than tropical species.

When the acclimation temperature was 22°C, the oxygen consumption rate increased to 50%, indicating higher oxygen supply requirements at this temperature. Different species of invertebrates including the sea urchins *Mesocentrotus franciscanus*, *Strongylocentrotus purpuratus*, *S. droebachiensis*, *S. fragilis*, limpet *Patella (Scutellastra) granularis*, sea cucumber *Apostichopus japonicus*, periwinkle *Littorina saxatilis*, California sea hare *Aplysia californica*, intertidal snail *Tegula regina*, black sea urchin *Arbacia stellata* and ark shell *Scapharca subcrenata*—have had higher rates of oxygen consumption when exposed to higher temperatures (Ulbricht and Pritchard 1972; Percy 1972; Ulbricht 1973; Branch and Newell 1978; Dong et al. 2011; Sokolova and Pörtner 2003; Re et al. 2013; Salas-Garza et al. 2014; Díaz et al. 2017; Jiang et al. 2020). Hochachka and Somero (2002) found that, for organisms such as *K. kelletii*, as environmental temperatures increase, there is a corresponding increase in metabolic rate which means that more energy is spent on maintenance and is thus unavailable for growth, and reproduction. This will not only affect the survivability of that particular individual, but also have a negative effect on future generations. Dong et al. (2011) and Miller et al. (2013) claim that this pattern suggests that high thermal tolerance may come with significant fitness and energy costs.

Thermal metabolic scope is an organism's potential to do any work requiring energy and is strongly correlated with maximum growth rates; this parameter is a good indicator of fitness and animal welfare in natural and aquaculture conditions in terms of growth performance and reproduction (Paschke et al. 2018). The highest thermal metabolic scope of *K. kelletii* was obtained at the acclimation temperature of 13°C using TIMR methodology. These results agree with the preferred temperatures of 13.4°C that we obtained. On the other hand, the thermal aerobic scope at temperatures starting at 22°C represents or perhaps slightly exceeds the upper limit of the thermal range, beyond the aerobic capacity of Kellet's whelk adults. Le et al. (2017) found similar results in geoduck clams *Panopea zelandica* exposed to 11 to 23°C. This means that both species are sensitive to increases in temperature due to climate change.

CONCLUSIONS

This study demonstrated that *Kelletia kelletii*

adults exhibit thermoregulatory behavior because *Kellett's* whelks actively distinguish and select the most suitable thermal environment to maximize their metabolic efficiency. In addition, the width of its thermal window corresponds to that of an organism that has an intermediate degree of eurythermality. At an acclimation temperature of 22°C, the oxygen consumption rate increased to 50%, indicating that oxygen supply requirements are higher at this temperature. The thermal metabolic scope of *K. kelletii* was obtained at the acclimation temperature of 13°C using TIMR methodology. These results agree with the preferred temperatures obtained of 13.4°C, which effectively support these thermal intervals in which metabolic conditions were found better. This species is vulnerable to increases in environmental temperatures over 22°C, which affect its metabolic rate and energy metabolism along with factors such as growth, reproduction and distribution.

Acknowledgments: The authors are grateful to the technical staff of CICESE involved in performing this research to Diana Fischer and Noah Last for English revision and edition.

Authors' contributions: F. Díaz and AD. Re-Araujo contributed to the study design, performed the laboratory work, collected the data, analyzed the information and wrote the paper; E. Carpizo-Ituarte, Z. Garcia Esquivel and E. Larios-Soriano contributed to the study design, analyzed the information and revised the paper; L. Perez-Carrasco and E. Lerma performed the laboratory work and collected the data.

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).

REFERENCES

- Angeles-Gonzalez LE, Martínez-Meyer E, Yañez-Arenas C, Velazquez-Abunader I, Garcia-Rueda A, Díaz F, Tremblay N, Flores-Rivero MA, Gebauer P, Rosas C. 2020. Using realized thermal niche to validate thermal preferences from laboratory studies. How do they stand? *Ecol Indic* **118**:106741. doi:10.1016/j.ecolind.2020.106741.
- Beamish FWH, Trippel EA. 1990. Heat Increment: A static dynamic dimension in bioenergetic models? *Trans Am Fish Soc* **119**:649–661. doi:10.1577/1548-8659(1990)119<0649:HIASOD>2.3.CO;2.
- Bennett JM, Calosi P, Clusella-Trullas S, Martínez B, Sunday J, Algar AC, Araújo MB, Hawkins BA, Keith S, Kühn I, Rahbek C, Rodríguez L, Singer A, Villalobos F, Ángel Olalla-Tárraga M, Morales-Castilla I. 2018. GlobTherm, a global database on thermal tolerances for aquatic. *Sci Data* **5**:1–7. doi:10.1038/sdata.2018.22.
- Bennett WA, Beiting TL. 1997. Temperature tolerance of the sheepshead minnow, *Cyprinodon variegatus*. *Copeia* **1**:77–87. doi:10.2307/1447842.
- Branch GM, Newell RC. 1978. A comparative study of metabolic energy expenditure in the limpets *Patella cochlear*, *P oculus* and *P. granularis*. *Mar Biol* **49**:351–361. doi:10.1007/BF00455030.
- Cerezo Valverde J, Martínez López FJ, García García B. 2006. Oxygen consumption and ventilatory frequency responses to gradual hypoxia in common dentex (*Dentex dentex*): basis for suitable oxygen level estimations. *Aquaculture* **256**:542–551. doi:10.1016/j.aquaculture.2006.02.030.
- Chan BKK, Lima FP, Williams GA, Seabra R, Wang HY. 2016. A simplified biomimetic temperature logger for recording intertidal barnacle body temperatures. *Limnol Oceanogr-Met* **14**:448–455. doi:10.1002/lom3.10103.
- Cherry DS, Dickinson KL, Cairns Jr J. 1997. Preferred avoided and lethal temperatures of fish during rising temperature conditions. *J Fish Res Board Can* **34**:239–246. doi:10.1139/f77-035.
- Cowles RB, Bogert CM. 1944. A preliminary study of the thermal requirements a desert reptile. *Bull Am Mus Nat Hist* **83**:265–296.
- Cox DK. 1974. Effect of three heating rates on the critical thermal maximum of bluegill. In: Gibbons JW, Sharitz RR (eds) *Thermal Ecology*. AEC Symposium Series. Springfield, pp. 158–163.
- Cumillaf JP, Blanc J, Paschke K, Gebauer P, Diaz F, Re D, Chimal ME, Vasquez J, Rosas C. 2016. Thermal biology of the sub-polar-temperate estuarine crab *Hemigrapsus crenulatus* (Crustacea: Decapoda: Varunidae). *Biol Open* **5**:220–228. doi:10.1242/bio.013516.
- Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proc Natl Acad Sci USA* **105**:6669–6672. doi:10.1073/pnas.0709472105.
- Díaz F, Del Rio-Portilla M, Sierra E, Aguilar M, Re-Araujo A. 2000. Preferred temperature and critical thermal maxima of red abalone *Haliotis rufescens*. *J Therm Biol* **25**:257–261. doi:10.1016/S0306-4565(99)00032-7.
- Díaz F, Re A, González R, Sánchez L, Leyva G, Valenzuela F. 2007. Temperature preference and oxygen consumption of the largemouth bass *Micropterus salmoides* (Lacépède) acclimated to different temperatures. *Aquac Res* **38**:1387–1394. doi:10.1111/j.1365-2109.2007.01817.x.
- Díaz F, Re AD, Galindo-Sanchez CE, Carpizo-Ituarte E, Perez-Carrasco L, Gonzalez M, Licea A, Sanchez A, Rosas C. 2017. Preferred temperature critical thermal maximum and metabolic response of *Arbacia stellata* (Blainville, 1825; Gmelin, 1791). *J Shellfish Res* **36**:219–225. doi:10.2983/035.036.0124.
- Díaz F, Re AD, Medina Z, Re G, Valdez G, Valenzuela F. 2006. Thermal preference and tolerance of green abalone *Haliotis fulgens* (Philippi, 1845) and pink abalone *Haliotis corrugata*

- (Gray, 1828). *Aquac Res* **37**:877–884. doi:10.1111/j.1365-2109.2006.01506.x.
- Díaz F, Re AD, Salas A, Galindo-Sanchez CE, Gonzalez M, Sanchez A, Rosas C. 2015. Behavioral thermoregulation and critical thermal limits of giant keyhole limpet *Megathura crenulata* (Sowerby 1825) (Mollusca; Vetigastropoda). *J Therm Biol* **54**:133–138. doi:10.1016/j.jtherbio.2013.05.007.
- Díaz F, Salas A, Re AD, González M, Reyes I. 2011. Thermal preference and tolerance of *Megastrea (Lithopoma) undosa* (Wood 1828) (Gastropoda: Turbinidae). *J Therm Biol* **36**:34–37. doi:10.1016/j.jtherbio.2010.10.004.
- Díaz-Herrera F, Buckle-Ramirez F, Baron-Sevilla B, Farfan C. 1996. Behavioral thermoregulation of *Bulla gouldiana* (Gastropoda: Opisthobranchia: Cephalaspidea). *J Therm Biol* **21**:319–322.
- Dong Y, Yu S, Wang QZ, Dong S. 2011. Physiological responses in a variable environment: Relationship between metabolism hsp and thermotolerance in an intertidal-subtidal species. *PLoS ONE* **6**:e26446. doi:10.1371/journal.pone.0026446.
- Eme J, Bennett WA. 2009. Critical thermal tolerance polygons of tropical marine fishes from Sulawesi, Indonesia. *J Therm Biol* **34**:220–225. doi:10.1016/j.jtherbio.2009.02.005.
- Ern R, Huong do TT, Phuong NT, Madsen PT, Wang T, Bayley M. 2015. Some like it hot: thermal tolerance and oxygen supply capacity in two eurythermal crustaceans. *Sci Rep* **5**:10743. doi:10.1038/srep10743.
- Ern R, Huong DT, Phugon NT, Wang T, Bayley M. 2014. Oxygen delivery does not limit thermal tolerance in a tropical eurythermal crustacean. *J Exp Biol* **217**:809–814. doi:10.1242/jeb.094169.
- Flynn EE, Todgham AE. 2018. Thermal windows and metabolic performance curves in a developing Antarctic fish. *J Comp Physiol B Biochem System Environ* **188**:271–282. doi:10.1007/s00360-017-1124-3.
- Fry FEJ. 1947. Effects of the environment on animal activity. University of Toronto Studies of Biological Series 55, Publication Ontario Fisheries Research Laboratory.
- Gilroy A, Edwards SJ. 1998. Optimum temperature for growth of Australian abalone: preferred temperature and critical thermal maximum for blacklip abalone *Haliotis rubra* (Leach), and greenlip abalone *Haliotis laevigata* (Leach). *Aquac Res* **29**:481–485. doi:10.1046/j.1365-2109.1998.00241.x.
- Guderley H, Pörtner HO. 2010. Metabolic power budgeting and adaptive strategies in zoology: examples from scallops and fish. *Can J Zool* **88**:753–763. doi:10.1139/Z10-039.
- Hahn KO. 1989. Biotic and abiotic factors affecting the culture of abalone. In: Hahn KO (ed) *Handbook of culture of abalone and other marine gastropods*. CRC Press Inc. Boca Raton, Florida, pp. 113–134.
- Hecht T. 1994. Behavioural thermoregulation of the abalone *Haliotis midae*, and the implications for intensive culture. *Aquaculture* **26**:171–181. doi:10.1016/0044-8486(94)90258-5.
- Herrlinger TJ. 1981. Range extension of *Kelletia kelletii*. *The Veliger* **24**:78.
- Hines A, Anderson S, Brisbin M. 1980. Heat tolerance in black abalone *Haliotis cracherodii* Leach, 1814: Effects of temperature fluctuation and acclimation. *The Veliger* **23**:113–118.
- Hochachka PW, Somero GN. 2002. *Biochemical adaptation: mechanisms and process in physiological evolution*. Oxford University Press, New York, USA.
- Huey RB, Kearney MR, Andrew K, Holthum JAM, Jess M, Williams SE. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos Trans R Soc Lond B Biol Sci* **367**:1665–1679. doi:10.1098/rstb.2012.0005.
- Jiang Y, Jiao H, Sun P, Yin F, Tang B. 2020. Metabolic response of *Scapharca subcrenata* to heat stress using GC/MS-based metabolomics. *PeerJ* **8**:e8445. doi:10.7717/peerj.8445.
- Johnson JA, Kelsch SW. 1998. Effects of evolutionary thermal environment on temperature-preference relationships in fishes. *Environ Biol Fishes* **53**:447–458. doi:10.1023/A:1007425215669.
- Karelitz S, Uthicke S, Foo SA, Barker MF, Byrne M, Pecorino D, Lamare MD. 2017. Ocean acidification has little effect on developmental thermal windows of echinoderms from Antarctica to the tropics. *Glob Chang Biol* **23**:657–672. doi:10.1111/gcb.13452.
- Kelsh SW. 1996. Temperature selection and performance by bluegills: evidence for selection in response to available power. *Trans Am Fish Soc* **125**:948–955. doi:10.1577/1548-8659(1996)125<0948:TSAPBB>2.3.CO;2.
- Kordas RI, Harley CDG. 2016. Demographic responses of coexisting species to *in situ* warming. *Mar Ecol Prog Ser* **546**:147–161. doi:10.3354/meps11620.
- Le DV, Alfaro AC, Ragg NLC, Hilton Z, King N. 2017. Establishing the thermal window for aerobic scope in New Zealand geoduck clams (*Panopea zelandica*). *J Comp Physiol B Biochem System Environ* **187**:265–276. doi:10.1007/s00360-016-1038-5.
- Lugo P, Díaz F, Re AD, Olivares F, Gonzalez R, Dueñas S, Liccea A. 2016. Thermoregulatory behavior and high thermal tolerance of *Californiconus californicus* (Reeve, 1844), *Conasprella perplexus* (GB Sowerby II, 1857) and *Conasprella ximenes* (Gray, 1839) inhabited of Pacific Ocean and Gulf of California. *Molluscan Res* **36**:247–254. doi:10.1080/13235818.2016.1172545.
- Madeira D, Narciso I, Cabral H, Vinagre C. 2012. Thermal tolerance and potential climate change impact in marine and estuarine organisms. *J Sea Res* **70**:32.41. doi:10.1016/j.seares.2012.03.002.
- Miller NA, Paganini AW, Stillman JH. 2013. Differential thermal tolerance and energetic trajectories during ontogeny in porcelain crabs, genus *Petrolisthes*. *J Therm Biol* **38**:79–85. doi:10.1016/j.jtherbio.2012.11.005.
- Morley SA, Lemmon V, Obermüller BE, Spicer JI, Clark MS, Peck LS. 2011. Duration tenacity: a method for assessing acclimatory capacity of the Antarctic limpet *Nacella concinna*. *J Exp Mar Biol Ecol* **399**:39–42. doi:10.1016/j.jembe.2011.01.013.
- Nelson SG, Simmons MA, Knight AW. 1985. Calorigenic effect on diet on the grass shrimp *Crangon franciscorum* (Crustacea: Crangonidae). *Comp Biochem Physiol Part A Mol Integr Physiol* **82**:373–376. doi:10.1016/0300-9629(85)90870-9.
- Nichelmann M. 1983. Some characteristics of the biological optimum temperature. *J Therm Biol* **8**:69–71. doi:10.1016/0306-4565(83)90079-7.
- Norin T, Malte H, Clark TD. 2014. Aerobic scope does not predict the performance of a tropical eurythermal fish at elevated temperatures. *J Exp Biol* **217**:244–251. doi:10.1242/jeb.089755.
- Noyola RJ, Caamal-Monsreal C, Díaz F, Re D, Sánchez-Zamora A, Rosas C. 2013. Thermopreference, tolerance and metabolic rate of early stages juvenile *Octopus maya* acclimated to different temperatures. *J Therm Biol* **38**:14–19. doi:10.1016/j.jtherbio.2012.09.001.
- Noyola RJ, Mascaró M, Díaz F, Re AD, Sánchez-Zamora A, Caamal-Monsreal C, Rosas C. 2015. Thermal biology of prey (*Melongena corona bispinosa*, *Strombus pugilis*, *Callinectes similis*, *Libinia dubia*) and predators (*Ocyurus chrysurus*, *Centropomus undecimalis*) of *Octopus maya* from the Yucatan Peninsula. *J Therm Biol* **53**:151–161. doi:10.1016/j.jtherbio.2015.11.001.
- Padilla-Ramirez S, Díaz F, Re AD, Galindo-Sanchez CE, Sanchez-

- Lizarraga AL, Nuñez-Moreno LA, Moreno-Sierra D, Paschke K, Rosas C. 2015. The effect of thermal acclimation on the behavior, thermal tolerance and respiratory metabolism in a crab inhabiting a wide range of thermal habitats (*Cancer antennarius* Stimpson, 1856) the red shore crab. *Mar Freshw Behav Physiol* **48**:89–101. doi:10.1080/10236244.2015.1019212.
- Paschke K, Agüero J, Gebauer P, Díaz F, Mascaro M, Lopez-Ripoll E, Re D, Caamal-Monsreal C, Tremblay N, Pörtner HO, Rosas C. 2018. Comparison of aerobic scope for metabolic activity in aquatic ectotherms with temperature related metabolic stimulation: a novel approach for aerobic power budget. *Front Physiol* **9**:1438. doi:10.3389/fphys.2018.01438.
- Peck LS, Morley SA, Richard J, Clark MS. 2014. Acclimation and thermal tolerance in Antarctic marine ectotherms. *J Exp Biol* **217**:16–22. doi:10.1242/jeb.089946.
- Percy JA. 1972. Thermal adaptation in the boreo-artic echinoid, *Strogilocentrotus droebachiensis* (O.F. Müller 1776). I Seasonal acclimatization of respiration. *Physiol Biochem Zool* **45**:277–289. doi:10.1086/physzool.45.4.30155584.
- Pörtner HO. 2010. Oxygen and capacity limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. *J Exp Biol* **213**:881–893. doi:10.1242/jeb.037523.
- Pörtner HO, Farrell AP. 2008. Physiology and climate change. *Science* **322**:690–692. doi:10.1126/science.1163156.
- Pörtner HO, Peck MA. 2010. Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *J Fish Biol* **77**:1745–1779. doi:10.1111/j.1095-8649.2010.02783.x.
- Rangel RE, Johnson DW. 2018. Metabolic responses to temperature in a sedentary reef fish, the bluebanded goby *Lythrypnus dalli* Gilbert. *J Exp Mar Biol Ecol* **501**:83–89. doi:10.1016/j.jembe.2018.01.011.
- Re AD, Díaz F, Salas-Garza A, Gonzalez M, Cordero V, Galindo-Sanchez CE, Sanchez-Castrejon E, Sanchez-Zamora A, Licea A. 2013. Thermal preference, tolerance and temperature-dependent respiration in the California sea hare *Aplysia californica*. *Agric Sci* **4(6A)**:46–52. doi:10.4236/as.2013.46A007.
- Reynolds WW, Casterlin ME. 1979. Behavioral thermoregulation and the final preferendum paradigm. *Am Zool* **19**:211–224. doi:10.1093/icb/19.1.211.
- Rezende EL, Castañeda LE, Santos M. 2014. Tolerance landscapes in thermal ecology. *Funct Ecol* **28**:799–809. doi:10.1111/1365-2435.12268.
- Rodríguez-Fuentes G, Murúa-Castillo M, Díaz F, Rosas C, Caamal-Monsreal C, Sánchez A, Paschke K, Pascual C. 2017. Ecophysiological biomarkers defining thermal biology of the Caribbean lobster *Panulirus argus*. *Ecol Indc* **78**:192–204. doi:10.1016/j.ecolind.2017.03.011.
- Romero MR, Walker KM, Cortez CJ, Sanchez Y, Nelson KJ, Ortega DC, Smick SL, Hoese WJ, Zacherl DC. 2012. Larval diel vertical migration of the marine gastropod *Kelletia kelletii* (Forbes, 1850). *J Mar Biol* **2012**:1–9. doi:10.1155/2012/386575.
- Rosenthal RJ. 1970. Observations on the reproductive Biology of the Kellet's whelk *Kelletia kelletii*. *The Veliger* **12**:319–324.
- Sanda S, Hamasaki K, Dan S, Kitada S. 2019. Expansion of the northern geographical distribution of land hermit crab populations: colonization and overwintering success of *Coenobita purpureus* on the coast of the Boso Peninsula, Japan. *Zool Stud* **58**:25. doi:10.6620/ZS.2019.58-25.
- Salas-Garza A, Díaz F, Re AD, Galindo-Sánchez CE, Sanchez-Castrejon E, González M, Licea A, Sanchez-Zamora A, Rosas C. 2014. Preferred temperature, thermal tolerance and metabolic response of *Tegula regina* (Stearns, 1892). *J Shellfish Res* **33**:239–246. doi:10.2983/035.033.0123.
- Smith AM. 1991. The role of suction in the adhesion of limpets. *J Exp Biol* **161**:151–169.
- Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin AA. 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Mar Environ Res* **79**:1–15. doi:10.1016/j.marenvres.2012.04.003.
- Sokolova IM, Pörtner HO. 2003. Metabolic plasticity and critical temperatures for aerobic scope in a eurythermal marine invertebrate (*Littorina saxatilis*, Gastropoda, Littorinidae) from different latitudes. *J Exp Biol* **206**:195–207. doi:10.1242/jeb.00054.
- Somero GN. 2009. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J Exp Bio* **213**:912–920. doi:10.1242/jeb.037473.
- Stenseng EM, Braby CE, Somero GN. 2005. Evolutionary and acclimation-induced variation in the thermal limits of heart function in congeneric marine snails (genus *Tegula*): implications for vertical zonation. *Biol Bull* **208**:138–144. doi:10.2307/3593122.
- Stern S, Borut A, Cohen D. 1984. The effect of salinity and ion composition on oxygen consumption and nitrogen excretion of *Macrobrachium rosenbergii* (de Man). *Comp Biochem Physiol Part A Mol In Tegr Physiol* **79**:271–274. doi:10.1016/0300-9629(84)90428-6.
- Tomanek L, Somero GN. 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: implications for limits of thermotolerance and biogeography. *J Exp Biol* **202**:2925–2936.
- Ulbricht RJ. 1973. Effect of temperature acclimation on the metabolic rate of sea urchins. *Mar Biol* **19**:273–277. doi:10.1007/BF00348893.
- Ulbricht RJ, Pritchard AW. 1972. Effect of temperature on the metabolic rate of sea urchins. *Biol Bull* **142**:178–185. doi:10.2307/1540254.
- Vinagre C, Dias M, Cereja R, Abreu-Alfonso F, Flores AAV, Mendonça V. 2019. Upper thermal limits and warming safety margins of coastal marine species. Indicator baseline for future references. *Ecol Indc* **102**:644–649. doi:10.1016/j.ecolind.2019.03.030.
- Wang HY, Tsang LM, Lima FP, Seabra R, Ganmanee, M, Williams GA, Chan BKK. 2020. Spatial variation in thermal stress experienced by barnacles on rocky shores the interplay between geographic variation, tidal cycles and microhabitat temperatures. *Front Mar Sci* **7**:553. doi:10.3389/fmars.2020.00553.
- Zacherl D, Gaines SD, Lonhart SI. 2013. The limits to biogeographical distributions: insights from northward range extension of the marine snail *Kelletia kelletii* (Forbes, 1852). *J Biogeogr* **30**:913–924. doi:10.1046/j.1365-2699.2003.00899.x.
- Zheng Z, Jin C, Li M, Bai P, Dong S. 2008. Effects of temperature and salinity on oxygen consumption and ammonia excretion of juvenile miiuy croaker, *Miichthys miiuy* (Basilewsky). *Aquac Int* **16**:581–589. doi:10.1007/s10499-008-9169-7.