ORIGINAL RESEARCH

Thermal preference, tolerance, and thermal aerobic scope in clownfish *Amphiprion ocellaris* (Cuvier, 1830) predict its aquaculture potential across tropical regions

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Abstract The clownfish *Amphiprion ocellaris* is widely distributed in the coral reef ecosystems of tropical and subtropical regions of the West Indo Pacific, an area that hosts economically valuable species, and, thus, a suitable candidate for warm water aquaculture. This study determined the preferred temperature, critical threshold limits, represented by critical thermal maximum and critical thermal minimum, thermal window width, and aerobic metabolic scope of *A. ocellaris* clownfish acclimated to 20, 23, 26, 29, 32, and 35 °C. A positive response ($P < 0.05$) occurred when the preferred temperature significantly increased with increasing acclimation temperature. The preferred temperature obtained graphically was 30.0 °C. Acclimation temperature significantly affected the thermal tolerance which increased with acclimation temperature. The thermal window calculated for *A. ocellaris* was 301.5 °C^2. The thermal metabolic scope obtained in animals acclimated at the interval from 23 to 32 °C ($P > 0.05$) had a mean value of 4240.8 mg O$_2$ h^{-1} kg^{-1} w.w., revealing that *A. ocellaris* is a eurythermal species with a range of optimal physiological performance that closely matches the environmental conditions where it can be farmed. Therefore, the highest value of the thermal aerobic scopes corresponded to the intervals of the preferred temperature obtained for *A. ocellaris*. These results may partially explain their worldwide distribution pattern, as well as their aquaculture potential in tropical regions.

Keywords Thermoregulation · Thermal tolerance · Metabolic rate · Anemone fish · Tropical reefs · Aquaculture

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Introduction

Temperature is one of the most important factors for aquatic ectotherms. In most fish, body temperature is controlled by environmental temperature, so some organisms have developed thermoregulatory mechanisms to survive and optimize temperature-dependent physiological processes (Reynolds 1979; Goyer et al. 2014). Hence, temperature is recognized as one of the most important environmental factors, since it has control over energetics, growth, reproduction, and distribution of aquatic organisms, including fish (Angilletta et al. 2006).

Amphiprion ocellaris (clownfish) is widely distributed in coral reef ecosystems of tropical and subtropical regions. It belongs to the diverse and well-distributed family Pomacentridae with 28 known species that belong to two genera: *Amphiprion* and *Premnas* (Ajith Kumar and Balasubramanian 2009). *A. ocellaris* has a high demand in the international market as an ornamental fish for marine aquariums because of its beauty, small size, and maintenance in captivity (Ajith Kumar and Balasubramanian 2009). These colorful fish come from the coral reefs of the West Indo Pacific and live associated with anemones. However, due to the deterioration of their habitat and overfishing, their population has decreased drastically (Ajith Kumar and Balasubramanian 2009). The coral reef ecosystems where these fish live have experienced episodes of high temperatures in the last years (Nilsson et al. 2009; Harborne 2013; Madeira et al. 2016, 2017). In this context, knowing the thermal tolerance of clownfish should be a key aspect when restocking coral programs start operating in an attempt to replenish the ecosystem structure. For these reasons, the effort of restocking natural populations has been considered in the last few years by breeding them artificially to obtain mass production (Jung 2006; Ajith Kumar and Balasubramanian 2009; Abduh et al. 2011).

For rearing ornamental fish, the optimum temperature should be established as one of the most important environmental parameters for teleost fish growth, because it would allow us to examine the effect on growth of other variables, such as stocking density and nutritional requirements (Rushworth et al. 2011).

Thermoregulatory behavior of ectothermic fish is an important response that can be used to determine the optimum temperature range under which physiological and metabolic processes are optimum (Crawshaw 1977; Beitinger and Fitzpatrick 1979; Ward et al. 2010; Bellgraph et al. 2010; Xu et al. 2015). Recently, behavioral thermoregulation is receiving renewed attention, as it allows ectotherms to cope with climate change (Kearney et al. 2009; Huey et al. 2012; Sunday et al. 2014; Píasecnà et al. 2015).

In aquaculture, the critical thermal maximum (CTMax) and critical thermal minimum (CTMin) are crucial, since these limits are considered measures of thermal tolerance. Critical thermal limits are determined by raising or lowering temperature progressively from the acclimation temperature until muscle disorganization occurs in response to the thermal stressor. Both responses identify the temperature at which the first stress symptoms appear, and they are a direct reflection of how acclimation temperature modulates thermal tolerance in aquatic ectotherms (Becker and Genoway 1979; Paladino et al. 1980; Beitinger and Bennett 2000).

The aerobic scope (AS), calculated as the difference in O_2 consumption between the standard metabolic rate (SMR) and the maximum metabolic rate (MMR) (Chabot et al. 2016a, b; Farrell 2016; Halsey et al. 2018), represents the excess oxygen available to support biological fitness. Aerobic scope, like other physiological responses, has an optimum temperature range (Topt). Beyond this optimum point, temperature causes energy to be channeled to protect (*pejus* interval of temperature) or repair fish cellular integrity (Pörtner and Knust 2007; Pörtner 2010). In this context, the aerobic scope can be divided by the optimum range where the energy balance is positive and the maximum aerobic scope available for activities, such, as growth, development, reproduction, and storage. Once the temperature reaches a level where energy is continuously deriving from anaerobic metabolism, the endpoint will always be death (Pörtner 2010; Sokolova et al. 2012).

Halsey et al. (2015) mentioned that a strong and predictable relationship exists between activity and temperature in ectotherms, since a certain level of activity may be induced by thermal exposure. Paschke et al. (2018) stated that the temperature-induced metabolic rate (TIMR) method may be used to estimate the aerobic power budget of aquatic organisms to induce high metabolic rates, and it can be obtained through stimulation of organisms' activity using high, non-lethal temperatures that produce high metabolic rate (HMR). A minimum metabolic rate can be achieved when the activity is depressed by exposure to a temperature low enough to provoke a forced low metabolic rate (LMR).

Even though cultivation temperature was determined (28 ± 2 °C) (Jung 2006; Ajith Kumar and Balasubramanian 2009) in *A. ocellaris*, more information regarding temperature tolerance is needed to test if a relationship exists among thermal tolerance, preferred temperatures, and thermal metabolic scope (TMS).

This study aimed at determining the preferred temperature, thermal tolerance, and thermal window width, and comparing the TMS with the AS obtained by TIMR and chasing methods in juveniles of *A. ocellaris*, acclimated at different temperatures to optimize their culture conditions. At the same time, the study provides insight into the potential relationship among TMS, AS, and preferred temperature in an attempt to determine whether these physiological and behavioral indices match the same acclimation temperature. We hypothesized that a thermal metabolic scope (TMS), obtained by temperature-induced metabolic stimulation as the difference between HMR and LMR, could be used as a new indicator of the aerobic power budget for sedentary or sluggish organisms. This information could be useful to evaluate if energy deriving from respiratory metabolism reflects the optimum performance in tropical species, such as *A. ocellaris*.

Materials and methods

Origin of animals

Clownfish were obtained from a production laboratory of ornamental fish of the Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD), located in Mazatlán, Sinaloa, México. Organisms (N =540 juveniles) were transported by air to the Laboratory of Marine Biotechnology of Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE) with a wet weight ranging from 2.0 to 2.5 g. They were placed in 12 200-L tanks at 26 ± 1 °C for 9 days. Animals, N =40 individuals per tank, were acclimated in the laboratory to experimental temperatures of 20, 23, 26, 29, 32, and 35 ± 1 °C by decreasing or increasing temperature at a rate of 2 °C/day; for each experimental condition, two repetitions were made (N total = 480 organisms). Fish remained at these experimental temperatures for 21 days (Noyola Regil et al. 2015). The temperature in the experimental tanks was maintained using 1000-W heaters coupled to a thermocouple and connected to temperature controllers (Model Moeller ± 0.5 °C precision, San Diego, USA). An open seawater system allowed maintaining experimental tanks at 35 psu with 30% seawater exchange per day. Clownfish were fed a commercial diet (Skretting 0.8 mm) of 5% of their wet weight twice a day; any remaining food was daily siphoned from each tank.

Thermal biology

The final preferred temperature of clownfish was determined by the acute method described by Reynolds and Casterlin (1979), performing experiments in a tube (400 cm long and 20 cm in diameter with 20 segments). A Neslab thermoregulator (model HX 150 Portsmouth, NH, USA) (Fig. 1a) was connected to the right end of
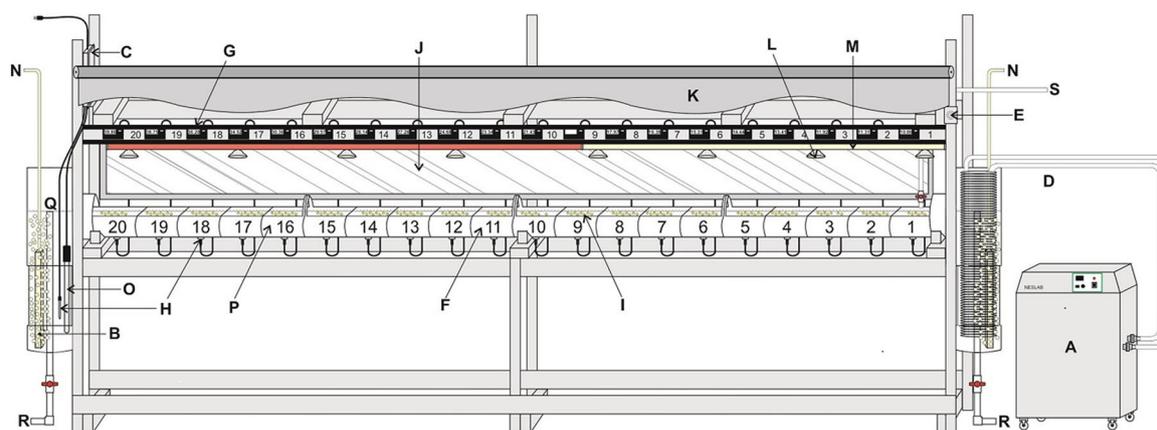
Fig. 1 Diagram of the horizontal temperature gradient apparatus used for determining the preferred temperature of *Amphiprion ocellaris*. **a** Digital recirculating chiller; **b** Airstone; **c** Heater temperature controller; **d** Titanium heat exchanger; **e** Dimmer **f** Gradient of 400 cm in length and 20 cm in diameter; **g** Digital thermometers; **h** Temperature sensor; **i** Airstones; **j** Mirror; **k** Curtain; **l** Fluorescent lamps; **m** Incandescent lamps; **n** Air line; **o** Titanium heater; **p** Gradient segments; **q** Water level; **r** Drain tubes; **s** Water drainage; **t** Refill line

the tube to cool water at 8 °C; a 1000-W heater (Fig. 1o) was placed at the left end and connected to a temperature-controlled heater (Model Moeller ± 0.5 °C precision, San Diego, USA) (Fig. 1c) to heat the water to 40 °C to allow generating a stable and linear 8.0 to 40.0 °C temperature gradient ($y = 9.12 + 1.39 \times$ Rsqr = 0.992; where x = segments of the gradient and y = temperature on the gradient segments (Fig. 1p). To eliminate vertical thermal stratification in the water column and to maintain a dissolved oxygen concentration of 9.4 mg L^{-1} in the cold end and 5.0 mg L^{-1} in the warm end, an air diffuser hose was placed along the tube (Fig. 1i). The water column depth in the gradient was 9 cm, and it was refilled at a rate of 11–12.0 L h^{-1} to maintain high water quality (Fig. 1t). The temperature was measured in each virtual segment with equidistant digital thermometers (Fig. 1g). A curtain was placed in front of the gradient to observe the organisms while avoiding the risk of stressing them (Fig. 1k). All fish were not fed 24 h before the experiment to avoid any interference by digestive activity (Nelson et al. 1985; Beamish and Trippel 1990). A total of 120 clownfish acclimated to 20, 23, 26, 29, 32, and 35 ± 1 °C were used to test the gradient. After the acclimation period, fish from each acclimation temperature were randomly selected and individually marked with plastic tags from 0.1 cm diameter 24 h before the experiment. Five fish of similar weight were marked and introduced into the gradient at the segment corresponding to their acclimation temperature. The location of the fish and temperature in each tube segment were recorded every 10 min for 120 min. Four repetitions were done per acclimation temperature (N =20), and experimental fish were used only once. The final preferred temperature was graphically determined by the intersection between preferred temperatures and the equality line that represented the same acclimation temperature range regarding the preferred temperature (Reynolds and Casterlin 1979).

For the control group, 15 individuals were placed in the gradient system (in groups of three and previously acclimated to different temperatures with the heater and chiller turned off (resulting in a constant temperature of 26 °C along the gradient, which was the temperature of the water entering the system). This control group 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 (CTMax) was determined in 15 fish for each acclimation temperature. At each time, a group of three fish was placed into a 40-L glass tank with constant aeration at their respective acclimation temperature for 30 min before the CTMax tests to reduce handling stress (Pérez et al. 2003). Then, the temperature was increased at a rate of 1 °C/min (Luttterschmidt and Hutchison 1997), using a 1000-W heater. The end point to CTMax was identified as the moment when the fish lost its ability to right itself (loss of equilibrium, LOE) (Beitinger and Bennett. 2000; Mora and Ospina 2001; Li et al. 2015, Vinagre et al. 2016) The temperature at which each fish reached LOE was recorded in the aquarium using a digital thermometer (Hanna Instruments HI 98509 temperature resolutions 0.1 °C Rhode Island USA).

Critical thermal minimum (CTMin) was assessed on 15 fish for each acclimation temperature using the methodology described in Noyola Regil et al. (2015). A stainless steel coil was connected to a chiller (PolyScience IP-35) inside the 40 L aquarium to allow temperature decline at a rate of 1 °C min^{-1}. Three individual fish were placed into an aquarium filled with water of the same acclimation temperature for 30 min before the CTMin test to reduce stress caused by handling (Pérez et al. 2003). An air stone was used to avoid thermal stratification. Criteria for the determination of the end point of CTMin were the same used for CTMax. The temperature at which each fish reached loss of equilibrium, LOE was recorded in the aquarium using a digital thermometer (Hanna Instruments HI 98509 temperature resolutions 0.1 °C Rhode Island USA). After the tests, fish were returned immediately to their acclimation temperature, and their survival was monitored for 96 h following the CTMax and CTMin tests. Each animal was used only once in the tests. The thermal window area was obtained with the CTMax and CTMin data following Bennett and Beitinger (1997) and expressed in °C^2.

Thermal metabolic scope and temperature-induced metabolic rate method

The temperature-induced metabolic rate (TIMR) methodology described by Paschke et al. (2018) was used to determine the thermal metabolic scope using CTMax where a temperature range at which locomotive activity is enhanced, since extreme temperature provokes high metabolic rates. Based on this fact, this study proposed that the weight-specific oxygen consumption upon stimulation of the organisms' activity caused high metabolic rates (HMR) when exposed to 95% of the CTMax (TIMRMax).

Similarly, a minimum metabolic rate could be obtained of the CTMin when the activity was depressed to 105% of the CTMin (TIMRMin) by exposure reaching a metabolic condition where low metabolic rate (LMR) was forced. It could be assumed that a thermal metabolic scope (TMS) induced by temperature might be obtained as HMR-LMR where TMS could be used as a new indicator of the aerobic power budget for sedentary or sluggish organisms.

For each acclimation temperature, ten 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 40-L aquarium equipped with a 1000-W heater and constant aeration at their respective acclimation temperature. The animals remained in these conditions for 30 min to recover from stress caused by handling; later, the temperature increased 1 °C/min^{-1} until it reached 95% of its CTMax; then, they were immediately transferred to an intermittent respirometric system (Díaz et al. 2007). Oxygen consumption was measured every 30 s for 5 min; this time period was chosen considering that more exposure time could provoke fatigue and anaerobic metabolism (Norin and Clark 2016), even though it was related to the high metabolic rate (HMR). Oxygen concentrations were measured with dipping probe oxygen mini-sensors (Loligo Systems, Copenhagen, DK) connected to a PC-controlled fiber optic trace oxygen transmitter (OXY-10 trace transmitter, PreSens Precision Sensing GmbH, Regensburg, DE).

For the determination of TIMRMin, clownfish were exposed to temperature reduction at a rate of 1 °C min^{-1}, similarly to that described to obtain CTMin. When organisms reached CTMin of 105%, they were immediately transferred to an intermittent respirometric system (Díaz et al., 2007) with a temperature similar to that of CTMin (105%). Oxygen consumption was measured every 30 s for 5 min, which corresponded to the low metabolic rate (LMR). Oxygen concentrations were measured with dipping probe oxygen mini-sensors (Loligo Systems, Copenhagen, DK) connected to a PC-controlled fiber optic trace oxygen transmitter (OXY-10 trace transmitter, PreSens Precision Sensing GmbH, Regensburg, DE).

Records of three chambers filled with water without fish were used as a control to account for microbial oxygen consumption in the filtered seawater to make the necessary corrections. Respiration rate was calculated as shown in the following equation:

$$MO_2 = (O_{2(A)} - O_{2(B)}) \cdot (V/t) / M,$$

where MO_2 is respiration rate (mg O_2 h^{-1} g^{-1} w.w.); $O_{2(A)}$ is the initial oxygen concentration in the chamber (mg O_2 L^{-1}); $O_{2(B)}$ is the final oxygen concentration in the chamber (mg O_2 L^{-1}); V is water volume in the chamber minus volume of water displaced by the animal; t is the time elapsed during measurement (h); and M is the body mass of the experimental animal (g w.w.).

The TMS was calculated according to the novel methodology in this study as the difference between the HMR and LMR, where HMR = high metabolic rate and LMR = low metabolic rate (both as mg O_2 h^{-1} kg^{-1} w.w.). The factorial metabolic scope was also calculated as HMR/LMR for comparative purposes.

Statistical analysis

Statistical comparisons were made for each factor as thermoregulatory behavioral, CTMin, CTMax, HMR, and LMR. Data were first assessed to confirm normality (Shapiro's test) and homogeneity of variances (Levene's test). After assumptions of normality and homogeneity of variances were unable to be completed, data were analyzed using Kruskal–Wallis test ($P \leq 0.05$). Later, Dunn´s multiple comparison post hoc tests ($P \leq 0.05$), and non-parametric tests were used to determine significant differences among each physiological response obtained from experimental temperatures. All statistical computations were made by Sigma-Plot software (V. 12.5).

Results

Thermal biology

When water temperature was maintained constant in the gradient (control treatment), juveniles of *A. ocellaris* were found to be distributed spontaneously along the gradient (400 cm) after 2 h, indicating that they showed
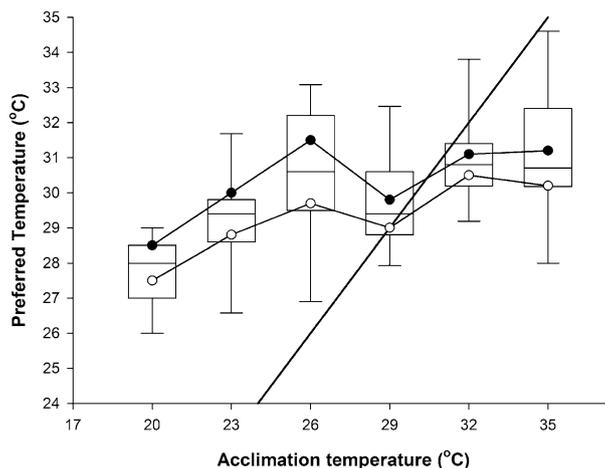
Fig. 2 Preferred temperature of *Amphiprion ocellaris* acclimated to different temperatures. The zone, bordered by circles, represents a 95% confidence interval of the median. The bars include 50% of the organisms' distribution. The continuous construction line of 45° represents the point where the preferred and acclimation temperatures are equal
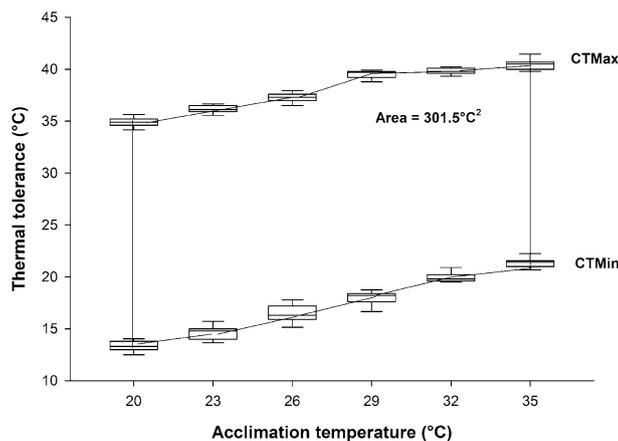

Fig. 3 Thermotolerance represented by critical thermal minima (CTMin) and critical thermal maxima (CTMax) and the thermal window of *Amphiprion ocellaris* acclimated to different temperatures

no preference for any particular segment. Acclimation temperature affected the thermal preference of clownfish ($H = 356.8$; $N = 120$; $P < 0.05$). The preferred temperature increased by acclimation temperature with low values in fish acclimated at 20 °C (28 ± 1.5 °C) and high values in those acclimated at 35 °C (30.7 ± 2.1 °C). A final thermal *preferendum* of 30 °C was calculated (Fig. 2). A direct relationship among acclimation temperature, CTMax, and CTMin was observed ($P < 0.001$). CTMax increased from 34.9 ± 0.5 °C to 40.1 ± 0.7 °C in fish acclimated from 20 to 35 °C, whereas CTMin values increased from 13.3 ± 0.5 to 21.4 ± 0.5 °C as acclimation temperatures increased (Fig. 3). With these values, a thermal window width for clownfish was obtained as 301.5 °C^2 (Fig. 3).
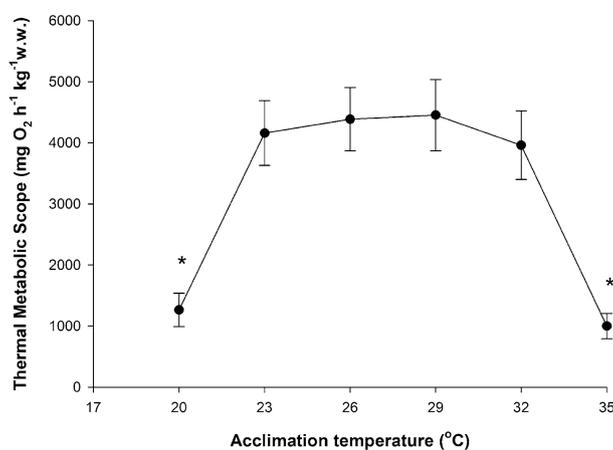
Thermal metabolic scope: TIMR method

The low metabolic rate (LMR) was not significantly affected by acclimation temperature in the range of 20–32 °C with an interval of 1031.6 mg O$_2$ h^{-1} kg^{-1} w.w., but, at 35 °C, the level significantly increased ($P < 0.05$), reaching a value of 1501.7 mg O$_2$ h^{-1} kg^{-1} w.w. The high metabolic rate (HMR) significantly declined ($P < 0.05$) from 2331.0 to 2501.0 mg O$_2$ h^{-1} kg^{-1} w.w. at lower or higher acclimation temperatures; at intermediate acclimation temperatures from 23 to 32 °C, an interval from 5012.0 to 5471.0 was
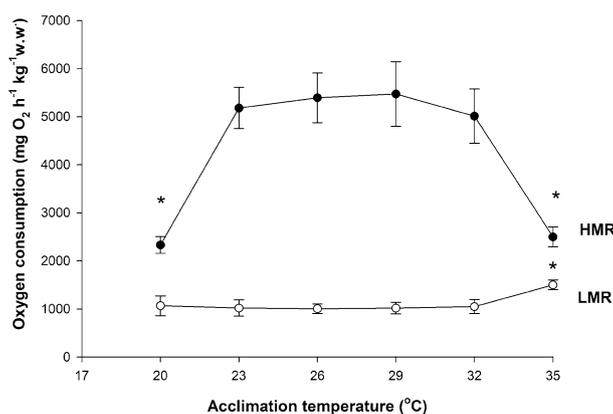
Fig. 4 High metabolic rate (HMR closed circles) and low metabolic rate (LMR open circles) of *Amphiprion ocellaris* obtained with the temperature-induced metabolic rate (TIMR) method acclimated to different temperatures. The asterisk above the points indicates significant differences ($P < 0.05$)

Fig. 5 Thermal metabolic scope (TMS) of *Amphiprion ocellaris* acclimated to different temperatures. The asterisk above the points indicates significant differences ($P < 0.05$)

displayed (Fig. 4). In consequence, a mean value of HMR was calculated as 5241.5 mg O_2 h^{-1} kg^{-1} w.w. (Fig. 4). When the thermal metabolic scope (TMS) was calculated from (HMR-LMR) in animals acclimated at the interval from 23 to 32 °C ($P > 0.05$), a mean value of 4240.8 mg O_2 h^{-1} kg^{-1} w.w. was obtained. TMS in animals acclimated at 20 and 35 °C had a lower value of 1264.0 and 1000.0 mg O_2 h^{-1} kg^{-1} w.w. respectively, when compared with the rest of the treatments (Fig. 5). The factorial aerobic scope had an interval from 1.7 to 5.4

Discussion

This study used thermal preference and TMS in an attempt to assess if behavior and physiological indexes can be used to define the optimum thermal range for this species. Data on preferences showed that clownfish exhibited an optimum thermal range from 27 to 32 °C, with a final preferred temperature of 30.3 °C. These temperatures were close to those obtained in the other coral reef fish. While studying with three Hawaiian fish, Medvick and Miller (1979) obtained the final preferred temperatures of 30.2 °C for the damselfish *Abudefduf abdominalis*; 29.2 °C for the surgeonfish *Acanthurus triostegus*; 27.2 °C for the butterfish *Chaetodon multicinctus*; whereas Nay et al. (2015) found a final preference of 29.5 °C for the cardinalfish *Chaeilodipterus quinquelineatus*. Habary et al. (2017) reported a mean preferred temperature of 28.9 °C for *Chromis viridis*.

All of these results show that fish optimal performance (from 27 to 30 °C) is coupled with the thermal tolerance accepted limits for coral reefs (16–30 °C) of the Caribbean and Great Barrier Reef (Coles and Riegl 2013) with the optimal thermal interval from 28 to 30 °C for zooxanthellae and anemones from the Pacific Ocean, Indian Ocean, and Red Sea (Hobbs et al. 2015).

When clownfish thermal preferences were compared with TMS, the preferred temperature corresponded with the maximum TMS in animals acclimated at 26, 29, and 32 °C, indicating that the thermal preference and maximum physiological performance for this species are linked to these temperature ranges These results suggest that these intermittent swimmer fish have a range of thermal variation in the reef, and they could use the energy of thermal aerobic scope to stay safe from predators.

Our findings with clownfish agree with those of Nilsson et al. (2009), Rummer et al. (2014), and Habary et al. (2017) who reported a significant reduction in aerobic scope at temperatures higher than 32 °C for other reef fish species, such as *Ostorhinchus cyanosoma, O. doderlenei, Cheilodipterus quinquelineatus, Chromis atripectoralis, Dascillus aruanus, D. melanurus, Pomacentrus moluccensis,* and *C. viridis.* In this sense, we can assume that a reduction in thermal aerobic scope, as observed in *A ocellaris,* could affect the ability of clownfish to feed, grow, and reproduce; therefore, it would have implications for long-term population sustainability, because 32 °C is below the *pejus* condition (Pörtner 2010). This temperature agrees with the envelope of blanching threshold for corals of 32 °C observed in the Caribbean and Great Barrier Reef (Coles and Riegl 2013).

The TIMR method was suitable to obtain information on the effects of temperature on the metabolic capacities of well-defined sedentary organisms, such as clownfish, offering a novel approach as a complement to the existing methods.

The factorial scope of *A. ocellaris* showed a moderate degree of aerobic potential at six acclimation temperatures with values ranging from 1.5 to 3.6, except at 29 °C where a relatively high value was obtained. The values obtained for the factorial scope of the triplefin *Forsterygion lapillum* (Khan and Herbert 2012), the barramundi *Lates calcarifer* (Norin et al., 2014), as well as for three species of damselfish and two of cardinalfish, both inhabiting coral reefs (Nilsson et al. 2009; Rummer et al. 2014), had intervals ranging from 1.2 to 4.25 corresponding to half of those reported for athletic fish species; these values were comparable to those obtained in intermittent swimmers (Schurmann and Steffensen 1997; McKenzie et al. 2001). Therefore, *A. ocellaris* can be considered an intermittent swimmer based on the values obtained for its factorial scope.

A summary of thermal tolerance for various fish species acclimated to different temperatures has been displayed, observing two fish groups: those with high thermal tolerance, represented by *Cattla cattla, Labeo rohita, Cirrhinus mrigala,* and *Horabagrus brachysoma* (Das et al. 2004, 2005; Dalvi et al. 2009), and those with a lower thermal tolerance represented by *Cyprinus carpio, Pangasius pangasius, Apogon novemfasciatus, Dascyllus aruanatus, Anabas testudineus, Lutjanus guttatus, Elacatinus oceanops, E. lobelia,* and *Argyrosomus regius* (Chatterjee et al. 2004; Debnath et al. 2006; Eme and Bennett 2009; Sarma et al. 2010; Larios-Soriano 2014; Di Santo and Lobel 2017; Kir et al. 2017).

The nine-band cardinalfish *Apogon novemfasciatus* and the white-tailed humbug *Dascyllus aruanatus,* inhabiting patch reefs and seagrass areas, exhibited CTMax values of over 40 °C and CTMin less than 13 °C, at the respective high and low acclimation range (Eme and Bennett 2009). Although these temperatures could be high, the threshold temperatures for the Arabian Gulf have been demonstrated to be 35–36 °C, regarding the worldwide thermal events that have caused recent declines in coral reef globally, indicating that, in warming scenarios, such temperatures could eventually be recorded in other coral reefs.

A thermal vulnerability (TV) has been defined as the difference between CTMax and maximum habitat temperature, providing an estimate of how close clownfish could live within this warming scenario (Vinagre et al. 2016). Taking into consideration, the CTMax values obtained of fish acclimated at 26, 29, and 32 °C (optimal thermal range) and a maximum thermal regime of 33 °C in a warming scenario, TV values of 4.3, 6.7, and 6.8 °C could be obtained, respectively. These values showed that clownfish might not be vulnerable to warming if they could survive without anemones or being less specific in their anemone selection.

This study has determined, for the first time, a value of $301.5 °C^2$ for the thermal window in *A. ocellaris*; to our knowledge, no similar reports have been published for *A. ocellaris* or any other type of clownfish where the thermal tolerance has been measured. This value was similar to that found by Larios-Soriano (2014) for *Lutjanus guttatus* ($336.5 °C^2$) and *Cyprinus carpio* ($311.6 °C^2$) (Chatterjee et al. 2004); both species are similar to clownfish and live in relatively narrow thermal environments. In contrast, Eme and Bennett (2009)

obtained thermal windows with areas ranging from 408 to 442.7 $°C^2$ in other species, such as the white-tailed humbug and nine-banded cardinalfish. Interestingly enough, the thermal window of clownfish was notoriously lower than that of those species, suggesting that clownfish are less eurythermal than those fish. According to Eme and Bennett (2009), these differences could be related to humbug and cardinal fish adaptations, acquired in more thermally fluctuating habitats in open reef zones (Eme and Bennett 2009).

This study evidenced that the thermal range where TMS was maximum (23 to 32 °C), it was within the preferred temperature range of clownfish, suggesting that thermal preference and TMS can be used as a tool for monitoring thermal regimes of this fish species. Moreover, we can conclude that *A. ocellaris* is well adapted to tolerate high temperatures, including those predicted during warming. Clownfish have a high metabolic scope that shows its maximum performance in a wide thermal range (Vinagre et al. 2016). Nevertheless, clownfish are dependent on anemones to survive, and the existing evidence has shown that anemones are highly sensitive to warming (Lasker et al. 1984). Further studies should evaluate thermal tolerance of anemones and clownfish at the same time; their capability to survive and reproduce in the absence of anemones should be tested in an attempt to assess the ecological significance of the wide thermal tolerance of clownfish.

Knowing the preferences and thermal tolerance of a species is important to define adequate culture conditions, as it allows determining the thermal requirements of organisms in a short period. The information regarding lethal temperature and the species' physiological optimum is important to infer the level of survival in a particular climate space. Therefore, the results obtained in this study are important to improve or establish clownfish culture. Based on the previous information, places with high temperatures not exceeding 30 °C and avoiding abrupt changes in temperature throughout the year should be chosen for rearing clownfish.

Authors contribution GVB Conducted the experiments and analyzed the data and writing the material and methods; FD and ADR Designed and consucted experiments, deaw the figures and writing results and discussion; LIC Proportioned the fish and reviewed the manuscript; LERI Revision the manuscript; MIAP Writing the Introduction; CR Cotributing to writing the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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