

# Physiological and behavioral responses of temperate seahorses (*Hippocampus guttulatus*) to environmental warming

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**Abstract** The aim of the present study was to evaluate, for the first time, the effect of environmental warming on the metabolic and behavioral ecology of a temperate seahorse, *Hippocampus guttulatus*. More specifically, we compared routine metabolic rates, thermal sensitivity, ventilation rates, food intake, and behavioral patterns at average spring temperature (18 °C), average summer temperature (26 °C), temperatures that they endure during summer heat wave events (28 °C), and in a near-future warming scenario (+2; 30 °C) in Sado estuary, Portugal. Both newborn juveniles and adults showed significant increases in metabolic rates with rising temperatures. However, newborns were more impacted by future warming via metabolic depression (i.e., heat-induced hypometabolism). In adult stages, ventilation rates also increased significantly with environmental warming, but food intake remained unchanged. Moreover, the frequency of swimming, foraging, swinging, and inactivity did not significantly change between the different thermal scenarios. Thus, we provide evidence that, while adult seahorses show great resilience to heat stress and are not expected to go through any physiological impairment and behavioral change with the projected near-future warming, the early stages display greater thermal sensitivity and may face greater metabolic challenges with potential cascading consequences for their growth and survival.

## Introduction

Presently, ocean warming is an omnipresent matter on every debate on marine system impacts (Halpern et al. 2008; Petit et al. 1999). There has been an increase in the mean global sea surface temperature of 0.13 °C per decade since 1979, and an increase in ocean interior temperature >0.1 °C since 1961 (Solomon et al. 2007). The climate systems are unpredictable, but forecasts point to a global mean temperature rise of 2 °C by 2100 (Santos et al. 2002, Lenton et al. 2008; Miranda et al. 2002). Moreover, heat waves, which presently last for a period of 1–2 weeks in the Portuguese coast (Miranda et al. 2002), will become more intense, more frequent and last for longer periods in a warmer climate scenario (Solomon et al. 2007).

Ocean temperature has a great influence on the biology and ecology of marine organisms and may lead to cascade effects on population, community, and ecosystem dynamics. Many species are sensitive to temperatures just a few degrees higher than those they usually experience in nature. A rise in temperature as small as 1 °C can have important and rapid effects on mortality and geographic distribution of some organisms (Kennedy et al. 2002; Perry et al. 2005; Brierley and Kingsford 2009). In general, fishes are ectothermic, that is, their internal temperature varies with the surrounding environmental temperature, which results in increased metabolic rates with environmental warming (Roessig et al. 2004). An increase in metabolism enhances growth rates, promotes a faster embryogenesis, and reduces the period of incubation (Pimentel et al. 2012; Rosa et al. 2012). Moreover, digestion becomes a faster process, and the gastric secretions are augmented, as well as the direct absorption of nutrients (Love 1970). However, environmental warming also elicits increased oxygen demand. Both temperature and oxygen availability might act as

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synergistic stressors and be too overwhelming to fish's cardiorespiratory processes. Above a certain temperature, also known as pejus temperature ( $T_p$ ), oxygen delivery is maximal and cannot further rise to cover elevated metabolic demands, and the ability of an organism to increase aerobic metabolism is compromised (Pörtner et al. 2004, 2005). Although survival is not immediately threatened beyond  $T_p$ , the ability to perform higher functions (e.g., feeding, growth, and reproduction) becomes limited (Pörtner and Knust 2007), which will influence the overall fitness and survival success of the species, especially on a long-term perspective.

Even though the consequences of ocean warming may be less severe in migratory fishes than in sessile animals, the peculiar characteristics of seahorses make them particularly sensitive to disturbances of their natural environment and a very interesting model for climate change studies. Migration from warm to cold waters may be a less effective strategy against ocean warming in fishes with patchy distribution (associated with structurally complex habitats) and reduced mobility. Moreover, seahorses live in shallow coastal waters, which will be more impacted by global warming in the future than the open seas and oceans (Philipart et al. 2011). Seahorses are already threatened worldwide by habitat degradation and overfishing, whereat additional stress from rapid climate change will add to its already high conservational value.

There is growing interest in studies that focus on the impact of climate change on marine animals, but there are no reports of this matter on seahorses, except in the research field of aquaculture techniques. All these studies point that temperature may affect survival, growth, feeding, behavior, reproduction, and even skin color of seahorses (Wong and Benzie 2003; Foster and Vincent 2004; Lin et al. 2006, 2009; Sheng et al. 2006; Koldewey and Martin-Smith 2010). Moreover, there are already some signs that seahorse populations may not respond well to extreme temperatures. In the Swartvlei estuary in South Africa, at least 3,000 *Hippocampus capensis* were found dead and trapped in small pockets of water, where water temperature reached 32 °C (Russell 1994).

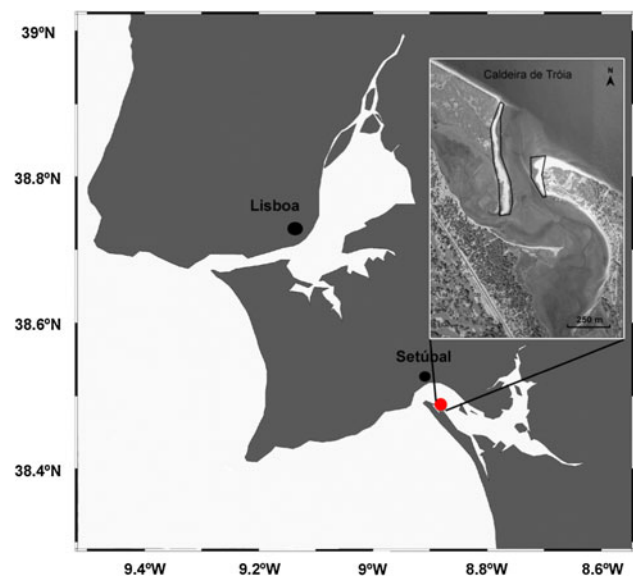
Due to their attractive appearance and peculiar ecology facts, like male pregnancy and monogamy, seahorses are considered flagship species for conservation campaigns (Foster and Vincent 2004), which makes them ideal species to draw attention to climate change issues. With the present study, we attempt to investigate the effects of ocean warming on the metabolic rates, feeding, and behavior patterns of the temperate seahorse *H. guttulatus*. Our data will contribute to a better understanding of the climate change effects on seahorses, taking a step forward on the prediction of animal response to the future expected conditions.

## Materials and methods

### Specimen collection and stocking conditions

Twenty adult seahorses (fifteen females and five males, four of them pregnant), *Hippocampus guttulatus*, were collected by beach seine near Caldeira de Tróia in the Sado estuary, Portugal (Fig. 1), between September and October 2011. The seahorses were transferred to the aquaculture facilities in the Guia Marine Laboratory and held in a recirculating system composed of 170-L glass aquaria (140 × 35 × 35 cm). All fishes were wet-weighted and identified with color bead necklaces loosely hold. Green plastic structures were provided as holdfasts for the seahorses.

During the first 4 weeks, fish were acclimatized to captive conditions and no experimental essays were run. The water parameters were as follows: temperature 20–22 °C, salinity 34–35, and pH 8.0–8.1 (similar to those found in the wild during the sampling period). Water quality was assured by a protein skimmer, bio-balls, and a UV sterilizer. Ammonia, nitrites, and nitrates concentrations were analyzed weekly and kept below detection levels (0.1, 0.3, and 10.0 mg L<sup>-1</sup>, respectively). Photoperiod was defined as 14-h light:10-h dark cycles. *H. guttulatus* were fed ad libitum twice a day with frozen enriched *Artemia* and *Mysis*, except for the day prior to the experimental essays. Aquaria were cleaned daily and 10 % water changes were made every week. After the initial 4-week period, female seahorses were acclimatized to four different temperature scenarios: (1) the average spring temperature (18 °C), (2) the average summer temperature



**Fig. 1** Map of the sampling area (red circle) in Caldeira de Tróia, located in the mouth of the Sado estuary, Portugal

(26 °C), (3) the temperature that they endure during summer heat wave events (28 °C), and (4) the projected temperature during heat waves in a near-future warming scenario (+2; 30 °C). Although the pregnant males were always kept at the initial acclimation conditions, their recently newborn juveniles were transferred to the four different temperature scenarios before the respirometry experiments (performed 1 day after hatching). At the end of the experiments, tags were removed and all adult seahorses were released to their original habitat without injuries.

### Oxygen consumption rates

#### *Adult seahorses*

All fifteen females were used to determine the oxygen consumption rates (routine metabolic rates) in the four thermal scenarios. Animals were placed in a flow-through respirometry setup (Loligo Systems, Denmark; more details in Rosa and Seibel 2008, 2010). To avoid bacterial contaminations, the seawater was filtered (0.2 µm) and treated (50 mg L<sup>-1</sup> streptomycin) before it was pumped at a constant flow rate (average 140 mL min<sup>-1</sup>) from a water-jacketed, gas equilibration column through the respirometers. Respirometers were immersed in a large thermostatted bath (Lauda) and acclimatized for 2 h to the specific water temperature. All respirometers had holdfasts on the bottom to ensure a normal animal behavior. The water in the column was bubbled continuously to maintain incoming water at high O<sub>2</sub> values (normoxia, 21 % O<sub>2</sub>). Each Clarke-type O<sub>2</sub> electrode was connected to a Strathkelvin Instruments 928 Oxygen Interface. Before and after each run, the experimental setup was calibrated and checked for electrode drift and microbial oxygen consumption. Each experiment was 6 h long: 2 h for acclimation and the following 4 h for oxygen measurement. During the experimental runs, fish were continuously observed in order to detect abnormal behaviors that could indicate stress. All experiments were carried out in darkness and at atmospheric pressure.

#### *Juvenile seahorses*

To determine the oxygen consumption rates (routine metabolic rates) of newborn seahorses in each of the four temperature scenarios, juveniles were incubated in sealed water-jacketed respirometry chambers (RC300 Respiration cell, Strathkelvin, North Lanarkshire, Scotland) containing filtered seawater mixed with antibiotics (50 mg L<sup>-1</sup> streptomycin) to avoid bacterial respiration (according to Rosa et al. 2009, 2012). For each temperature, five animals were used. Water volumes were adjusted in relation to animal mass in order to minimize locomotion and stress but still

allow spontaneous and routine activity rates of the newborns. Bacterial controls were conducted in parallel to correct for possible bacterial respiratory activity. The respiration chambers were placed in water baths (Lauda, Lauda-Königshofen, Germany) to control temperature. To record oxygen concentrations, water samples were taken from each syringe, with a Hamilton gastight 500-µL syringe, and injected in a water-jacketed Clarke-type O<sub>2</sub> electrode connected to a multi-channel oxygen interface (Strathkelvin, North Lanarkshire, Scotland). The duration of respiratory runs varied from 4 to 6 h.

### Thermal sensitivity and ventilation rate change

For each developmental stage (adult and juvenile), the temperature coefficient ( $Q_{10}$ ) for routine metabolic rates and ventilation rates was determined using the standard equation:

$$Q_{10} = [R(T_2)/R(T_1)]^{10}/(T_2 - T_1),$$

where  $R(T_1)$  and  $R(T_2)$  represent the routine metabolic rates or ventilation rates at temperatures  $T_1$  and  $T_2$ , respectively.  $Q_{10}$  values were calculated for the temperature intervals of 18–26, 18–28, and 18–30 °C.

### Ventilation rates, food intake, and behavioral patterns

Ten adult seahorses were individually placed in 27-L glass aquaria in a recirculating rearing system, and their ventilation rates, feeding rates, and activity patterns were measured at each of the four temperature scenarios. Seahorses were fed 100 frozen enriched *Artemia* twice a day. Gill ventilation rates were measured by counting the number of opercular beats per minute, before feeding. Food intake was determined by collecting and counting the leftovers at the end of the day. Behavioral patterns were analyzed for each seahorse during 15 min twice a day (30 min after each feeding), based on the ethogram described in Table 1. It is worth noting that these analyses were not performed in the recently newborn seahorses.

### Statistical analysis

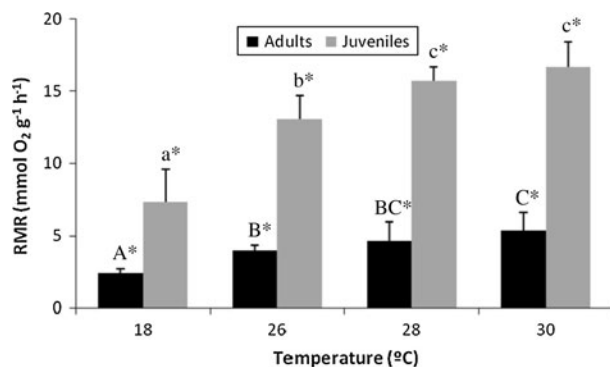
A two-way ANOVA was carried out in order to detect significant differences in routine metabolic rates between developmental stages (adult and newborn juveniles) and temperatures. The effect of temperature on ventilation rates, food intake, and behavioral patterns of adult seahorses was evaluated using repeated measurements ANOVA. ANOVAs were followed by Tukey's HSD or unequal N HSD post hoc tests. All statistical analyses were performed for a significance level of 0.05, using Statistica 10.0 software.

**Table 1** Ethogram of *H. guttulatus* activity patterns

Category	Behavior description
Swimming (S)	The seahorse swims, moving actively the dorsal and pectoral fins
Feeding (F)	The seahorse tilts the body toward the aquarium floor in search for food, points the snout toward the prey, and swallows the prey with a suction force while a clicking sound can be heard
Swinging (Sg)	The seahorse remains attached to the holdfast, with slight movements of the head or body
Inactivity (I)	The seahorse remains resting, without performing any kind of movement, while attached or unattached to the holdfast

## Results

Routine metabolic rates (RMR) were significantly affected by temperature and developmental stage (Fig. 2; Table 2). Mean RMR increased with temperature from 2.40 (18 °C) to 5.37  $\mu\text{mol O}_2 \text{g}^{-1} \text{h}^{-1}$  (30 °C) in adult seahorses and from 7.36 (18 °C) to 16.68  $\mu\text{mol O}_2 \text{g}^{-1} \text{h}^{-1}$  (30 °C) in newborn juveniles (see also scaling effect in “Appendix”). The  $Q_{10}$  values related to adult and newborn RMR ranged mainly around normal values (2.0–2.5), except for juveniles at the highest temperature interval (Fig. 3). Between 28 and 30 °C, the pronounced  $Q_{10}$  drop below 1.5 indicates



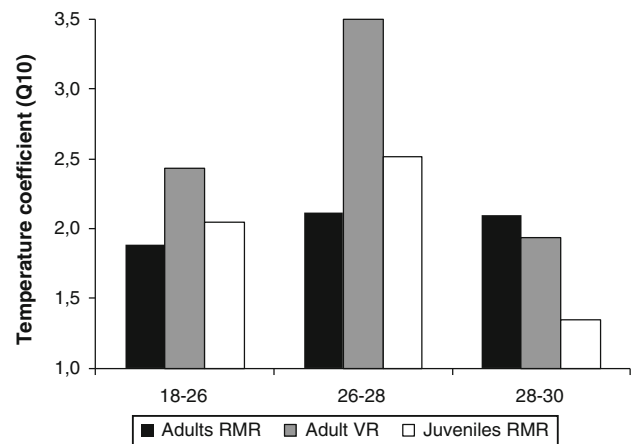
**Fig. 2** Impact of environmental warming on the routine metabolic rates (RMR,  $\mu\text{mol O}_2 \text{g}^{-1} \text{h}^{-1}$ ) of juvenile and adult seahorses, *H. guttulatus*. Values represent mean  $\pm$  SD ( $n = 5$  for juveniles and  $n = 15$  for adults). Different letters and asterisks represent significant differences ( $p < 0.05$ ) between temperatures and life stages, respectively. For more statistical details, see Table 2

**Table 2** Results of two-way ANOVA evaluating the effect of temperature (T) and life stage (S; juveniles and adults) on metabolic rates of seahorses, *H. guttulatus*

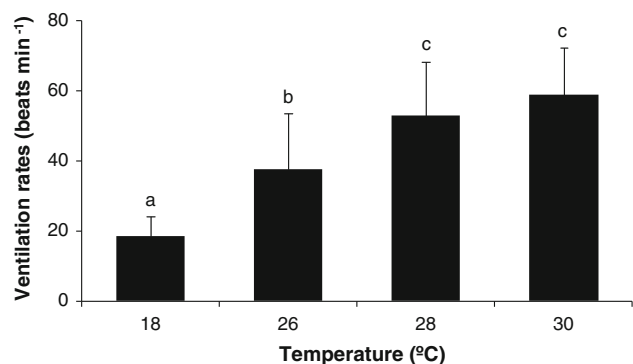
	df	MS	F	p
Metabolic rates				
Temperature (T)	3	111.00	83.87	0.000
Stage (S)	1	1,245.81	941.34	0.000
T $\times$ S	3	32.38	24.47	0.000
Error	72	1.32		

metabolic suppression due to thermal limitation of oxygen supply in newborn juveniles (Fig. 4).

Ventilation rates of adult seahorses were significantly affected by temperature (Fig. 3; Table 3). Opercular beats increased with rising temperature from 18 (18 °C) to 59 beats  $\text{min}^{-1}$  (30 °C). The temperature coefficient ( $Q_{10}$ ) ranged between 1.9 and 3.5 (Fig. 3). Unexpectedly, food intake (Fig. 5; Table 3), swimming, feeding, swinging,



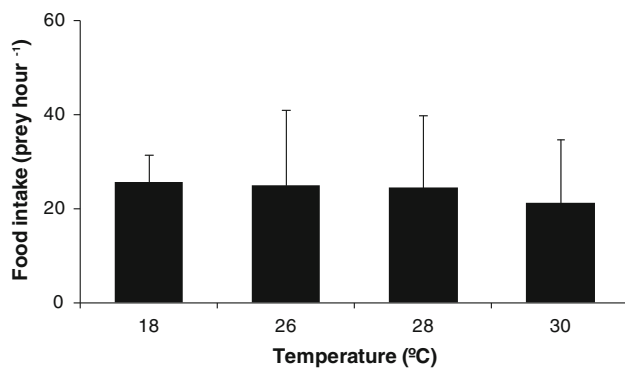
**Fig. 3** Impact of environmental warming on the thermal sensitivity ( $Q_{10}$  values) of the routine metabolic rates (RMR) and ventilation rates (VR) adult and juvenile seahorses, *H. guttulatus*



**Fig. 4** Impact of environmental warming on the ventilation rates (beats  $\text{min}^{-1}$ ) of adult seahorses, *H. guttulatus*. Values represent mean  $\pm$  SD ( $n = 10$ ). Different letters represent significant differences ( $p < 0.05$ ) between temperatures. For more statistical details, see Table 3

**Table 3** Results of repeated-measures ANOVA evaluating the effect of temperature on ventilation rates, food intake, and activity patterns (frequencies of swimming, feeding, swinging, and inactivity) of seahorses, *H. guttulatus*

	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p</i>
<b>Ventilation rates</b>				
Temperature	3	3,288.50	32.01	0.000
Error	27	102.72		
<b>Food intake</b>				
Temperature	3	37.86	0.95	0.429
Error	27	39.73		
<b>Swimming frequency</b>				
Temperature	3	104.42	0.48	0.698
Error	27	216.96		
<b>Feeding frequency</b>				
Temperature	3	438.79	1.88	0.156
Error	27	233.03		
<b>Swinging frequency</b>				
Temperature	3	649.90	2.93	0.051
Error	27	221.66		
<b>Inactive mode</b>				
Temperature	3	703.22	1.80	0.172
Error	27	391.39		

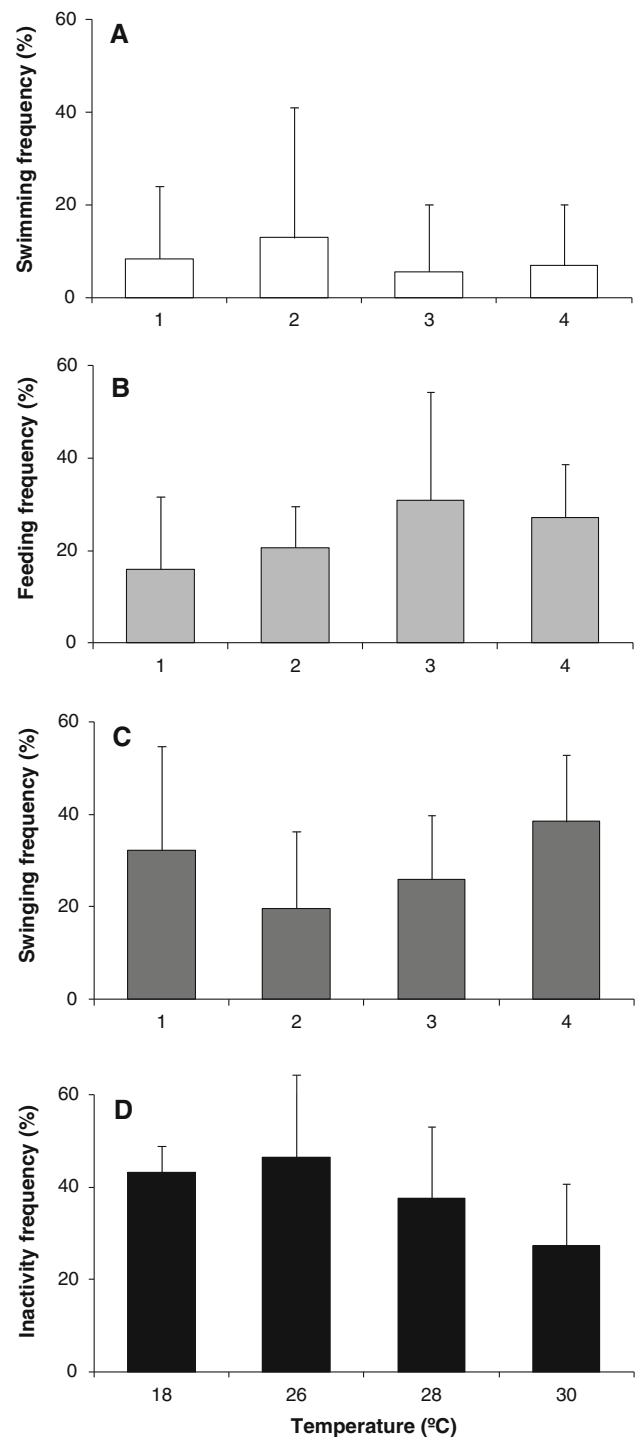


**Fig. 5** Impact of environmental warming on the food intake (prey h<sup>-1</sup>) of adult. For more statistical details, see Table 3

and inactivity frequencies remained unchanged (Fig. 6; Table 3) with increasing temperature.

## Discussion

*H. guttulatus* is generally found in shallow inshore waters, in depths below 12 m (Lourie et al. 2004). In the Portuguese coast, they are found mainly in estuary mouths and lagoon systems, being subject to great temperature fluctuations throughout the day and year. This eurythermic animal is therefore able to acclimatize and adapt to temperature oscillations, but will it be prepared for a warmer environment?



**Fig. 6** Impact of environmental warming on the behavioral patterns (%) of adult seahorses, *H. guttulatus*: **a** swimming frequency, **b** feeding frequency, **c** swinging frequency, and **d** inactivity frequency. For more statistical details, see Table 3

As expected, *H. guttulatus* metabolism and ventilation rates increased with temperature. In adults, such increase resulted in normal values of  $Q_{10}$  (around 2 and 3.5; Dejours 1973; Schmidt-Nielsen 1997; Ford et al. 2004), even for the highest temperature interval tested (28–30 °C), which

suggests that they were not under severe thermal stress. Given the poor swimming skills and low mobility of adult seahorses, their great thermal tolerance is extremely important and will allow them to face future increases in water temperature.

Although *H. guttulatus* has been shown to tolerate well high temperatures until at least 30 °C, extreme temperatures may, however, have a negative impact on several aspects of seahorse life. Temperature has been shown to have a positive effect on seahorse reproduction, but only until the optimal temperature is reached. In the tropical seahorse *H. kuda*, the developmental time of gonads decreased, while the gonadosomatic index and fecundity, fertility and initial juvenile survival rates increased with temperature, but not indefinitely. Beyond 30 °C, all the parameters start showing a reverse negative trend (Lin et al. 2006).

The increase in the metabolic rates of adult seahorses with warming was accompanied by an increase in the ventilation rates, but unexpectedly, no changes were observed in the food intake and activity patterns. In general, food intake increases with temperature until a certain limit is reached, as observed in *H. trimaculatus* juveniles (Sheng et al. 2006). Moreover, activity patterns are also expected to change with temperature. Yet, it is important to note that this seahorse species spent most of the time attached to the holdfast with the prehensile tail, completely still or slightly moving the head (Faleiro et al. 2008). Compared to its sympatric conspecific *H. hippocampus* that prefers more open habitats, *H. guttulatus* has proved to be more sensitive and to prefer dense and vegetated habitats with high holdfast availability (Curtis and Vincent 2005).

In contrast to adult seahorses, *H. guttulatus* juveniles proved to be more sensitive to warming. The abrupt decline in their  $Q_{10}$  to values below 1.5 indicates active metabolic suppression of early stages when exposed to temperatures higher than 28 °C. Such temperatures were already outside the temperature tolerance window of the early stages (beyond the pejus temperature), and therefore, their fitness and survival success will be compromised due to oxygen deficiency (Pörtner et al. 2004, 2005). Several studies have shown that, although a rise in water temperature can be positive and result in faster growth and greater survival of newborn juveniles (Lin et al. 2006, 2008, 2009, 2010), these factors may become compromised after a certain temperature is reached. Growth, condition, and survival of newborn *H. erectus* decreased after 28–29 °C (Lin et al. 2008). Beyond 30 °C, the survival of juvenile *H. kuda* was compromised (Lin et al. 2006), and the feeding rates of

*H. trimaculatus* juveniles decreased (Sheng et al. 2006). 3.5-month-old *H. whitei* juveniles also displayed faster growth but a lower condition factor and hepatosomatic and gonadosomatic indexes at higher temperatures (Wong and Benzie 2003).

From a physiological point of view, these findings may be explained in the light of metabolic suppression. It is a defense mechanism to unsuitable external factors, including temperature among others. In this way, the organism puts some biological processes in standby as a strategy for energy saving, prioritizing the survival of the individual. One example is the protein synthesis, which has a significant energy cost and suffers a significant decrease when the organism needs to save energy for more crucial processes (Boutilier 2001; Guppy and Withers 1999; Rosa and Seibel 2008, 2010). However, metabolic suppression might jeopardize the normal development and increase vulnerability to other stressors like predation, starvation, or diseases (Pörtner and Knust 2007; Wang and Overgaard 2007).

Summing up, with this study, we provide evidence that, while adult seahorses show great resilience to heat stress and are not expected to go through any physiological impairment and behavioral change with the projected near-future warming, the early stages display greater thermal sensitivity and will face greater metabolic challenges with potential cascading consequences for their growth and survival. Even though seahorse life history characteristics such as short generation time, rapid growth, and early maturation may enable them to have a certain level of adaptability and resilience to environmental disturbance (Curtis and Vincent 2006), other characteristics such as small home ranges and low mobility might be harmful in a climate change scenario, since they are generally confined to a small area and have no capacity of long distance migrations (Foster and Vincent 2004; Curtis and Vincent 2006). The greater sensitivity of newborn juveniles to higher temperatures and their great vulnerability to ocean warming indicates that early stages can be the bottleneck for population adaptability in a climate change perspective.

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## Appendix

See Table 4.

**Table 4** Size range, mass-specific oxygen consumption rates ( $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) at different temperatures (18; 26; 28, and 30 °C) as a function of body size in *H. guttulatus*

	18 °C	26 °C	28 °C	30 °C
Mass (g)	0.010–18.000	0.016–18.000	0.013–18.000	0.013–18.000
Rate ( $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ )	1.855–9.203	3.473–14.942	3.418–16.645	3.658–19.317
<i>a</i>	3.597	6.313	7.115	8.125
<i>b</i>	−0.166	−0.185	−0.200	−0.172
<i>N</i>	20	20	20	20
<i>r</i> <sup>2</sup>	0.828	0.957	0.899	0.884

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