UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL

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

Maria Luísa Aurélio

DISSERTAÇÃO

MESTRADO EM ECOLOGIA MARINHA

2012

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Dissertação orientada por:

Prof. Doutor Rui Rosa

Prof. Doutor Luís Narciso

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A felicidade da abelha e do golfinho deve-se, tão só, a existirem.

Para o Homem, é sabê-lo e maravilhar-se com isso

Jacques Yves Cousteau

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INDEX

Resumo.....	5
Abstract.....	10
Introduction.....	11
Material and Methods.....	13
Results.....	17
Discussion.....	23
Acknowledgements.....	25
References.....	26
Annex I.....	30

RESUMO

O presente estudo teve como objectivo investigar, pela primeira vez, os efeitos do aumento da temperatura nas taxas metabólicas e de alimentação e nos padrões comportamentais do cavalo-marinho *Hippocampus guttulatus*. Esta investigação irá contribuir para um melhor entendimento do impacto das alterações climáticas, não só em cavalos-marinhos, mas também noutros organismos marinhos.

O clima está a mudar, e num futuro próximo os ecossistemas acompanharão essa mudança. Desde a época pré-industrial até aos nossos dias, as pressões antropogénicas têm contribuído para o aumento dos níveis de dióxido de carbono (CO_2) na atmosfera e as previsões apontam para que continue a aumentar. Este acumular das emissões pós-industriais na atmosfera está a afectar o balanço térmico do planeta e os níveis de carbonatos nos oceanos. Actualmente o aumento das temperaturas é um assunto recorrente em qualquer debate acerca de impactos dos sistemas marinhos. A temperatura da água do mar está a aumentar a um ritmo sem precedentes. A cada dez anos, desde 1979, tem sido registado um aumento da temperatura média da superfície do mar de $0.13\text{ }^{\circ}\text{C}$, bem como um aumento da temperatura média do oceano superior a $0.1\text{ }^{\circ}\text{C}$ desde 1961. Apesar de o clima ser considerado um sistema imprevisível, as previsões actuais apontam para um aumento global da temperatura de $2\text{ }^{\circ}\text{C}$ até 2100. Estima-se que as ondas de calor, que actualmente têm uma duração de uma a duas semanas serão, até 2100, mais intensas, mais frequentes e mais duradouras.

A temperatura do oceano tem uma forte influência na ecologia dos organismos marinhos, podendo provocar impactos significantes ao nível das populações, comunidades e ecossistemas. Um aumento da temperatura em $1\text{ }^{\circ}\text{C}$ pode causar efeitos consideráveis na mortalidade e distribuição geográfica de alguns organismos, perturbando todo o ecossistema marinho.

A maioria dos peixes são animais ectotérmicos, isto é, a sua temperatura interna varia consoante a temperatura ambiente, o que resulta num aumento das taxas metabólicas numa situação de aumento da temperatura média da água. Um dos principais papéis nas reacções bioquímicas envolvidas nos processos metabólicos é desempenhado pela temperatura, de facto, um aumento de $10\text{ }^{\circ}\text{C}$ pode duplicar ou triplicar as taxas de reacção – o que se reflecte em valores de sensibilidade térmica (Q_{10}) de 2-3. Um aumento nas taxas metabólicas dos peixes promove o desenvolvimento de embriões, reduz o período de gestação e aumenta as taxas de crescimentos. O aumento das taxas

metabólicas com o aumento da temperatura afecta também as taxas de alimentação, a digestão dá-se mais rapidamente, as secreções gástricas aumentam, bem como a absorção directa de nutrientes, o que leva a um esgotamento mais rápido das reservas em caso de indisponibilidade de alimento. Acima de uma determinada temperatura, a temperatura *pejus* (T_p), a necessidade de oxigénio atinge um pico, não sendo suficiente para cobrir as necessidades metabólicas do organismo. Apesar de a sobrevivência não ser directamente afectada além da T_p , a capacidade de desempenhar outros mecanismos fisiológicos torna-se mais reduzida, o que, a longo-prazo, será prejudicial para a sobrevivência das espécies.

Animais como os cavalos-marinhos são particularmente sensíveis a perturbações no seu habitat, dada a sua reduzida mobilidade, o que os torna num interessante modelo para investigações no âmbito das alterações climáticas. Para além disso, os cavalos-marinhos vivem em águas costeiras pouco profundas, sendo que estas áreas fechadas serão provavelmente mais afectadas pelo aquecimento global do que o mar aberto. As espécies de cavalos-marinhos estão ameaçadas um pouco por todo o mundo, deste modo, o impacto da súbita subida das temperaturas só irá aumentar o seu já elevado valor conservacionista. Todas as espécies de cavalos-marinhos encontram-se já listadas na Lista Vermelha de Espécies Ameaçadas do IUCN, bem como no Apêndice II do CITES. Há, actualmente, um interesse crescente em estudos que focam o impacto das alterações climáticas nos organismos marinhos, no entanto, não existem registos de investigações sobre este assunto em cavalos-marinhos, exceptuando na área do desenvolvimento de técnicas de aquacultura. Destes, todos apontam para um impacto da temperatura ao nível da sobrevivência, crescimento, alimentação, comportamento, reprodução, e até coloração dos animais.

No presente estudo, comparámos as taxas metabólicas e a sensibilidade térmica, de adultos e juvenis a quatro temperaturas. Para os adultos, comparámos ainda as taxas de ventilação, as taxas de alimentação e os padrões comportamentais às mesmas quatro temperaturas. As temperaturas foram escolhidas de modo a reflector: a temperatura média durante a Primavera (18 °C), a temperatura média no Verão (26 °C), a temperatura máxima registada em períodos de ondas de calor (28 °C) e a temperatura prevista num cenário de aquecimento global (28 °C + 2 °C, 30 °C) no estuário do Sado, em Portugal.

O cavalo-marinho *H. guttulatus* encontra-se no nosso país maioritariamente em sistemas estuarinos ou lagunares. Estes, sendo peixes estuarinos, adaptam-se facilmente a

variações de temperatura ao longo do dia e do ano, mas terão a mesma capacidade de adaptação num cenário de alterações climáticas?

Como esperado, os nossos resultados revelaram um aumento das taxas metabólicas e de ventilação com o aumento da temperatura, sem, no entanto, se demonstrarem stress térmico, com valores de sensibilidade térmica normais, mesmo para o intervalo de temperaturas mais elevadas (28 °C – 30 °C). Este elevado grau de adaptabilidade a variações de temperatura é uma mais-valia para um animal com fracas capacidades natatórias e reduzida mobilidade. No entanto, a longo prazo, a exposição a temperaturas extremas poderá ter um impacto mais severo na ecologia da espécie.

Surpreendentemente, o aumento da temperatura não provocou o resultado expectável nas taxas de alimentação nem no comportamento dos cavalos-marinhos adultos. Seria esperado que as taxas de alimentação acompanhassem o aumento da temperatura, e que o comportamento sofresse perturbações. Tal poderá ser explicado, mais uma vez, pela capacidade de aclimação a grandes variações de temperatura, e pelo comportamento passivo desta espécie.

Por outro lado, os juvenis *H. guttulatus* revelaram-se mais sensíveis ao aumento da temperatura, com um aumento significativamente superior das taxas metabólicas quando comparado com os adultos. De facto, o declínio abrupto para valores de Q_{10} inferiores a 1.5, indica supressão metabólica quando expostos a temperaturas superiores a 28 °C, o que sugere que estas temperaturas se encontram fora do intervalo de tolerância térmica dos juvenis. Quando um organismo é sujeito a temperaturas fora do seu intervalo de tolerância fica mais vulnerável a factores externos, dando prioridade a processos osmoregulatórios, e delegando para segundo plano outros processos fisiológicos menos cruciais para a sua sobrevivência. No entanto, este estado de dormência do organismo pode ser prejudicial ao normal desenvolvimento dos juvenis e aumentar a sua vulnerabilidade à predação, indisponibilidade de alimento, ou doenças.

Os resultados obtidos neste estudo indicam que, apesar de os cavalos-marinhos adultos revelarem uma alta resiliência face ao stress térmico, os juvenis mostram uma elevada sensibilidade ao aumento das temperaturas. As características dos cavalos-marinhos, como a reduzida mobilidade, tornam-nos mais vulneráveis às perturbações subjacentes às alterações climáticas no seu habitat, uma vez que não têm capacidade para migrações de longas distâncias, permanecendo confinados a áreas restritas. Esta maior vulnerabilidade dos juvenis ao aumento da temperatura sugere que esta fase do ciclo de

vida poderá causar um efeito gargalo nas populações num cenário de aquecimento global.

Palavras-chave: cavalo-marinho; *Hippocampus guttulatus*; aquecimento dos oceanos; alterações climáticas; metabolismo; comportamento

**Physiological and behavioral responses of temperate seahorses
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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 °C, 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 suppression. In adult stages, ventilation rates also increased significantly with environmental warming, but unexpectedly 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 will face greater metabolic challenges with potential cascading consequences for their growth and survival.

Keywords: seahorse; *Hippocampus guttulatus*; ocean warming; climate change; metabolism; behavior

Introduction

The climate is changing and, in the future, the ecosystems may have changed as well. As a result of human activities, carbon dioxide (CO₂) has greatly increased in the atmosphere, and is expected to continue increasing. The cumulative post-industrial CO₂ emissions are affecting the heat balance of the earth and the carbonate equilibrium of the oceans. The increasing levels of oceanic CO₂ and the global tendency for rising temperatures may act like synergistic effects on marine ecosystems, which might be exacerbated when combined with effects like oxygen deficiency (resulting from global warming) and anthropogenic eutrophication (Pörtner et al. 2004a). These combined factors may be dangerous for marine fauna, by reducing animal performance and harming populations.

Presently, ocean warming is an omnipresent matter on every debate on marine system impacts (Halpern et al. 2008; Petit et al. 1999; Solomon et al. 2007). Surface temperature is rising at an unprecedented rate in human experience. 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 greater than 0.1 °C since 1961 (IPCC 2007). The climate systems are unpredictable but forecasts point to a global mean temperature rise of 2 °C by 2100 (Lenton et al. 2008; Santos et al. 2002; Miranda et al. 2002). Heat waves, which presently last for a period of one to two weeks (Miranda 2002), will become more intense, more frequent and last for longer periods in a warmer climate scenario (IPCC 2007).

Water 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). In fact, rising temperatures are already affecting the abundance and distribution of species, and compromising the entire marine ecosystem (Brierley and Kingsford 2009; Perry et al. 2005).

In general, fishes are ectothermic, i.e. their internal temperature varies with the surrounding environmental temperature, which results in increased metabolic rates in a warming scenario (Roessig 2004). Temperature is one of the major factors influencing

the biochemical reactions involved in metabolic processes, so much that, a warming of 10 °C can double or triple the reaction rates, resulting in thermal sensitivity (Q_{10}) values of 2-3 (Seibel and Drazen 2007; Gillooly et al. 2001). An increase in fish metabolic rates increases the growth rates, promotes the development of embryos and reduces the period of incubation (Love 1970). Increased metabolic rates at higher temperatures also affect the feeding rates: digestion becomes a faster process, the gastric secretions increase, as well as the direct absorption of nutrients, which leads to an enhanced depletion in the absence of available food resources in warmer waters (Love 1970). Above a certain temperature, also known as pejus temperature (T_p), oxygen delivery is maximal and cannot further rise to cover elevated metabolic demands, hence, the ability of an organism to increase aerobic metabolism is compromised (Pörtner et al. 2004b, 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 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 warmer to colder 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 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. All seahorse species are listed in the IUCN Red List of Threatened Species and in the Appendix II of CITES. There is growing interest in studies that focus 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 3000 *Hippocampus capensis* were found dead and

trapped in small pockets of water, where water temperature reached 32 °C (Russel 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 increasing water temperature on the metabolic rates, feeding rates and behavior patterns of the temperate seahorse *Hippocampus guttulatus*. Hopefully, 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, which may support the development of new models and strategies, not only for seahorses as for other marine organisms as well.

Material and Methods

Specimen collection and stocking conditions

Seahorses, *H. guttulatus*, were collected near Caldeira de Tróia in the Sado estuary, Portugal (Figure 1), in October 2011. The seahorses were transferred to the aquaculture facilities in the Guia Marine Laboratory and kept in a recirculating system composed of 170 L glass aquaria (140x35x35 cm). Green plastic structures were provided as holdfasts for the seahorses. Water parameters were: temperature 20–22 °C, salinity 34–35 and pH 8.2–8.3. 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⁻¹, 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.

All fishes were wet-weighted and identified with color bead necklaces loosely hold around the neck with an elastic cotton string. During the first four weeks, adult fish were acclimatized to captive conditions and no experimental essays were run. After that, adult seahorses and newborn juveniles, from males captured pregnant and that gave birth in captivity, were acclimatized to four different temperature scenarios: i) the

average spring temperature (18 °C), ii) the average summer temperature (26 °C), iii) the temperature that they endure during summer heat wave events (28 °C), and iv) the projected temperature during heat waves in a near-future warming scenario (+2 °C; 30 °C). At the end of the experiments, tags were removed and all seahorses were released on their original habitat without injuries.

Figure 1. Map of the sampling area in Caldeira de Tróia, located in the mouth of the Sado Estuary, Portugal.

Oxygen consumption rates

Adult seahorses

A total of 15 specimens were used to determine the oxygen consumption rates (routine metabolic rates) of adult seahorses in each of the four temperature scenarios. Animals were placed in an intermittent flow-through respirometry set-up (Loligo Systems,

Denmark; see Rosa and Seibel 2008, 2010). To avoid bacterial contaminations, the seawater was filtered (0.2 μm) and treated (50 mg L^{-1} streptomycin) before it was pumped at a constant flow rate (average 140 mL min^{-1}) 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. During acclimation, the water in the column was bubbled continuously to maintain incoming water at high O_2 values (normoxia, 21% O_2). After acclimation, the flow-through system was turned off and a Clarke-type O_2 electrode was put in each respirometer. Each electrode was connected to a Strathkelvin Instruments 928 Oxygen Interface. The system Strathkelvin was calibrated using air-saturated seawater. 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

A total of 5 specimens were used 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^{-1} streptomycin) to avoid bacterial respiration. For each temperature (18 °C, 26 °C, 28 °C and 30 °C), 5 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 gas-tight 500 μL syringe, and injected in a water-jacketed Clarke-type O_2 electrode connected to a multi-channel oxygen interface (Strathkelvin, North Lanarkshire, Scotland). The duration of respiratory runs varied from 3 to 4:30 h. After the experiments, juveniles were weighed on a precision balance.

Thermal sensitivity

For each developmental stage (adult and juvenile), thermal sensitivity (Q_{10}) 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 at temperatures T_1 and T_2 , respectively. Q_{10} values were calculated for the temperature intervals of 18–26 °C, 18–28 °C and 18–30 °C.

Ventilation rates, food intake and behavioral patterns

Ten 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 (18 °C, 26 °C, 28 °C and 30 °C). Acclimation to each temperature was done through a 2 h gradual increase in water temperature. 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.

Table 1. Ethogram of *Hippocampus 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 towards the aquarium floor in search for food, points the snout towards 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.

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 behavioural patterns of adult seahorses was evaluated using repeated measurements ANOVA. ANOVAs were followed by Tukey 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.

Results

Metabolic rates and thermal sensitivity

Routine metabolic rates (RMR) were significantly affected by temperature and developmental stage (Figure 2; Table 2). Mean RMR increased with temperature from 2.40 ± 0.33 (18 °C) to 5.37 ± 1.21 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ (30 °C) in adult seahorses, and from 7.36 ± 2.22 (18 °C) to 16.68 ± 1.73 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ (30 °C) in newborn juveniles. The Q_{10} values of adult and newborn seahorses ranged mainly around normal values (2.0-2.5), except for juveniles at the highest temperature interval (Figure 3). Between 28 and 30 °C, the pronounced Q_{10} drop below 1.5 indicates metabolic suppression in newborn juveniles.

See Annex I for metabolic scaling relationships.

Figure 2. Impact of environmental warming in the routine metabolic rates (RMR, $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$) of juvenile and adult seahorses, *Hippocampus guttulatus*. Values represent mean $\pm$ S.D (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, *Hippocampus guttulatus*.

	df	MS	F	p
Metabolic rates				
Temperature (T)	3	111.00	83.87	0.000
Stage (S)	1	1245.81	941.34	0.000
TxS	3	32.38	24.47	0.000
Error	72	1.32

Figure 3. Impact of environmental warming in the thermal sensitivity (Q_{10} values) of adult and juvenile seahorses, *Hippocampus guttulatus*.

Ventilation rates, food intake and behavioral patterns

Ventilation rates of adult seahorses were significantly affected by temperature (Figure 4; Table 3). Opercular beats increased with rising temperature from 18 (18 °C) to 59 beats min^{-1} (30 °C). Unexpectedly, food intake (Figure 5; Table 3) and behavioral patterns (Figure 6; Table 3) remained almost unchanged with increasing temperature.

Figure 4. Impact of environmental warming in the ventilation rates (beats min⁻¹) of adult seahorses, *Hippocampus guttulatus*. Values represent mean $\pm$ S.D (n=10). Different letters represent significant differences (p<0.05) between temperatures. For more statistical details see Table 3.

Figure 5. Impact of environmental warming in the food intake (prey hour⁻¹) of adult seahorses. Different letters represent significant differences (p<0.05) between temperatures. For more statistical details see Table 3.

Figure 6. Impact of environmental warming in the behavioral patterns (%) of adult seahorses, *Hippocampus guttulatus*: A) swimming frequency, B) feeding frequency, C) swinging frequency, and D) inactivity frequency. 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, *Hippocampus guttulatus*.

	df	MS	F	p
Ventilation rates				
Temperature	3	3288.50	32.01	0.000
Error	27	102.72		
Food intake				
Temperature	3	37.86	0.95	0.429
Error	27	39.73		
Swimming frequency				
Temperature	3	104.42	0.48	0.698
Error	27	216.96		
Feeding frequency				
Temperature	3	438.79	1.88	0.156
Error	27	233.03		
Swinging frequency				
Temperature	3	649.90	2.93	0.051
Error	27	221.66		
Inactivity				
Temperature	3	703.22	1.80	0.172
Error	27	391.39		

Discussion

Hippocampus guttulatus is generally found in shallow inshore waters, in depths below 12 meters (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. In Portugal, the mean coastal temperature in estuarine waters is 18 °C in spring and 26 °C in summer, although during summer heat waves it can reach 28 °C. This eurythermic animal is therefore able to acclimatize and adapt to temperature oscillations, but will he be prepared for a warmer environment?

Like most fishes, seahorses are ectothermic and their body temperature fluctuates with external temperature. As expected, *H. guttulatus* metabolism increased with water temperature. In adults, the increase of metabolic rates from 2.40 at 18 °C to 5.37 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ at 30 °C resulted in normal values of thermal sensitivity (Q_{10} values around 2), 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 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 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. After 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, as expected, 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. The lack of differences in food intake and behavior between the different temperature scenarios may be explained by the referred ability of the species to adapt to great temperature fluctuations, as they endure in their habitat. Furthermore, as observed

by Faleiro et al. (2008), this species spent most of the time attached to the holdfast with the prehensile tail, completely still or slightly moving the head or body. Compared to his sympatric conspecific *H. hippocampus* that prefers more open habitats, *H. guttulatus* has revealed to be much less active 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 much more sensitive to warming. The metabolic rates of newborn juveniles increased significantly more with temperature than those of adult seahorses. Indeed, the abrupt decline in their thermal sensitivity to values below 1.5 indicates active metabolic suppression of early stages when exposed to temperatures higher than 28 °C. Our data suggest that such temperatures are already outside the temperature tolerance window of the early stages (i.e., beyond the critical temperature) and, therefore, their fitness and survival success will be compromised due to oxygen deficiency (Pörtner et al. 2004b, 2005). Increased metabolic rates cause an increase in oxygen demand, but higher temperatures result in less dissolved oxygen in the water. Both factors combined might thus act as synergistic stressors and be too overwhelming to the cardiorespiratory processes that suffer a decline in the efficiency to sustain muscular activity, growth and, at a critical point, the survival of the fish (Pörtner and Knust 2007).

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 presented 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 at the light of metabolic suppression.

Metabolic suppression is a defense mechanism to unsuitable external factors, including temperature among others. In this way, the organism puts some biological processes in stand-by 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). 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). 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 long-term ocean warming indicates that early stages can be the bottleneck for population adaptability in a climate change perspective.

Seahorses are charismatic fishes that have the potential to become symbols of estuarine area protection. Their unique aspect and curious life strategy turns them into perfect flagship species for marine conservation issues, so, it is important to understand their vulnerabilities and their responses to the near-future scenario of climate change.

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Annex

I

Table I. Size range, mass-specific oxygen consumption rates ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$) at different temperatures (18 °C; 26 °C; 28 °C and 30 °C) as a function of body size in *Hippocampus 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
N	20	20	20	20
r^2	0.828	0.957	0.899	0.884