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Effect of a gradually increasing temperature on the behavioural and physiological response of juvenile *Hippocampus erectus*: Thermal preference, tolerance, energy balance and growth

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ABSTRACT

The physiological and behavioural responses of ectotherms to temperature is strongly dependent on the individuals' previous thermal history. Laboratory based studies investigating the mechanisms of thermoregulation in marine ectotherms, however, rarely consider key temporal elements of thermal exposure, such as the rate at which temperature changes. We tested the hypothesis that juvenile seahorses, *Hippocampus erectus*, from a tropical coastal lagoon in Yucatan, Mexico, would exhibit variations in physiological and behavioural descriptors of thermoregulation when submitted to contrasting regimes during 30 days: temperature constant at 25 °C (C 25); gradually increasing 1 °C every 5 days from 25 to 30 °C (GI 25–30); and constant at 30 °C (C 30). Immediately after exposure, critical maximum temperature, thermal preference, oxygen consumption, partial energy balance, growth rate and survival of seahorses were measured. Seahorses exposed to GI 25–30 showed a significantly higher critical thermal maxima (37.8 ± 0.9 °C), preference (28.7 ± 0.4 °C), growth ($1.10 \pm 0.49\%$) and survival (97.6%) than those exposed to C 30 (36.5 ± 1 , 29.4 ± 0.3 °C, $0.48 \pm 0.32\%$, 73.8%, respectively). Both high temperature regimes induced metabolic depression, but ramping resulted in a greater amount of energy assimilated ($278.9 \pm 175.4 \text{ J g}^{-1} \text{ day}^{-1}$) and higher energy efficiency for growth (89.8%) than constant exposure to 30 °C ($115.4 \pm 63.4 \text{ J g}^{-1} \text{ day}^{-1}$, 65.3%, respectively). Gradually increasing temperature allowed physiological mechanisms of thermal adjustment to take place, reflecting the capacity of juvenile *H. erectus* to respond to environmental change. Despite its advantage, this capacity is limited in time, since the cumulative effect of thermal exposure affected metabolic performance, eventually compromising survival. The study of seahorse response to thermal variations in the context of ocean warming needs to consider the temporal elements of thermal exposure to foresee its vulnerability under future scenarios.

1. Introduction

Temperature partially defines the limits of the distribution of marine ectotherms through the physiological performance of individuals exposed to thermal variations of different spatial and temporal scales (Stillman and Somero, 2000; Somero, 2010; Tepolt and Somero, 2014). From an evolutionary perspective, the selective pressure of temperature has resulted in the development of long-lasting adaptive capacities to live in specific habitats (Crawshaw, 1977;

Pigliucci, 1996). However, there are considerable differences in the degree to which response to temperature occurs and is reversible (Pigliucci et al., 2006), hence the need to define phenotypic plasticity as the limits of resilient changes in phenotype under a fluctuating environment (Bradshaw, 1965). Acclimation has been considered a reversible form of phenotypic plasticity (Beitinger et al., 2000; Madeira et al., 2012) operating at short time scales (Terblanche et al., 2007; Rachalewski et al., 2018), and has been an experimental condition frequently examined in a broad variety of thermal studies (Tepolt and

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Somero, 2014; Padilla-Ramirez et al., 2015; Madeira et al., 2016; Vinagre et al., 2016).

It has been long known that the physiological and behavioural response of ectotherms to temperature is strongly dependent on the individuals' previous thermal history (Frisk et al., 2012; Rezende et al., 2014; Macnaughton et al., 2018). Seasonal and latitudinal variations in temperature determine the thermal tolerance of populations (Levinton and Monahan, 1983; Wehrtmann and Kattner, 1998; Morley et al., 2012; Bennett et al., 2018), and laboratory based experiments have often manipulated acclimation temperatures to examine thermal tolerance in a variety of marine species (Eme and Bennett, 2009; Noyola et al., 2015). Such manipulations have mostly consisted of abrupt changes and constant exposure to temperature followed by measures of critical thermal limits. These measures are based on either the time of survival at each temperature or the proportion of surviving individuals at a certain time (Lutterschmidt and Hutchison, 1997a). Since abrupt thermal shifts are rare under natural conditions, authors have recommended exposing organisms to gradually changing temperatures (ramping), particularly when ecologically relevant thermal limits are being investigated (Terblanche et al., 2007). The magnitude and rate in which temperature changes, as well as the starting point and duration of sub-lethal thermal conditions are important elements when the underlying mechanisms of thermo-regulation are being investigated (Terblanche et al., 2011).

Temperature directly controls the speed of enzymatic reactions involved in the degradation and utilization of ingested energy, thereby modulating all metabolic processes (Roessing et al., 2004). An increment in metabolism can increase growth rate, promote embryogenesis and reduce the incubation period of species (Pimentel et al., 2012; Rosa et al., 2012). However, ambient warming can also produce higher oxygen consumption, hence important changes in energy balance (McElroy et al., 2012; Yoon et al., 2013). Furthermore, temperature and dissolved oxygen can become synergistic stressors producing an overwhelming effect on the individual that endangers its survival (Pörtner, 2010; Aurelio et al., 2013).

According to recent theories (Pörtner and Knust, 2007), the aerobic scope (difference between active and standard oxygen consumption) is related to temperature in a way that each species has adapted to reach a maximum aerobic scope within an *optimum* thermal range. With increasing (or decreasing) temperature, aerobic scope is preserved at the expense of increasing oxygen supply to the mitochondria, and the thermoregulatory capacity will consequently depend on the time of exposure to temperatures that pertain to an interval known as *pejus* (Pörtner, 2010). In a thermal continuum, increasing temperatures in this interval will produce a decrease in growth and reproduction as the vulnerability of the population raises (Magozzi and Calosi, 2015). At extreme temperatures, individuals reach a further thermal limit (*pessimum*) from which anaerobic mechanisms are activated (i.e. aerobic scope ~ 0) to supply energy for cell repair and basic life maintenance (Sokolova et al., 2012). These ideas constitute a conceptual model that has proved very useful to identify and explain the biochemical and molecular mechanisms involved in response to temperature. Nevertheless, empirical studies need to incorporate the relationship between time and its close interaction with thermal exposure explicitly in order to describe and predict the limits of thermal tolerance realistically.

As in other coastal species (Madeira et al., 2016; Pratchett et al., 2017), many aspects of the population dynamics of fish from the genus *Hippocampus* are strongly controlled by water temperature (Teixeira and Musick, 2001; Foster and Vincent, 2004). Despite the recent prominence of studies on thermal tolerance, few in the last years have focused on the effect of temperature on physiological and behavioural traits of seahorses (e. g. Planas et al., 2012; Aurelio et al., 2013) and gene expression of HSP and reproduction (e. g. Johnson, 2017; Qin et al., 2018). Most, however, are confined to the aquaculture context (e.g. Wong and Benzie, 2003; Lin et al., 2008; Lin et al., 2010). A few reports on syngnathids from temperate regions (e.g. *Syngnathus acus*,

Russell, 1994; *S. scovelli*, Power and Attrill, 2003; *H. guttulatus*, Lin et al., 2006) have considered a possible thermal threshold $\sim 30^\circ\text{C}$ after which fish growth, reproduction and escape behaviour (camouflage) decline and the individuals' survival may be at risk. Aurelio et al. (2013) suggested that early juvenile *H. guttulatus* have higher thermal sensitivity than adults and could display greater metabolic adjustments under extreme thermal stress. The lack of information on syngnathid populations from tropical regions, by contrast, is noteworthy.

Hippocampus erectus Perry 1810 is distributed along the West coast of America from the South of Nova Scotia, Canada, to the North coast of Brazil (Fritzsche and Vincent, 2002; Lourie, 2004). In the northern coast of Yucatan, *H. erectus* is exposed to marked temporal variations in temperature (CONANP, 2006). According to field data collected at Chelem coastal lagoon (Jiménez, 2012), adult seahorses (particularly pregnant males) occur throughout the year but are more abundant from December through March, when mean temperature varies from 18 to 26 °C. Adults are more abundant at sites with moderate coverage of submerged vegetation (i.e. seagrass *Halodule* and *Syringodium*, *Dasycladus* spp. and red algae). Juvenile seahorses (< 95 cm total height) are more abundant from April to July, when mean temperature varies from 25 to 30 °C, and are associated with dense submerged vegetation commonly found at the shallow margins of the lagoon.

Ongoing studies on the thermal biology of juvenile *H. erectus* from Chelem Lagoon (Mexico) have shown this tropical population tolerates wide thermal variations and has a great capacity to recover from acute thermal exposures (Del Vecchio et al., unpublished data). Results have shown juvenile *H. erectus* have a critical thermal maximum ranging from 33.1 ± 1.4 to $36.2 \pm 1.2^\circ\text{C}$, and a minimum ranging from 7.0 ± 0.7 to $11.9 \pm 2.1^\circ\text{C}$, depending on whether previous acclimation temperature was 18 and 30 °C, respectively (Mascaró et al., 2015). The maximum lethal limits were established at 35.4 ± 0.5 and $38.7 \pm 0.5^\circ\text{C}$ for the respective acclimation temperatures, but no deaths were observed when temperature decreased to $< 4^\circ\text{C}$ at a rate of 1°C per minute (Amaral, 2013). In addition, thermal preference in juvenile seahorses was $25.7 \pm 0.9^\circ\text{C}$ whether seahorses had been previously acclimated to either temperature (Huipe-Zamora, 2015). Most outstanding was that seahorses kept at 30 °C over long time periods (> 30 days) showed a significantly lower growth rate than those kept at 18 °C, suggesting that 30 °C is outside the *optimum* (*sensu* Pörtner, 2010), and its detrimental effect will depend markedly on time of exposure.

The present study aimed to compare the cumulative effect of a high and sustained exposure, to a gradually increasing temperature on the thermal tolerance and preference of juvenile *H. erectus* from a tropical coastal lagoon. Our intention was to relate the thermoregulatory response to measures of seahorse growth, survival and partial energy balance, including respiration, energy ingested, produced and assimilated (for definitions see Lucas and Watson, 2014). Experiments were set to demonstrate that abrupt thermal change, combined with constant exposure to temperatures near *pejus*, might result in high energy demanding thermoregulatory mechanisms. A persistent exposure to a sub-lethal temperature should modify thermal tolerance and preference, and reflect in the aerobic metabolism by reducing energy supply for growth. By contrast, a gradual increase to a similar final temperature could allow seahorses to compensate for the energy cost associated with thermoregulation.

2. Materials and methods

2.1. Origin and maintenance of seahorses

This study followed the Guide for the Care and Use of Experimental Animals in Research and Teaching of the Faculty of Superior Studies-Cuautitlán (<http://www.cuautitlan.unam.mx/>) at Universidad Nacional Autónoma de México. All efforts were made to minimize stress in experimental subjects and meet standard levels of animal welfare (see below for conditions). Taking into account that the effect of high

temperature was the objective of this study, overall seahorse mortality was relatively low (12.9%) and changes in feeding and mobility were the only visual signs of stress detected (i.e. seahorses did not show changes in colour or disease).

Juvenile *H. erectus* were obtained from two pregnant males captured at Chelem Lagoon (21°17'N and 89°40'W) on the northern coast of Yucatan, Mexico, under permit No. SGPA/DGVS/12741/13. Males were individually placed in glass aquaria (50 cm width × 28 cm depth × 70 cm height; approx. 90 L), kept at 25 °C until the offspring were born and were later liberated in the same location where they had been captured. New born *H. erectus* were then evenly distributed in acrylic aquaria that increased in capacity from 20 L (0–20 days since birth) to 90 L (20–90 days since birth) to keep seahorse density at ~1.25 individuals L⁻¹. Aquaria were connected to a seawater recirculation system equipped with a mechanical (nylon monofilament bag) and a biological filter (with sand, coral fragments, and artificial live rock fragments), and a protein skimmer. A plastic structure was introduced in each aquarium to serve as holdfast for juvenile, and no other substrate was used.

Seahorses from 0 to 20 days were fed *Artemia salina* nauplii (2–3 mL l⁻¹) previously enriched with DHA-protein-Selco®. Juveniles from 21 to 90 days were fed a mixture of 75% similarly enriched metanauplii (*A. salina*) and 25% frozen amphipods (*Elasmopus pectenicrus*). Food rations (20% of seahorse wet weight day⁻¹) were offered at 10:00, 14:00 and 18:00 h, and food remains and faecal matter were removed daily. During maintenance, seahorses were kept under a photoperiod of 12 h dark - 1 h dim light - 10 h light - 1 h dim light, constant salinity (33 ± 1) and temperature (25 ± 0.5 °C) to assure all experimental subjects had a similar thermal history. Every third day, 25% of the water in the system was replaced with filtered seawater to keep ammonia content at minimum and adjust salinity levels. Seahorses were approximately 3 months old and had a mean wet weight of 0.92 ± 0.05 g (n = 126) at the beginning of the experiment. Both male and female juveniles were indistinctively used in the present study to avoid excessive manipulation and errors in sex identification.

2.2. Experimental design

Eighty *H. erectus* were randomly assigned to each of three groups and exposed to different thermal regimes during a 30-day experimental period (Fig. 1): constant water temperature of 25 °C (C 25); gradual increments of 1 °C every 5 days from 25 to 30 °C (GI 25–30); and

constant water temperature at 30 °C (C 30). Seahorses in C 30 required a preparation period to raise water temperature at a rate of 1 °C day⁻¹ for 4 days from 25 to 29 °C. During this time, seahorses in treatments C 25 and GI 25–30 were maintained at 25 °C. The fifth day of the preparation period was also the first day of the experimental period, adding up to a total of 34 days (Fig. 1). Water temperature was increased by means of two 300 W submersible heaters (BioPro H-100) and a chiller (Resun® CL-600) directly connected to the water reservoir in the recirculation system.

Thermal regimes were chosen to contrast a sustained exposure to a high but sublethal temperature of 30 °C (Mascaró et al., 2015) to one where temperature increases slowly from 25 °C and does not exceed 30 °C. Exposure for no more than 30 days was established to assure the survival of individuals and descriptors to be measured. A constant regime at 25 °C was included as a control, since this was the temperature preferred by juvenile *H. erectus* and is considered optimum for its maintenance under controlled conditions (Huipé-Zamora, 2015). It should be noted that both the magnitude and rate of increase in the temperatures used in this study are well within those experienced by juvenile seahorses at Chelem (Mariño-Tapia and Enríquez, 2010; Table S1).

Both throughout the preparation and experimental period (Fig. 1), seahorses were kept in 90 L glass aquaria (27 aquaria, with 8–9 individuals per aquarium) and were fed and maintained as described previously. All seahorses were individually identified using combinations of three coloured plastic beads held around the neck by a thin nylon cord. Immediately after the last day of the experimental period, growth rate and survival, thermal preference, critical thermal maximum, oxygen consumption, and components of partial energy balance of seahorses in all three treatments were evaluated.

2.3. Growth and survival

The wet weight of 42 seahorses in each thermal regime was individually registered before and after the 30-day experimental period with a digital balance (OHAUS Scout II SC200; g ± 0.005). The daily growth coefficient (DGC % day⁻¹) of each individual was calculated as:

$$DGC \% d^{-1} = [(\ln W_f - \ln W_i)/t]*100$$

where W_f and W_i are seahorse final and initial wet weight, and t is the number of days. Seahorse survival was assessed three times a day during the experimental period, and any dead individuals removed and

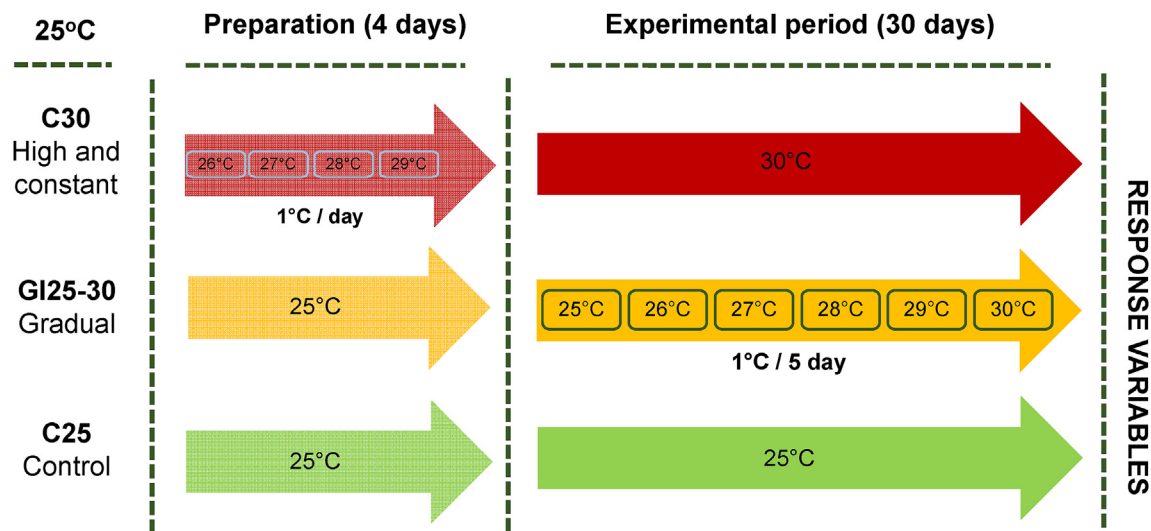


Fig. 1. Experimental design used to evaluate the effect of exposing juvenile *H. erectus* to three thermal regimes (C 25: constant water temperature at 25 °C; GI 25–30: gradual increments of 1 °C every 5 days from 25 to 30 °C; and C 30: constant water temperature at 30 °C) on several response variables measured at the end of the experimental period: growth rate and survival, thermal preference, critical maximum temperature, oxygen consumption, and components of partial energy balance.

excluded from further analysis.

2.4. Thermal preference

Thermal preference was determined using the acute method (Reynolds and Casterlin, 1979). A horizontal gradient made of a PVC tube (400 cm long and 20 cm wide) was divided into 20 virtual segments (14 cm long) and filled with filtered seawater (15 cm deep; Díaz et al., 2006). A temperature gradient from $20 \pm 1.5^\circ\text{C}$ to $35 \pm 1.2^\circ\text{C}$ was formed by connecting a 1200 W heater (MOELLER®) and a chiller (PolyScience® FT25) on opposite sides of the tube, and water temperature at each segment was measured using a digital thermometer (Cooper® DPP400W). A diffuser hose was placed along the tube to keep dissolved oxygen at $\sim 6.5\text{ mg O}_2\text{ L}^{-1}$ ($> 90\%$ saturation) throughout the gradient and avoided stratification in the water column. Plastic holdfasts similar to those used previously were placed in the centre of each segment along the bottom of the tube, and light was kept homogeneous, thereby offering similar conditions in all segments throughout the gradient.

Seahorses were individually placed in the gradient at the virtual segment corresponding to the temperature to which they were last exposed in each thermal regime (Fig. S1). The location and temperature in the segment selected by each individual after 30 min was recorded. Preliminary trials recording the location selected by juvenile *H. erectus* in a similar thermal gradient (Huipé-Zamora, 2015) showed that seahorses stayed within the selected locations after the first 30 min and until at least 1 h. Thus, a single preferred temperature was obtained for each individual seahorse, adding to a total of $n = 20$ independent replicas in each temperature regime.

In order to rule out the possibility that the locations selected by *H. erectus* were the result of preferences other than temperature, another 10 seahorses from each treatment were introduced in the tube under constant temperature and water flow conditions (either 25 or 30°C , depending on the thermal regime; Fig. S1). The location of seahorses after 30 min was registered and compared correspondingly. Additionally, heater and chiller were randomly assigned to each side of the gradient to avoid interference with room disturbance, and seahorses were fasted for 24 h previous to trials to avoid any bias associated with feeding behaviour.

2.5. Critical thermal maxima

In order to quantify the effect of temperature regimes on the maximum thermal tolerance of juvenile *H. erectus*, seahorses were exposed to a rapid increase of water temperature following procedures and definitions described by Lutterschmidt and Hutchison (1997b). Forty-eight seahorses ($n = 16$ independent replicas from each treatment) were individually placed in a cylindrical glass chamber (350 mL) with a plastic holdfast and a digital thermometer (Cooper DPP 400 W; $\pm 1^\circ\text{C}$). Chambers were kept in a thermally controlled bath (25 L glass aquarium) set at 25 or 30°C (depending on the thermal regime) and equipped with a submersible pump (260 L h^{-1}) and an air diffuser to avoid water stratification. Water temperature in the bath was rapidly increased to impede acclimation using a 1000 W heater ($\sim 1^\circ\text{C minute}^{-1}$; Lutterschmidt and Hutchison, 1997b), and observations of seahorse behaviour and temperature were recorded every minute. The criteria for critical thermal maxima (CTM) estimation was the loss of equilibrium and pronounced shaking or spasms (Mascaró et al., 2015). Three control trials were conducted where another 24 seahorses ($n = 8$ from each treatment) were placed in chambers in a similar manner but water was maintained constant at the temperature to which they were last exposed in each thermal regime (Fig. 1). Observations of seahorses from each treatment and its respective control were compared to assure that changes in behaviour were strictly due to thermal exposure. Survival was monitored for 24 h after trials and seahorses were returned to their respective aquaria.

2.6. Food ingestion and oxygen consumption

Trials to measure food intake and oxygen consumption in juvenile *H. erectus* were carried out simultaneously using a continuous flow respirometer that consisted of 9 respirometry chambers (250 mL) connected to a well aerated re-circulating system. Twenty-four seahorses ($n = 8$ independent replicas from each treatment) were individually placed in chambers that were filled with filtered seawater at a constant flow of $\sim 0.1\text{ L min}^{-1}$. Chambers were, in turn, placed in a thermo-regulated water bath set at 25 or 30°C (depending on the thermal regime). One chamber was left without a seahorse and used as a control during measurements. Dissolved oxygen was recorded both at water entrance and exit in each chamber using oxygen sensors attached to flow-cells that were connected by optical fibre to an Oxy 10 mini-amplifier (PreSens®, Germany). Sensors were calibrated at either 25 or 30°C with saturated seawater (33 salinity; 100% dissolved oxygen) and at a 5% anhydrous sodium sulphite solution (0% dissolved oxygen). The wet weight of each individual was registered immediately after trials (OHAUS Adventurer AR-2140) and dry weight was estimated considering 70% of water content (Lin et al., 2009). Oxygen consumption ($\text{VO}_2\text{ mg g}^{-1}\text{ h}^{-1}$) was calculated as the difference in dissolved oxygen concentrations between water entrance and exit and was corrected using time and water flow measurements.

Seahorses were introduced in respirometry chambers at 7:00 am and starved during 24 h prior to trials. This was done to prevent any interference with previous feeding and assure they were habituated to chambers. Routine metabolism (R_{rout}) was obtained from VO_2 measures taken every minute from 7:00 to 10:00 am the following morning. Seahorses were then fed enriched *Artemia* and amphipods (75 and 25%, respectively) in a single ration of 20% their wet weight. All seahorses were given 6 h to feed, after which uneaten food was removed, dried and weighed to determine intake. Ingestion (I) was calculated as the difference between the food offered and that which remained in each chamber. A value of 20291 J g^{-1} of dry weight of food obtained previously with a calorimeter was used to transform ingestion to energy units. Overall seahorses spent $\sim 36\text{ h}$ in respirometry chambers.

The apparent heat increment (R_{AHI} or specific dynamic action; Beamish and Trippel, 1990) was estimated from the difference between R_{rout} and the maximum value of VO_2 attained after feeding (R_{max}), considering the time needed to reach this value (TRP). Oxygen consumption measurements during the feeding phase were taken every minute until oxygen consumption returned to pre-feeding values. A similar amount of food was introduced in the chamber without a seahorse to control respiration by algae and bacteria associated with food. The conversion factor of 13.6 J mg^{-1} was used to transform fasting and postprandial VO_2 to J g^{-1} (Lucas and Watson, 2014). The R_{AHI} value was calculated taking into consideration that seahorses were fed three times a day during the extent of the experiment. The metabolic rate R_{AHI} ($\text{J g}^{-1}\text{ dry weight day}^{-1}$) was thus calculated as:

$$R_{\text{AHI}} = [(R_{\text{max}} - R_{\text{rout}}) * 3\text{TRP}]$$

2.7. Partial energy balance

Energy budget was estimated using the following equations (Lucas and Watson, 2014):

$$\text{Ass} = R_{\text{tot}} + P_{\text{g}}$$

where Ass is assimilated energy, R_{tot} indicates respiration ($R_{\text{tot}} = R_{\text{rout}} + R_{\text{AHI}}$), and P_{g} is the energy invested in growth or produced. This was obtained using the actual growth rate of seahorses during the experimental time (30 days). A value of 14938 J g^{-1} of seahorse dry weight obtained previously with a calorimeter was used to transform the growth data into production units (Huipé-Zamora, 2015). All components of energy balance were expressed as $\text{J g}^{-1}\text{ dry weight day}^{-1}$. Assimilated, respiratory and production gross efficiencies were

calculated as $Ass/I \times 100$, $R_{tot}/I \times 100$ and $P_g/I \times 100$, respectively. Respiratory and production net efficiencies were calculated as $R_{tot}/Ass \times 100$ and $P_g/Ass \times 100$, respectively.

2.8. Phenotypic plasticity

The phenotypic plasticity of juvenile *H. erectus* regarding thermal tolerance was estimated as the difference between the mean critical thermal maximum of seahorses under the C 30 and C 25 (high and constant temperature exposure) and C 25–30 and C 25 (gradually increasing temperature exposure) following definitions and calculations in Magozzi and Calosi (2015).

2.9. Statistical analyses

Differences in thermal preference, critical thermal maxima and growth of seahorses between the three temperature regimes were assessed using General Linear Mixed Models with aquaria as a random nested factor (Zuur et al., 2009). When the random effect was not significant (Likelihood ratio test), the term was dropped from the model and a one-way ANOVA, followed by post hoc comparisons of means was applied. Analysis of the residuals was used to validate the statistical models (Zuur et al., 2007). Kaplan-Meier analysis and a log-rank non-parametric test was applied to survival data to compare daily changes in the probability of survival of seahorses from the different thermal regimes. Because this test cannot be applied to nested data, a log-rank test was used to discard significant variations in the probability of survival amongst aquaria separately. All calculations were done using packages “nlme” and “survival” in R (R Core Team, 2013).

Differences in energy balance of seahorses under the three thermal regimes was examined using Principal Coordinate Analysis (PCoA) applied on a dissimilarity matrix of Euclidian distances between different samples (Legendre and Legendre, 1998). The variables considered were production (P_g), total respiration (R_{tot}) and ingestion (I). Prior to ordination, the data was transformed (square root) and normalized by centring and dividing between the standard deviation of each transformed variable (Legendre and Legendre, 1998). A permutational multiple ANOVA was applied on the dissimilarity matrix to detect statistical differences between thermal regimes using 9999 permutations of residuals under the reduced model (Anderson, 2001; McArdle and Anderson, 2001). The underlying design had aquaria as a random factor ($b = 3$ levels) nested within thermal regime as a fixed factor ($a = 3$ levels). Multivariate analysis and graphs were performed using PRIMER-e v 6.1.14 PERMANOVA + v 1.0.1 ©.

3. Results

3.1. Growth and survival

The daily growth coefficient of juvenile *H. erectus* was significantly affected by temperature regime ($F = 19.9$; $p < 0.001$). Seahorses under gradually increasing temperatures (GI 25–30) had the highest mean value (Fig. 2), which was statistically similar to that of seahorses kept at 25 °C constant (C 25; $t = 0.48$; $p = 0.63$). Juvenile *H. erectus* exposed constantly to 30 °C (C 30) showed significantly lower growth ($t = -5.36$; $p < 0.001$; Fig. 2). Growth in juveniles from different aquaria nested within each thermal regime could not be statistically distinguished ($L. ratio = 3.85 \cdot e^{-8}$; $p = 0.99$).

Similarly, survival of seahorses under treatments C 25 and GI 25–30 had high and similar overall survival (95.3 and 97%, respectively; Table 1), and the probability of survival decreased slowly and steadily after day 10 (Fig. 3). By contrast, overall survival of seahorses under treatment C 30 was markedly lower (74%), and the probability of survival decreased sharply after day 15. Results of the log-rank non-parametric test showed significant differences between Kaplan-Meier curves indicating that the probability of survival decreased at a

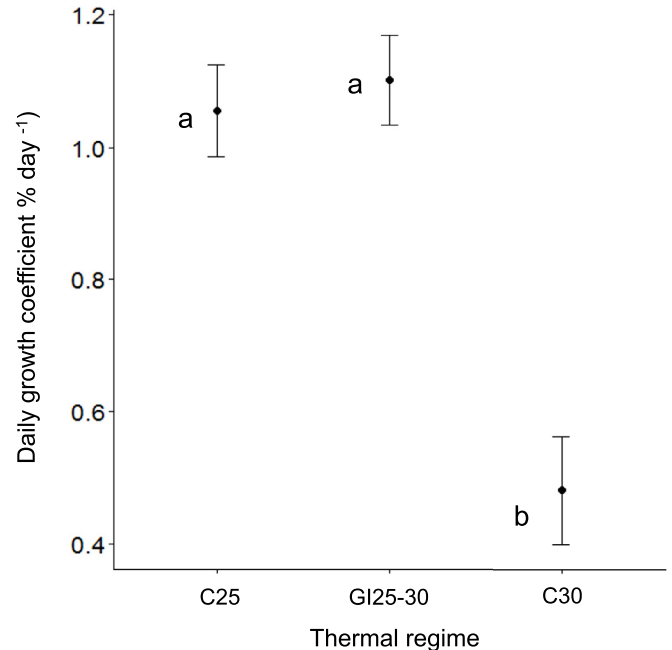


Fig. 2. Daily growth coefficient (DGC % day⁻¹) of juvenile *H. erectus* exposed during 30 days to three different thermal regimes: C 25: constant water temperature at 25 °C; GI 25–30: gradual increments of 1 °C every 5 days from 25 to 30 °C; and C 30: constant water temperature at 30 °C. Values are mean \pm residual standard error; different letters represent values that differed statistically ($p < 0.05$).

Table 1

Daily growth coefficient, survival, thermal preference and critical maximum temperature measured in juveniles *H. erectus* under three different thermal regimes: constant water temperature at 25 °C (C 25); gradual increments of 1 °C every 5 days from 25 to 30 °C (GI 25–30); and constant water temperature at 30 °C (C 30). Values are mean \pm standard deviation, with n replicates. Different letters indicate statistical differences at $p < 0.05$.

	Thermal regime (°C)		
	C 25	GI 25-30	C 30
Initial weight (g)	0.89 \pm 0.17	0.93 \pm 0.3	0.91 \pm 0.25
n	42	42	42
Final weight (g)	1.15 \pm 0.23	1.3 \pm 0.45	1.06 \pm 0.33
n	40	41	31
Daily growth coefficient (%)	1.05 \pm 0.41 _a	1.10 \pm 0.49 _a	0.48 \pm 0.32 _b
n	38	40	27
Survival (%)	95.3	97.6	73.8
Preferred temperature (°C)	27.8 \pm 0.3 _a	28.7 \pm 0.4 _a	29.4 \pm 0.3 _b
n	20	19	20
Critical thermal maxima (°C)	31.9 \pm 1.4 _a	37.8 \pm 0.9 _b	36.5 \pm 1 _c
n	16	14	10

different rate depending on thermal regime ($\chi^2 = 12.9$; $p < 0.01$; Fig. 3). No differences in the probability of survival of seahorses from different aquaria could be demonstrated ($\chi^2 = 16.4$; $p = 0.3$).

3.2. Thermal preference

Exposure to the three different thermal regimes also modified the thermal preference of seahorses ($F = 4.51$; $p < 0.05$). Individuals exposed constantly to C 30 preferred segments with significantly higher temperatures than those both under GI 25–30 and C 25 ($t = 1.64$; $p < 0.001$; Fig. 4), the latter being statistically similar ($t = 0.93$; $p = 0.10$). Thermal preference did not vary significantly amongst seahorses from different aquaria ($L. ratio = 1.6$; $p = 0.21$). In control trials, where temperature was maintained constant, seahorses showed no

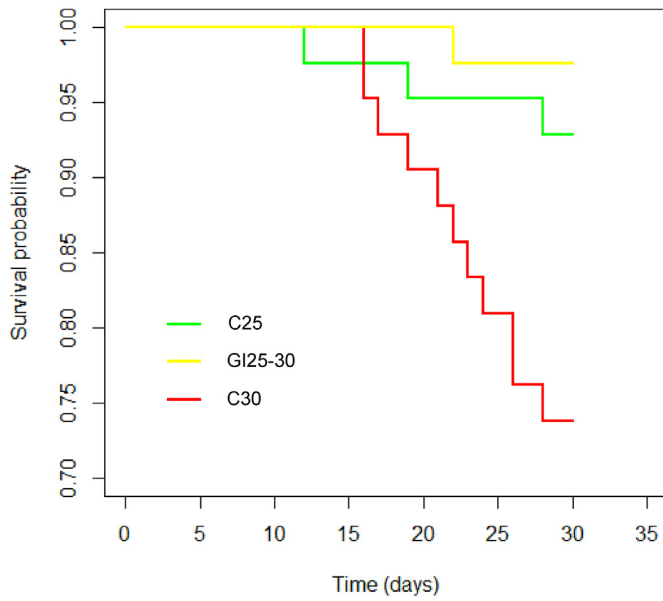


Fig. 3. Kaplan-Meier curves showing the probability of survival of juvenile seahorses *H. erectus* exposed during 30 days to three different thermal regimes: C 25: constant water temperature at 25 °C; GI 25–30: gradual increments of 1 °C every 5 days from 25 to 30 °C; and C 30: constant water temperature at 30 °C. Note the scale in the y-axis has been modified for visualization purposes.

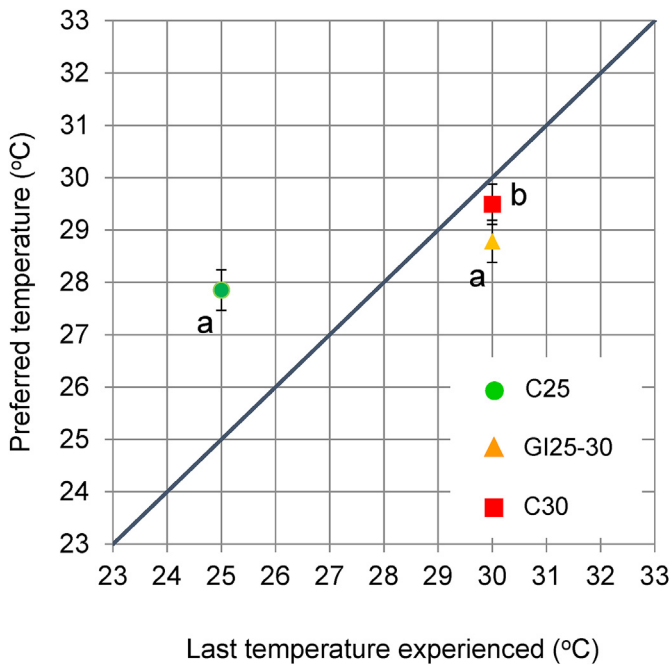


Fig. 4. Relation between preferred temperature (mean \pm residual standard error) and temperature at which juvenile *H. erectus* were last exposed under three different thermal regimes: C 25: constant water temperature at 25 °C; GI 25–30: gradual increments of 1 °C every 5 days from 25 to 30 °C; and C 30: constant water temperature at 30 °C. The straight line represents the isotherm, i.e. when the temperature of preference and exposure are the same. Different letters represent values that differed statistically ($p < 0.05$).

preference for any particular segment in the horizontal tube, thereby confirming that selection of segments under a choice situation was temperature-driven.

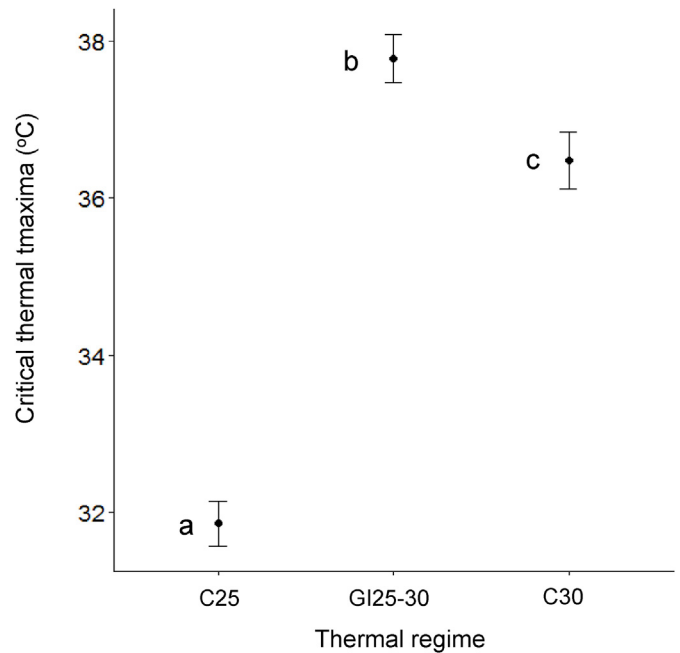


Fig. 5. Critical thermal maxima (°C) of juvenile *H. erectus* exposed to three different thermal regimes: C 25: constant water temperature at 25 °C; GI 25–30: gradual increments of 1 °C every 5 days from 25 to 30 °C; and C 30: constant water temperature at 30 °C. Values are mean \pm residual standard error; different letters represent values that differed statistically ($p < 0.05$).

3.3. Critical thermal maxima

The thermal tolerance of juvenile *H. erectus* was significantly determined by previous exposure to temperature ($F = 105.81$; $p < 0.001$; Fig. 5). Seahorses exposed to GI 25–30 showed a significantly higher CTM than seahorses at both C 25 ($t = 14.12$; $p < 0.001$) and C 30 ($t = 2.72$; $p < 0.01$; Fig. 5). In addition, the CTM of seahorses in C 30 was significantly higher than in C 25 ($t = -10.02$; $p < 0.001$; Fig. 5). No statistical differences in the CTM of seahorses from different aquaria nested within each thermal regime could be detected ($L. ratio = 1.6 \cdot e^{-8}$; $p = 0.99$). Observation of seahorses in control trials where individuals were manipulated in the same manner, but temperature was kept constant, revealed no abnormal behaviour. Only two animals from C 30 and one from GI 25–30 died within the 24 h surveillance period after CTM determinations were conducted (6.3% of the total).

3.4. Food ingestion and oxygen consumption

Exposure to high temperature decreased the amount of food ingested by juvenile *H. erectus*, both in absolute energy (Table 2) and relative to seahorse wet weight. Seahorses kept at C 25 ingested $15.67 \pm 3.32\%$ of their total wet weight (1.09 ± 0.15 g; mean \pm standard deviation), whereas those exposed to GI 25–30 and C 30 ingested 10.76 ± 3.48 and $10.18 \pm 1.44\%$ of their total wet weight (1.15 ± 0.18 g and 1.14 ± 0.2 g, respectively).

Oxygen consumption by seahorses was strongly affected by temperature regime. Routine oxygen consumption in treatment C 25 was markedly higher than in both C 30 and GI 25–30 (Table 2). Oxygen consumption during apparent heat increment (R_{AHI}), however, was relatively similar between treatments. Total respiration was, thus, highest in seahorses exposed to C 25, followed by those exposed to C 30, and lowest in seahorses exposed to GI 25–30.

Table 2

Partial energy balance components (J g^{-1} dry weight day^{-1}) measured in juveniles *H. erectus* under three different thermal regimes: constant water temperature at 25 °C (C 25); gradual increments of 1 °C every 5 days from 25 to 30 °C (GI 25–30); and constant water temperature at 30 °C (C 30). Values are means \pm standard deviation. I: energy ingested with food; P_g : energy invested in growth; Ass: energy assimilated ($R_{\text{tot}} + P_g$); R_{rout} : routine respiration; R_{AHI} : apparent heat increment; R_{tot} : total respiration ($R_{\text{rout}} + R_{\text{AHI}}$); Ass/I, R_{tot} /I and P_g /I: assimilated, respiratory and production gross efficiency; P_g /Ass and R_{tot} /Ass: production and respiratory net efficiency.

	Thermal regime (°C)		
	C 25	GI 25-30	C 30
	n = 4	n = 6	n = 6
Ingestion (I)	6221.3 \pm 1796.1	4268.1 \pm 657.5	4505.8 \pm 1673.4
Production (P_g)	226.7 \pm 69	250.4 \pm 176.8	75.3 \pm 56
Assimilation (Ass)	299.1 \pm 76.5	278.9 \pm 175.4	115.4 \pm 63.4
Respiration (R)			
R_{rout}	65.8 \pm 21.1	21.9 \pm 18.3	34.8 \pm 41
R_{AHI}	6.6 \pm 7.7	6.6 \pm 2.8	5.3 \pm 1.5
R total	72.4 \pm 27.3	28.5 \pm 17.6	40.1 \pm 4.2
Percentages			
Ass/I (%)	4.8	6.5	2.6
R_{tot} /I (%)	1.2	0.7	0.9
P_g /I (%)	3.6	5.9	1.7
P_g /Ass (%)	75.8	89.8	65.3
R_{tot} /Ass (%)	24.2	10.2	34.7

3.5. Partial energy balance

Despite ingesting relatively less food, seahorses exposed to GI 25–30 showed higher assimilated gross efficiency than those exposed to both C 25 and C 30 temperature, the latter being markedly low (Table 2). In addition, seahorses in the GI 25–30 showed the lowest respiratory and production gross efficiencies (Table 2). Whilst the respiratory gross efficiency of seahorses was similar in both C 25 and C 30 treatments, the latter were much less efficient in gross production (Table 2). As a result, seahorses under a gradually increasing temperature used 89.8% of energy assimilated for production and only 10.2% for respiration, whereas seahorses kept under optimal thermal conditions used 75.8% of assimilated energy for production and 24.2% for respiration. By contrast, seahorses kept under a high and constant temperature used only 65.5% of assimilated energy for production and 35.5% for respiration (Table 2).

The PCoA on the components of partial energy balance indicated that 83% of the total variation of data was explained by the first two coordinates (Fig. 6). Samples representing each individual seahorse were ordered following all three energy balance components. Samples in C 25 were associated to a high ingestion and respiration, and an intermediate production. Samples in GI 25–30 were associated to a low ingestion and respiration, but a relatively higher production. Samples in C 30 had relatively low values in ingestion and respiration, but the lowest production values (Fig. 6). Results of the permutational multiple ANOVA showed significant differences between samples of all three treatments ($pseudo-F = 3.23$; $p < 0.05$; with 9418 unique permutations), indicating that the observed differences in the energy balance components of seahorses can be attributed to their exposure to different thermal regimes. Here again, the random effect of aquaria nested within each thermal regime was not statistically significant ($pseudo-F = 1.85$; $p = 0.09$; with 9950 unique permutations).

3.6. Phenotypic plasticity

The phenotypic plasticity regarding thermal tolerance in seahorses exposed to C 30 was calculated as 4.6 °C, whereas in seahorses under GI 25–30 this value was 5.9 °C.

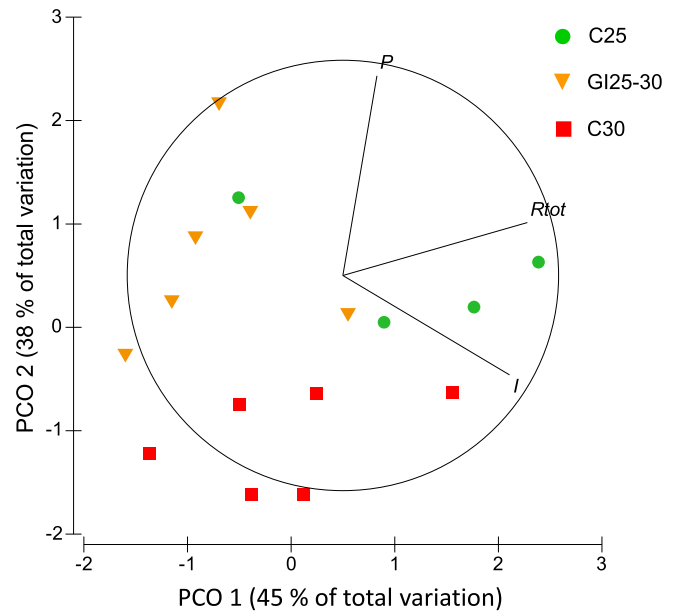


Fig. 6. Principal Coordinate Analysis ordination on components of partial energy balance measured in juvenile *H. erectus* exposed to three different thermal regimes: C 25: constant water temperature of 25 °C; GI 25–30: gradual increments at 1 °C every 5 days from 25 to 30 °C; and C 30: constant water temperature at 30 °C. Components were food ingestion (I), total respiration (R_{tot}) and Production (P) and were all expressed as J g^{-1} dry weight day^{-1} . Data were transformed (square root) and normalized by centring and dividing between the standard deviation (Legendre and Legendre, 1998) previous to analysis.

4. Discussion

Results in the present study demonstrate that exposure to different temperatures over time has a marked cumulative effect on the thermal tolerance, preference and survival of juvenile *H. erectus*, and modifies the proportion of assimilated energy that is allocated to growth and other energy-demanding processes. Overall, the physiological and behavioural indicators measured in seahorses exposed to a gradually increasing temperature significantly differed from those measured in animals exposed to a high and constant thermal regime. The latter can be considered a long-term exposure with a long-lasting effect, since it was enough to reduce the probability of survival and growth among juvenile *H. erectus*. The gradually increasing thermal regime, by contrast, had a short-term effect on seahorse physiology and behaviour, extending the limits of thermal tolerance and preference, and maintaining survival and growth rates similar to those of seahorses under optimum thermal conditions, at least temporarily.

Previous authors have emphasised that gradual changes in temperature allow the time necessary for the adaptive physiological mechanisms to thermal stress to be expressed (Terblanche et al., 2007; Overgaard et al., 2011). Examples of these mechanisms are increased cardiac and ventilatory performance (Frederich and Pörtner, 2000; Sartoris et al., 2003; Farrell et al., 2009); an increased oxygen carrying capacity of blood proteins (Giomi and Pörtner, 2013); and the activation of anti-oxidative enzymes (Freire et al., 2011; Rodríguez-Fuentes et al., 2017). Such expression depends on the rate of thermal change (Chown et al., 2009; Mitchell and Hoffmann, 2010) and cannot be observed in animals that are statically exposed to extreme temperatures (Terblanche et al., 2011). Results herein are evidence that a rate of temperature change of 1 °C every 5 days triggered such mechanisms thereby displaying the phenotypic plasticity of *H. erectus* to thermal changes. This plasticity allowed seahorses to increase their thermal tolerance and preference, and modify their metabolic performance,

reflecting the capacity of *H. erectus* to respond to these environmental changes.

The thermal history of an individual has been pointed out as one of the main factors influencing critical thermal limits in marine ectotherms (Lutterschmidt and Hutchison, 1997a; Dallas and Rivers-Moore, 2012). Both the temperature and duration of previous acclimation are known to modify the physiological and behavioural response to further thermal exposure (Vinagre et al., 2016; Sánchez-García et al., 2017). Results in the present study were no exception, given that the CTM of seahorses previously maintained at a constant 25 °C was significantly lower than of those exposed to higher temperatures. Moreover, the differences observed amongst seahorses in the C 30 and GI 25–30 regimes constitute evidence that the rate in which temperature increases determines the thermal tolerance and preference in this species. Juvenile *H. erectus* subject to gradual increments of 1 °C were capable of an immediate and progressive response that occurred during the 5-day interval following each step. Such a time lapse probably enabled the kinetic stimulation of biochemical reactions by moderate warming (Madeira et al., 2016; Sánchez-García et al., 2017). This may have triggered compensatory physiological and biochemical mechanisms that were activated only after certain temperature-time exposures, thereby leading seahorses through the transition between a fully functional acclimation capacity to a condition of acclimation in protection (see Pörtner, 2010; Sokolova et al., 2012; Tepolt and Somero, 2014; Farrell, 2016).

A widening of the thermal polygon as a result of experimental exposure to a varying thermal regime has been found in other marine ectotherms but appears to be a species-specific feature (Díaz et al. 2006, 2007, 2013, 2015; Noyola et al., 2015; Flynn and Todgham, 2018). Garcia-Rueda (2017) found that acclimation to fluctuating temperatures increased the range of thermal tolerance in *Callinectes sapidus* from the southern Gulf of Mexico but not in *Octopus maya* from the Yucatan Peninsula. The author explained this could be related to the ubiquitous nature of the eurythermal crab species, in contrast with the tropical octopus endemic to Yucatan. The extensive latitudinal distribution of *H. erectus* along the estuaries of the West coast of the American Atlantic (Fritzsche and Vincent, 2002; Lourie, 2004) has been used to explain the relatively large thermal polygon (171–302 °C²) and high phenotypic plasticity (4.7 °C) estimated for the population in Chelem (Mascaró et al., 2015). Based on their findings the authors believed that populations of *H. erectus* in the southern Gulf of Mexico could be close to the upper thermal limit of the species, at which phenotypic plasticity reaches a threshold. In the present study, the phenotypic plasticity calculated for seahorses maintained under a constant thermal regime was 4.6 °C but rose to 5.9 °C when temperature was increased gradually, suggesting that not only the magnitude but the rate of thermal increase plays an important role in the adaptive response to temperature.

Differences in the experimental thermal regimes also had a pronounced effect on the metabolic performance and use of metabolic energy in juvenile *H. erectus*. The low ingestion rates amongst seahorses in C 30 and GI 25–30 indicated that exposure to temperatures above the optimal may limit the consumption of enough food to satisfy energetic demands. Appetite loss has been shown to be an immediate response to thermal (Noyola et al., 2013) and other conditions of environmental stress (Vernberg and Piyatiratitivorakul, 1998; Hulathduwa et al., 2007), and food avoidance constitutes a behavioural mechanism displayed by animals in order to save metabolic energy that would otherwise be used for the highly demanding postprandial processes. However, that seahorses exposed to temperatures above 25 °C ingested less food did not entirely explain the differences in growth rate observed throughout this study, and these were probably the result of events taking place after food ingestion.

According to recent thermal theory, oxygen supply at the cell is maximal under optimal thermal conditions but will become limited as individuals are exposed to temperatures beyond a critical threshold or

pejus (Pörtner et al., 2004). As temperature increases, the physiological and biochemical mechanisms that provide the mitochondria with oxygen to complete oxidative phosphorylation become insufficient, and the final phase of aerobic metabolism is impaired (Pörtner et al., 2004). In the present study, routine oxygen consumption was high in seahorses exposed to 25 °C, reflecting an optimum condition; but decreased markedly in individuals subject to higher temperatures. These results suggest that both a gradually increasing and a high and constant thermal exposure correspond to conditions beyond *pejus* for juvenile *H. erectus*, since both induced metabolic depression. However, the amount of oxygen consumed by seahorses during apparent heat increment was similar in all three thermal regimes, suggesting that the observed reduction in the respiratory metabolism affected functions other than those strictly directed to food processing and absorption.

Many fish and invertebrates that inhabit fluctuating intertidal areas resort to metabolic depression and anaerobiosis as a means to tackle extreme thermal and other stressing events (i.e. desiccation, anoxia, extreme salinity and starvation; Guppy and Withers, 1999; Magozzi and Calosi, 2015). To contend with thermal stress, energy metabolism is re-organized (Sommer and Pörtner, 1999; Sokolova and Pörtner, 2003) and changes take place in the mitochondria and cell membrane in order to re-establish the equilibrium through the regulatory functions of antioxidant enzymes (Hochachka and Somero, 2002). Results herein add to several reports on the degree in which metabolic adjustments result from thermal stress in marine ectotherms from tropical regions (Sokolova et al., 2011; Padilla-Ramirez et al., 2015) and underline the high and complex dependence of metabolism on temperature (Johnston and Dunn, 1987).

Despite the reduced total respiration of seahorses both in C 30 and GI 25–30, the distinct effect of a gradual thermal exposure was best seen in an increased production of biomass to levels similar to those observed in seahorses under optimal thermal conditions. Not only did seahorses under ramping increase the total amount of energy assimilated but they were more efficient in the use of that energy for growth, as shown by the relative measures of assimilation and production of seahorses in those treatments. The progressively increasing temperature might have provided enough time for seahorses exposed to moderate warmth to respond by giving precedence to biomass production, particularly at the beginning of the ramp. As time went by, and temperature reached ~30 °C, part of the assimilated energy may have been gradually re-directed to maintenance activities. Whilst the temperature-time combination in which this shift in energy direction occurred is still unknown, ramping resulted in seahorses with a low routine metabolism, but growth rates as high as those under optimal conditions. Thus, a future characterization of the physiological and biochemical stages through which seahorses transit from an optimal to moderate and severe stress conditions should take into account a temperature-time element intrinsically involved in thermal exposure.

Compared to optimal thermal conditions, the critical thermal maxima increased in seahorses exposed to constant 30 °C (Figs. 1–3), a behaviour previously reported in juvenile *H. erectus* (Mascaró et al., 2015). These results indicate that compensatory mechanisms were also activated among seahorses under constant thermal exposure, but these were clearly unsuccessful in the extent of the functional response they produced: not only did they fail in keeping the growth coefficient above 1% day⁻¹ but were ineffective in maintaining survival of seahorses at levels above 80% after 30 days. In other studies, juvenile *H. erectus* kept at such temperature for periods longer than 30 days have also eventually died (Huipe-Zamora, 2015). These results clearly set 30 °C as a thermal threshold in which seahorses may shift from a sublethal to a lethal condition depending on time of exposure.

Sokolova et al. (2012) explored the links between metabolism, energy balance and stress tolerance in aquatic invertebrates and proposed that energy-related biomarkers could be used to determine the conditions when metabolic transitions from moderate stress to extreme stress occur. Considering the results found in the present study, we have

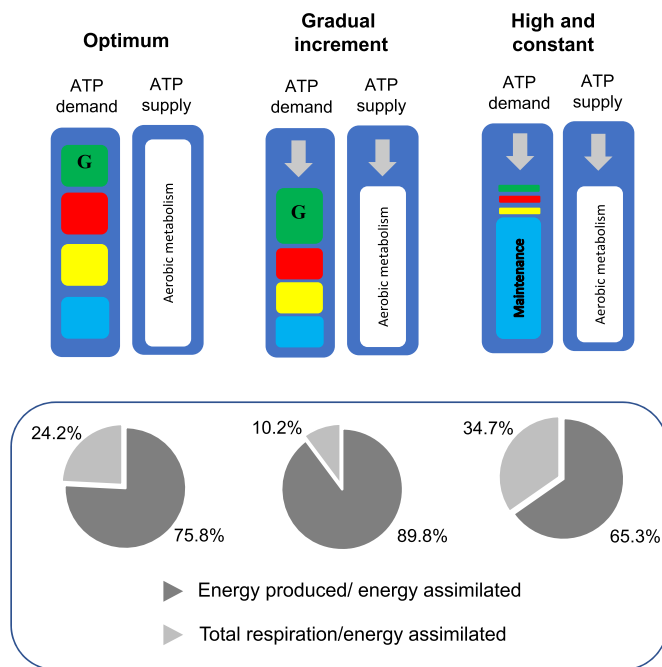


Fig. 7. Diagram adapted from Sokolova et al. (2012) showing an integrated interpretation of the proportion of assimilated energy directed to production and respiration as a response of juvenile *H. erectus* that have been exposed to optimum thermal conditions (constant at 25 °C); gradual increments of 1 °C every 5 days from 25 to 30 °C; and high and constant water temperature (30 °C).

modified the diagrammed summary presented by these authors to include the rate of thermal increase and time of exposure as important elements of the response to temperature in juvenile *H. erectus* (Fig. 7). When exposed constantly to 25 °C, seahorses used 76% and 24% of assimilated energy for production and respiration, showing that ATP supply via aerobic metabolism is sufficiently high to cover the costs of growth (G), maintenance and other ATP-demanding activities (coloured boxes). When exposed to a gradually increasing temperature, seahorses ingested less energy and decreased the aerobic metabolism in absolute numbers, but maintained high energy assimilation and directed 89% of it to production. The result was an increase in the daily growth coefficient. By contrast, when exposed constantly to a high temperature, seahorses reduced the aerobic metabolism and only 65% of assimilated energy was used for production, most of which was for maintenance. Because the response to high temperature is energetically demanding (Sokolova et al., 2012; Magozzi and Calosi, 2015), more energy was directed towards respiration and maintenance in those seahorses subject to the highest thermal stress (Fig. 7).

Overall, results herein provide evidence that species can passively exploit certain conditions within their range of thermal tolerance to increase their physiological performance (i.e. assimilation efficiency), and that a gradually increasing temperature can be considered one of these conditions. The advantages produced by such conditions, however, will be limited in time, since the cumulative effect of thermal exposure will progressively impact the metabolic performance (Pörtner and Knust, 2007). Whilst survival of the individual beyond a *pejus* temperature is not immediately endangered, the ability to carry out vital activities such as feeding and hiding from predators can be restricted and will be so increasingly with time (Pörtner et al., 2004).

Our results are in accordance with the idea that species tolerant to extreme thermal events can be at a greater risk when exposed chronically to sub lethal temperatures (Magozzi and Calosi, 2015; Vinagre et al., 2016), and suggest that phenotypic plasticity is reduced under conditions of sustained thermal stress. By contrast, a gradually increasing temperature enabled a further expansion of seahorse plasticity

in response to warming, a feature that could represent a selective advantage amongst species from thermally heterogeneous habitats. Such an advantageous response, however, appears to be limited to a short period of time before the effect of exposure becomes chronic. Evidence in this direction has been previously reported both in juvenile *H. erectus* (Lin et al., 2009) and the tropical species from the Indo-Pacific, *Hippocampus kuda* (Lin et al., 2006) in studies conducted to improve culture protocols. These showed that moderately high temperature can promote growth and survival, increase fecundity and gonadosomatic index and reduce the duration of gonad development in seahorses kept at temperature as high as 29 °C, but these benefits become compromised once thermal exposure reaches ca. 30 °C (Lin et al., 2008; Aurelio et al., 2013).

Results herein also contribute to understanding the response of *H. erectus* to temperature in an ecological context. Gradual thermal changes like the ones used in the ramp in the present study can be expected to occur in the coastal lagoons of the southern Gulf of Mexico and Yucatan peninsula, where *H. erectus* is distributed. In Chelem Lagoon, mean water temperature can rise from 18 to 30 °C from February to July and August, a period of approximately 6–7 months (Jiménez, 2012). At the peak of the dry season (April–May), mean temperature at the centre of the lagoon can fluctuate between 22 and 30 °C (Mariño-Tapia and Enríquez, 2010), whereas shallow areas can register up to 36–38 °C (CONANP, 2006). Juvenile *H. erectus* are abundant in shallow waters, where the relatively dense submerged vegetation produces moderate hypoxia conditions during the dark hours of the day. Under such temporally heterogeneous environment, seahorses can use their physiological plasticity to survive and may momentarily take advantage of favourable combinations of temperature and hypoxia that stimulate biomass production. The effect of temperature on seahorse growth and survival under other ecologically relevant magnitudes and rates of thermal increment and hypoxia is something that remains to be investigated.

Seahorses are distributed throughout tropical and temperate coastal regions both in the Atlantic and Indo-Pacific oceans. Whilst the evolutionary history of the genus *Hippocampus* is not yet fully understood, the global distribution of seahorses reflects a major dispersion event (Teske et al., 2004) taking place in shallow coastal waters characterized by marked thermal fluctuations. Under this scenario, seahorses probably developed physiological adaptations to compensate for thermal changes occurring at different temporal and spatial scales. Such environmental conditions could have promoted the dispersion of the group in its evolutionary history and help to explain the amplitude of the thermal tolerance found in tropical populations of *H. erectus* today.

Author contributions

JLH conducted the experiments and contributed to data analysis and the writing of the manuscript. CR contributed to designing and conducting the experiments, the interpretation of results and the revision of the manuscript. FD and KP contributed to the interpretation of results and the revision of the manuscript. NS contributed to the writing and revision of the manuscript. MM designed the experiments, analysed results, produced tables and figures, and wrote the manuscript.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2019.102406>.

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