diversity

MDPI

Article

Temperature Effects on Growth Performance, Fecundity and Survival of *Hippocampus guttulatus*

Jorge Palma [1,*], Miguel Correia [2,3], Francisco Leitão [1] and José Pedro Andrade [1]

[1] Centre of Marine Sciences (CCMAR), University of the Algarve, 8005-139 Faro, Portugal; fleitao@ualg.pt (F.L.); jandrade@ualg.pt (J.P.A.)

[2] Seahorse, Pipefish, and Seadragon Specialist Group, IUCN Species Survival Commission, 1196 Gland, Switzerland; m.correia@oceans.ubc.ca

[3] Project Seahorse, Institute for the Oceans and Fisheries, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada

* Correspondence: jpalma@ualg.pt

Abstract: This experiment aimed to determine the temperature limits beyond which seahorse growth and reproduction become suboptimal due to climate change. Four temperatures (16, 20, 24, and 28 °C) were tested to evaluate their effect on juvenile (1–56 days post-parturition (DPP)) and adult (one year old) long-snout seahorses, *Hippocampus guttulatus*. Additionally, the reproductive performance of adults was observed. Another experiment measured oxygen consumption (MO_2) in the same age groups and temperatures. Adults showed significantly higher growth rates at 20 and 24 °C compared to 16 and 28 °C. Adult mortality rates were 0%, 0%, 6.2%, and 62.5% at the respective temperatures. Juvenile growth performance was higher at 20 °C and 24 °C but significantly lower at 16 °C and null at 28 °C, with survival rates of 8%, 62%, 10%, and 0%, respectively. Oxygen consumption increased with temperature, ranging from 106.3 ± 3.1 to 203.3 ± 3.1 μmol O_2/g BW/h at 16 °C, and from 127.6 ± 3.5 to 273.3 ± 3.1 μmol O_2/g BW/h at 28 °C for adults and 1 DPP juveniles, respectively. The study highlights that juvenile and adult *H. guttulatus* have narrow thermal boundaries, beyond which reproduction, growth, and survival are seriously affected. Under climate change, the species appears unable to cope, potentially leading to their rapid disappearance.

Keywords: seahorses; *Hippocampus guttulatus*; temperature; growth; reproduction; oxygen consumption; climate change

check for updates

Citation: Palma, J.; Correia, M.; Leitão, F.; Andrade, J.P. Temperature Effects on Growth Performance, Fecundity and Survival of *Hippocampus guttulatus*. *Diversity* **2024**, *16*, 719. https://doi.org/10.3390/d16120719

Academic Editor: Elena Kupriyanova

Received: 5 August 2024
Revised: 6 November 2024
Accepted: 19 November 2024
Published: 25 November 2024

1. Introduction

Seahorses, like other small marine fish species, are an integral part of marine biodiversity and ecosystem function [1]. They are recognized as flagship and bioindicator species [2,3], making them ideal models for assessing risks from marine contaminants and environmental stressors [4–7]. Seahorses are considered as such due to their unique life history, characterized by sparse distribution, low mobility, site fidelity, small home ranges, low fecundity, mate fidelity, and lengthy parental care for small broods [1,8–10]. These traits may render them vulnerable to overfishing and environmental disruptions, including habitat damage and degradation [1]. Additionally, seahorses inhabit shallow, coastal areas worldwide, where anthropogenic disturbances tend to be most frequent and severe [3]. These constraints help explain why 2 seahorse species are listed as “Endangered”, 12 as “Vulnerable”, and 1 as “Near Threatened” on the 2024 IUCN Red List of Threatened Species, with only 10 listed as “Least Concern” and 17 still as “Data Deficient”. This reflects substantial gaps in knowledge, even for a heavily exploited fish group like seahorses.

The two European seahorse species, *H. guttulatus*, and *H. hippocampus*, which inhabit the Ria Formosa (southern Portugal), are also exposed to similar environmental stressors. Populations of the two seahorse species experienced a significant decline between 2001 and 2009, with a 94% reduction in *H. guttulatus* and a 73% reduction in *H. hippocampus*, due to unknown causes [4]. These authors did not link the population decline to any of the tested environmental factors. Instead, they suggested it could be related to differences in water quality across the lagoon, shifts in dominant seagrass species, or natural population fluctuations. Between 2012 and 2013, a slight population increase was observed [5], indicating that *H. guttulatus* populations fluctuated and were positively correlated with habitat loss. Previously, ref. [11] already identified human activities (e.g., fishing, illegal fishing, anchoring, and dredging) and natural changes in the lagoon's dynamics (e.g., silting and shifting currents) as the main causes of habitat loss. Later, in 2018, ref. [12] reported another significant decline in *H. guttulatus* populations, again linked to the same human activities identified by [11]. This decline continued until 2021, bringing population levels back to those observed by [4] in the earlier study (Palma, personal observation). The causes of population decline were directly related to anthropogenic disturbances but do not fully explain it, and the effect of climate change should be regarded as an additional stressor. Combined, these drivers of change have a major impact on these seahorse populations, which, due to their sensitive life history, may become powerless to cope with these impacts. Therefore, well-designed conservation plans are urgently needed to preserve the biodiversity of this ecosystem.

Sensitivity to regional climatic conditions and anthropogenic disturbances interact to strongly affect habitat loss and fragmentation [6], threatening biodiversity, decreasing dispersal rates, and increasing population mortality of marine species [7]. Climate change negatively impacts inshore marine habitats and their fauna, including seahorses, through changes in temperature, ultraviolet radiation (UVR), rainfall patterns, community composition, the status of coastal habitats, and storm activity [8]. These changes are likely to be more severe in enclosed waters, where local warming occurs [9]. According to NOAA Daily Sea Surface Temperature Analysis data, over the past 30 years, there has been a significant increase in coastal sea surface temperatures (°C/decade) throughout most of the Iberian Peninsula and the North African Atlantic coast (avg = 0.214 °C). Despite the unpredictability of climate systems, forecasts indicate a global mean temperature rise of 2 °C by 2100 [10]. Heat waves, which currently last for 1–2 weeks along the Portuguese coast [13], are expected to become more intense, more frequent, and of longer duration in warmer climate scenarios [14].

The Ria Formosa is a mesotidal, shallow coastal lagoon prone to extreme daily and seasonal temperature variations [15] and UVR exposure [16]. These conditions are expected to become more frequent and severe under the current climate change scenario. Previous data [17] showed a direct relationship between seawater temperature and seahorse habitat use. In some surveyed locations, seahorse abundance decreased during high temperature peaks (25 °C), whereas in locations with lower temperatures (20 °C), the abundance remained more stable. It was also observed that in locations with equal habitat complexity, seahorse abundance was always lower at higher temperatures. This population shift coincided with the breeding season. Since temperature can influence gonad development and the survival of larvae, post-settlement juveniles, and adults in many species [4], seahorses may be more susceptible to temperature fluctuations and unable to cope due to their life history and specific habitat requirements. Overall, temperature plays a critical role in shaping the distribution, life cycle traits, and biological activities of ectothermic animals, including fish. Elevated temperatures resulting from global warming disrupt homeostasis in fish, leading to metabolic imbalances and increased vulnerability to stress and disease [18]. While some fish species demonstrate resilience to a wide range of temperatures [19], others experience temperature fluctuations in their habitats but are more susceptible to stress, which negatively impacts their health [20]. Temperature has significant effects on the performance and biogeography of ectothermic animals, largely due to its impact on metabolism [21,22]. As temperature increases, there is an exponential rise in cellular respiration rates, which in turn elevates whole-animal oxygen consumption rates (i.e., the standard metabolic rate) to meet aerobic energy demands [23]. Measuring aerobic metabolic rate (often equated to oxygen consumption rate, MO_2) can help address hypotheses like the metabolic theory of ecology [24,25], which suggests that metabolic rate is a fundamental biological factor governing many ecological patterns. This approach is particularly relevant to fish biology and climate change research, as it helps to explore the hypothesis that aerobic scope is linked to whole-animal performance and fitness [26–28]. The theoretical foundation of this hypothesis suggests that the biochemical and physiological capacities of aquatic ectotherms have evolved to maximize aerobic scope within a certain temperature range to optimize performance related to fitness (e.g., growth, reproduction, and locomotion). As aerobic scope decreases at temperatures outside this range, performance declines [29]. The hypothesis implies that critical functions such as growth, locomotion, and reproduction are causally linked to aerobic scope, meaning that animals should experience optimal fitness when living within their optimal thermal range [29]. The rate of respiration, commonly measured by oxygen consumption, is often used to indicate the metabolic rate in aquatic organisms. Fish oxygen consumption can be categorized based on their physiological condition [30]. Basal metabolism represents the lowest energy expenditure required to sustain life [30,31]. However, due to spontaneous activity in fish, measuring basal metabolism is challenging, so the term "resting metabolism" is preferred over the minimum oxygen consumption rate [30,31]. Metabolic scope refers to the range within which an animal's aerobic metabolic rate can vary [32]. The metabolic scope related to feeding is defined as the difference between the maximum oxygen consumption observed during feeding and the oxygen consumption at a fasted state [33,34]. Thus, analyzing the oxygen consumption of a fish after feeding, when metabolic levels are higher, will provide more contextualized information about the maximum oxygen consumption limit, which is crucial in situations of rising temperatures and lower oxygen saturation in the water.

Metabolism generally scales allometrically with body size, where larger individuals have higher absolute metabolic rates but lower mass-specific metabolic rates [35]. In ectotherms, metabolic rate is primarily governed by temperature, the 'master variable', due to its control over enzyme kinetics [36]. However, it is also influenced by endogenous factors, such as body size [37] and physiological characteristics like thermal history [38]. The present study aims to improve our knowledge on this issue by gathering information to define the temperature effects on the growth, reproduction, and survival of *H. guttulatus*, as well as the fish metabolic response to different temperature scenarios.

2. Materials and Methods

2.1. Adult Growth and Reproduction

Before the trial began, *H. guttulatus* broodstock was kept under the same husbandry conditions, except for the temperature, which was allowed to follow natural wild conditions. The trial lasted for seven months, starting two months prior (March) to the expected start of the breeding season and ending in September. At the beginning of the experiment, 64 adult F4 generation *H. guttulatus* (32 males, 32 females) were selected and stocked in each of 4 units of 250 L white plastic flat-bottom rectangular tanks (one tank per treatment) at 16 animals (8 males, 8 females) per tank. Nautical rope (1 cm in diameter) resembling natural *Codium* spp. algae was added to the tanks to serve as holdfasts for the seahorses to attach to. The tanks were assembled in two semi-closed systems, with a constant water flow ($\approx$50 L h^{-1}) and moderate aeration. The temperature was controlled as follows: one tank system was connected to a chiller, and through different water inflows to the tanks, the water temperature was controlled at 16 ± 1.2 °C and 20 ± 1.5 °C in each of the two tanks. In the second tank system, water was heated and controlled at 24 ± 1.2 °C and 28 ± 1 °C in each of the two tanks. Salinity was not controlled and followed the natural seasonal pattern. Tanks were illuminated from above with 2 × 36 W fluorescent tubes, with an intensity of 600 ± 25 lux at the water surface, and a photoperiod controlled by a timer. The photoperiod was adjusted every two weeks to match the natural photoperiod. Water quality parameters (ammonia, nitrates, and nitrites) were recorded twice a week and kept stable throughout the experiment; ammonia values were always below detectable levels, nitrate < 0.3 mg L^{-1}, and nitrite < 1.25 mg L^{-1}.

During the experiment, seahorses were fed ad libitum once daily at 10:00 a.m. with the same dietary treatment, composed of two live mysid species, *Mesopodopsis slabberi* and *Diamysis lagunaris*, in slightly different proportions depending on the season. As the prey were supplied live, seahorses could feed on demand, which justified the single daily feeding. Each day, uneaten food and feces were removed from the tanks by siphoning every morning before feeding. All adult fish were sampled for length and weight at the start and end of the trial. During the experiment, seahorses were allowed to mate and reproduce freely. When broods occurred, juvenile seahorses from each brood were gently collected from the broodstock tanks with a beaker and counted.

2.2. Juvenile Growth

Once broods began to occur and juveniles were born, 180 juveniles from the same brood were collected from the 20 °C broodstock tank and randomly distributed into the rearing tanks. The rearing tanks were initially maintained at 20 °C, and only after the juveniles had been transferred was the temperature gradually increased or decreased to the target experimental temperatures over a 24 h period. The juvenile growth trial was conducted according to a completely randomized design, with three replicate tanks assigned per temperature. Fifteen juvenile *H. guttulatus* were stocked in each of three replicate 10 L glass rectangular tanks at a density of 1.5 fish per liter. In each tank, the lateral and back walls were covered with black adhesive to improve prey detection [39]. The front wall was left uncovered for any necessary observation. Husbandry conditions and the experimental design used in this experiment were the same as described by [40], except for temperature control. Rearing tanks were assembled in semi-closed systems to allow temperature control for each set of three replicate tanks (Figure 1).

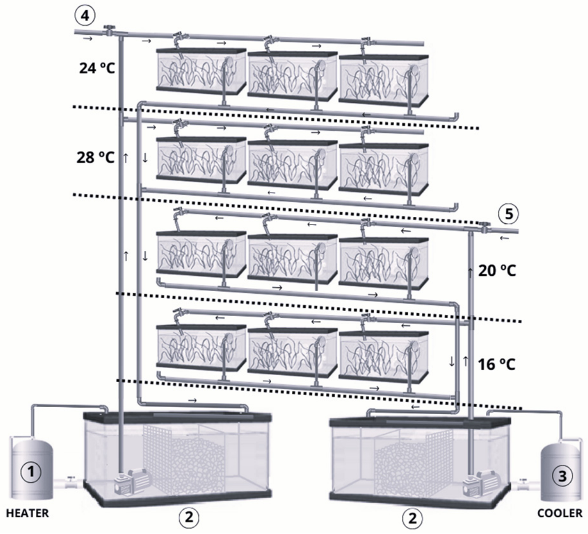


Figure 1. Rearing system: 1: heater, 2: water recycling units, 3: cooler, 4, 5: inflow water for temperature control. The arrows indicate the direction of water flow.

Four different temperatures (the same tested for adult seahorses) were tested: 16 ± 0.8 °C, 20 ± 0.8 °C, 24 ± 1 °C, and 28 ± 0.9 °C. Salinity and dissolved oxygen were kept, respectively, at 37.2 ± 0.2‰ and 7.3 ± 0.2 mg/L. Tanks were illuminated from above with two 36 W fluorescent tubes, with a light intensity of 900 ± 40 lux at the water surface and a photoperiod controlled by a timer at 16L:8D (06:00–22:00 h). Seawater quality data (ammonia, nitrates, and nitrites) were recorded biweekly. Values remained stable throughout the experiment; ammonia values were always below detectable levels, nitrate < 0.3 mg/L, and nitrite < 1.25 mg/L.

The juvenile grow-out trial lasted for 56 days, from 0 to 56 days post-parturition (DPP), during which juvenile seahorses were fed ad libitum with live copepods (*Oithona nana*). Juveniles were sampled every 14 days until the end of the experiment (56 DPP). To reduce stress during sampling, seahorses were measured using a modified approach: rather than the three measurements suggested by [41] (head, trunk, and tail lengths combined), the total length was calculated by summing the head length (from the tip of the snout to the midpoint of the cleithral ring) and the height of the fish (from the cleithral ring to the tip of the fully extended tail). The data were used to calculate the following: (1) weight gain (WG, mg d^{-1}) = (Wf − Wi)/d, where Wf is the final seahorse wet weight (mg), Wi is the initial wet weight (mg), and d is the number of days; (2) growth rate expressed as thermal-unit growth coefficient (TGC) per fish in each tank, TGC = $[(Wf^{(1/3)} - Wi^{(1/3)})/\Sigma(T \times d)] \times 100$, where Wf, Wi, and d, are as described above, and T is the water temperature; and (3) condition factor (CF) = (wet weight (g)/length $(cm)^3$) × 100.

2.3. Respirometry—Experimental Procedures

Seahorse oxygen consumption was determined at three different life stages: juveniles with 1, 14, and 28 DPP, and adults (12 months old). For the 14 and 28 DPP juveniles, a second batch was raised at 20 °C in two replicate tanks, as the initial group experienced complete mortality at 28 °C. At 14 DPP, in one tank, the water temperature was gradually increased to 28 °C over a 48 h period, followed by respirometry analysis. In the second tank, the same process was repeated, with the analysis conducted at 28 DPP. Oxygen consumption was measured by flow-through respirometry following an adapted protocol defined by [42]. In brief, before transferring to the respiratory chambers, fish were fed ad libitum; mealtime started at 10:30 h, and fish were fed for 45 min (group $n = 12$). Measurements started immediately after transfer (11:00 h) and continued for 22 h, with 8 h in the light and 14 h in the dark. To keep the stress level of the fish at a minimum, they were not disturbed during the respirometry measurements. The wet mass of each individual was determined immediately after removal from the chamber. The flow-through respirometry system consisted of six individual metabolic chambers (2.3 L capacity). Each fish was individually placed in a metabolic chamber. Since housing in respirometry chambers may induce stress responses [43,44], measurements taken during the first 3 h of the light period were discarded. The water inlet was always at an oxygen saturation level, while the oxygen concentration at the outlet was measured by a polarographic microelectrode model 8–730 (Microelectrodes Inc., Bedford, MA, USA). Measurements were controlled by a PC using the Oxilogger 2009 software.

The oxygen saturation level in the water reservoir was maintained by aeration. Air bubbling was achieved using a 10 cm rectangular air stone diffuser, while a small pump (Eheim® compact+) ensured thorough mixing of the water. A peristaltic pump (ISMATEC, model ISM920A, Switzerland) controlled the water flow in the chambers using Tygon® tubing (480-µm inner diameter). Each respirometry run consisted of sequential measurements from all the chambers. The magnetic valves controlled by the Oxilogger 2009 software determined in which chamber the oxygen consumption was being measured. At the start of each cycle, the oxygen dissolved in water was measured for 30 s to calibrate the software, followed by a 120 s washing step of seawater from the next chamber before starting the next measurement period (30 s). This step was always performed before and after the measurement in each chamber. Six oxygen consumption measurements were registered over a 30 s period (5 s each). A complete measurement cycle was performed in 18 min, comprising the oxygen probe calibration, a washing step, dissolved oxygen measurement in the water chamber, another washing step, and so forth in each of the six chambers in sequence. The water temperature in the outlet water of each chamber was measured with a temperature probe, and oxygen calibration was performed each time a measurement run started.

2.4. Statistical Analysis

After confirming normality and homoscedasticity, variance analyses were performed. Differences in juvenile seahorse length, wet weight, CF, TGC, and FCR were tested using nested ANOVA with post hoc Newman–Keuls (NK) multiple comparison test ($p = 0.05$).

Oxygen uptake rates were determined by calculating the slope for each sealed measurement period. The slope from each phase (expressed as μmol O_2/g BW/h) was corrected for background respiration by using the average of background measurements taken before and after the trial. These rates were further adjusted to account for the volume of the chamber and associated tubing, and then converted to a mass-specific rate of oxygen consumption (MO_2, in μmol O_2/g BW/h) based on the average of the weights recorded at the beginning and end of the experiment. The metabolic rate (MR) was modeled using a temperature-dependent mass-scaling equation, $MR = aM^{b}e^{cT}$, where a is the intercept, M represents body mass, b is the mass-scaling coefficient, c is the temperature-scaling coefficient, and T is temperature. To apply this model, both sides were ln-transformed and analyzed through linear regression. Data were expressed as mean ± SD of three runs of respirometry data, and statistical analysis was performed based on the average of the three runs per fish. A paired *t*-test was used to compare the oxygen consumption between the light and dark phases. Significance levels were set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism 10.0.

3. Results

3.1. Adult Seahorse Growth and Breeding Performance

Throughout the experimental trial (seven-month period), adult seahorses increased in both length and weight (Table 1). Growth parameters were proportionally higher in males than in females, irrespective of the rearing temperature. Significantly higher growth rates ($p < 0.05$) were observed in fish reared at 20 and 24 °C compared to those reared at 16 and 28 °C, resulting in a higher WG for fish reared at the intermediate temperatures (Table 1). Conversely, in all temperature treatments, both genders decreased their CF during the experiment.

Table 1. Growth parameters of adult males and females *H. guttulatus* at the start and at the end of the seven-month trial (CF—condition factor; WG—weight gain).

Growth Parameters	16 °C		20 °C		24 °C		28 °C	
	Females	Males	Females	Males	Females	Males	Females	Males
Initial length (cm)	16.3 ± 1.1^{a}	17.5 ± 0.7^{a}	16.5 ± 0.7^{a}	17.7 ± 0.4^{a}	16.3 ± 0.5^{a}	17.5 ± 0.8^{a}	16.8 ± 0.8^{a}	17.6 ± 0.5^{a}
Final length (cm)	17.7 ± 1.5^{a}	19.4 ± 1.3^{a}	18.6 ± 1.2^{a}	21.1 ± 0.6^{a}	18 ± 0.6^{a}	20.1 ± 0.4^{a}	18.3 ± 0.7^{a}	20.7 ± 0.8^{a}
Initial weight (cm)	12.3 ± 0.7^{a}	12.5 ± 1.8^{a}	12.4 ± 1.3^{a}	13.2 ± 0.7^{a}	12.3 ± 0.5^{a}	12.2 ± 1.6^{a}	12.4 ± 0.5^{a}	12.6 ± 0.5^{a}
Final weight (cm)	14.0 ± 0.2^{a}	16.9 ± 5.1^{a}	14.9 ± 3.1^{b}	20.5 ± 1.4^{a}	14.2 ± 0.8^{b}	18.1 ± 1^{a}	14.8 ± 1.3^{b}	19.7 ± 4.2^{a}
CF on start of the experiment	0.28 ± 0.1^{a}	0.23 ± 0^{a}	0.28 ± 0.1^{a}	0.24 ± 0^{a}	0.28 ± 0^{a}	0.23 ± 0^{a}	0.26 ± 0^{a}	0.23 ± 0^{a}
CF at the end of the experiment	0.25 ± 0.1^{a}	0.23 ± 0.1^{a}	0.23 ± 0^{a}	0.21 ± 0^{a}	0.24 ± 0^{a}	0.22 ± 0^{a}	0.24 ± 0^{a}	0.22 ± 0^{a}
WG (g/fish d^{-1})	0.03 ± 0.01^{a}	0.08 ± 0.02^{a}	0.04 ± 0.1^{b}	0.13 ± 0.07^{a}	0.03 ± 0.01^{b}	0.11 ± 0.03^{a}	0.04 ± 0.01^{b}	0.13 ± 0.05^{a}
Mortality (nº ind.)	0	0	0	0	0	1	3	7
% mortality	0		0		6.2^{b}		62.5^{a}	

CF—Condition Factor; WG—Weight Gain. Within gender analysis: Rows with different superscripts letters are significantly different ($p < 0.05$).

Adult mortality varied significantly at the four tested temperatures. No mortality was observed at 16 and 20 °C, but with increasing temperatures, the mortality rate increased from 6.2% (one fish) at 24 °C to 62.5% (10 fish) at 28 °C. Notably, the mortality rate among males was higher than that of females, representing 43.7% of the total mortality at 28 °C.

Regarding reproductive performance, no reproductive activity (including courtship behavior) was observed in seahorses kept at 16 °C. At 28 °C, only sporadic courtship behavior was observed, with no successful broods resulting from it. Conversely, seahorses kept at 20 and 24 °C produced 20 and 16 successful broods, respectively (2.5 and 2 successful broods per couple). The number of juveniles per brood decreased significantly ($p < 0.05$), from 410 ± 133 at 20 °C to 257 ± 157 at 24 °C.

3.2. Juvenile Growth

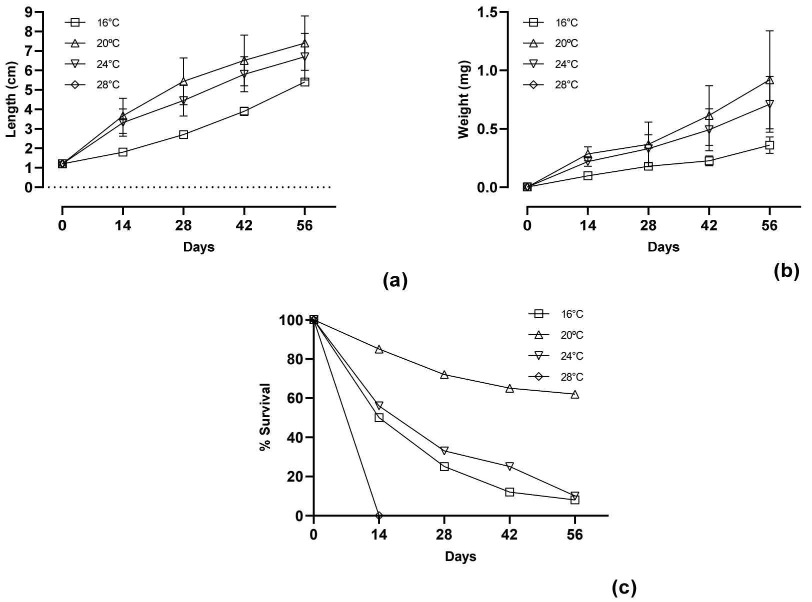
Data on length, weight, mean weight gain (WG), condition factor (CF), thermal-unit growth coefficient (TGC), and survival are reported in Table 2. At the end of the experiment

(56 DPP), significant differences ($p < 0.05$) in growth were observed between juveniles reared at 20 °C and 24 °C compared to those reared at 16 °C. However, no significant differences ($p > 0.05$) were observed between juveniles reared at 20 °C and 24 °C (Table 2). At 28 °C, all fish died before the first sampling at 14 DPP. This differential in the growth performance of fish reared at the intermediate temperatures was readily observed from the first sampling period (Figure 2a,b) until the end of the experiment. CF was the only unaffected growth parameter throughout the experiment, as no significant differences in CF were found among juvenile *H. guttulatus* reared at each of the four temperatures (Table 1). Survival ranged from 0% in fish reared at 28 °C to 62% in fish reared at 20 °C, with low intermediate values for fish reared at 16 °C (8%) and 24 °C (10%) (Figure 2c).

Table 2. Standard length (cm), body weight (g), thermal-unit growth coefficient (TGC), condition factor (CF), and feed conversion rate (FCR) (mean ± SD) of juvenile *H. guttulatus* at the end of the 56-day study under the four tested temperatures.

Growth Parameter	16 °C	20 °C	24 °C	28 °C
Standard length (cm)	5.4 ± 0.2 ^{b}	7.4 ± 1.3 ^{a}	6.7 ± 1.2 ^{a}	0
Body weight (g)	0.36 ± 0.07 ^{c}	0.92 ± 0.4 ^{a}	0.71 ± 0.24 ^{b}	0
WG (g.d^{-1})	0.006 ± 0.001 ^{b}	0.016 ± 0.002 ^{a}	0.013 ± 0.002 ^{a}	0
TGC	0.12 ^{c}	0.31 ^{a}	0.24 ^{b}	0
CF	0.22 ± 0.02 ^{a}	0.23 ± 0.03 ^{a}	0.24 ± 0.03 ^{a}	0
% survival	8 ^{b}	62 ^{a}	10 ^{b}	0

Initial weight = 0.002 ± 0.001 g: Initial length = 1.2 ± 0.2 cm, Initial Condition Factor = 0.12 ± 0.01. Rows with different superscripts are significantly different ($p < 0.05$).



(a)

(b)

(c)

Figure 2. (**a**) Length increase, (**b**) weight increase, and (**c**) percent survival of juvenile *H. guttulatus* reared at four tested temperatures (16, 20, 24, and 28 °C).

3.3. Respirometry Analysis

A distinct individual variation in oxygen consumption was observed after fish were moved to the respirometry chambers. Oxygen consumption values for each of the tested seahorse groups are detailed in Table 3. It was observed a constant decrease in oxygen consumption according to the seahorse age, from 203.3 ± 3.1 to 107.5 ± 3.7 µmol O_2/g BW/h between 1 DPP juveniles and adults (year old) at 16 °C, and from 273.3 ± 3.1 to 128.8 ± 3.8 µmol O_2/g BW/h between 1 DPP juveniles and adults (year old) at 28 °C during the light period. Likewise, the same pattern was observed during the dark period with a constant decrease in the oxygen consumption according to the seahorse age, from

198.7 ± 3.5 to 106.3 ± 3.1 µmol O_2/g BW/h between 1 DPP juveniles and adults (year old) at 16 °C, and from 127.6 ± 3.5 to 269 ± 6.2 µmol O_2/g BW/h between 1 DPP juveniles and adults (year old) at 28 °C. Regardless of the age, the oxygen consumption was observed to increase linearly with increasing temperatures. No matter the tested temperature, an allometric relation between fish size/age and oxygen consumption was observed, as newly hatched juveniles (1 DPP) consumed almost two times more oxygen than adult seahorses (one year old) (Figure 3).

Table 3. Average oxygen consumption (mean ± s.d. in µmol O_2/g BW/h) for each of the four age groups tested (DPP—days post-parturition).

		16 °C	**20 °C**	**24 °C**	**28 °C**
Light	1 DPP juveniles	203.3 ± 3.1^{d}	227.3 ± 4.9^{c}	242.3 ± 3.1^{b}	273.3 ± 3.1^{a}
	14 DPP juveniles	123.7 ± 3.7^{b}	128.4 ± 3.3^{b}	142.3 ± 3.5^{a}	156.4 ± 3.5^{a}
	28 DPP juveniles	119.4 ± 4.3^{b}	123.6 ± 3.2^{b}	132.8 ± 5.6^{a}	134.3 ± 4.7^{a}
	Adults (one year old)	107.5 ± 3.7^{c}	116.5 ± 4.7^{b}	126.3 ± 3.5^{a}	128.8 ± 3.8^{a}
Dark	1 DPP juveniles	198.7 ± 3.5^{c}	213 ± 4.1^{b}	213 ± 4.1^{b}	269 ± 6.2^{a}
	14 DPP juveniles	112.5 ± 4.8^{d}	125.2 ± 3.7^{c}	138.2 ± 3.6^{b}	151.6 ± 6.4^{a}
	28 DPP juveniles	117.7 ± 3.5^{b}	119.1 ± 4.6^{b}	127.1 ± 4.4^{a}	129.7 ± 3.8^{a}
	Adults (one year old)	106.3 ± 3.1^{c}	117.7 ± 3.4^{b}	131.5 ± 3.8^{a}	127.6 ± 3.5^{a}

Rows with different superscripts are significantly different ($p < 0.05$).

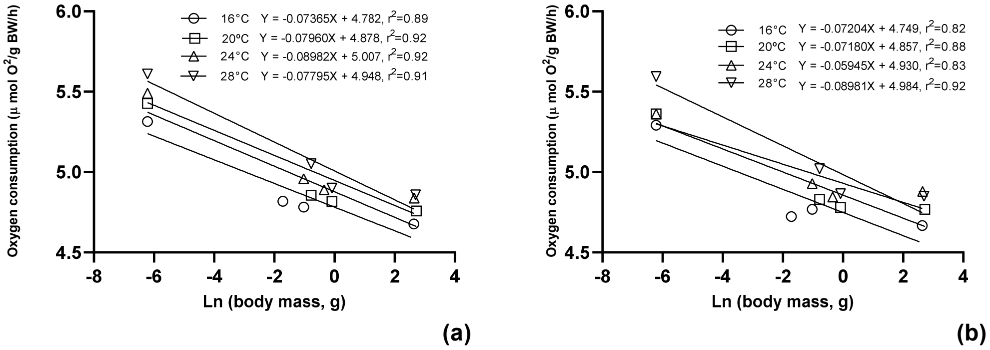


Figure 3. Metabolic rates (MR) (µmol O_2/g BW/h) during (**a**) light phase, and (**b**) dark phase of juveniles (1, 14, and 28 DPP) and adults for each of the four tested water temperatures (16, 20, 24 and 28 °C).

Regardless of age and tested temperature, with the exception of 1 DPP juveniles at 24 °C, the oxygen consumption was always higher, but not significantly different ($p > 0.05$) between the light period vs. the dark period.

4. Discussion

Fish thermal tolerance can be defined as heat and cold tolerance, which correspond to the highest summer water temperature and the coldest winter water temperature within their ranges [45]. While species can withstand temporary cold and heat peaks, the prolonged occurrence of these extreme temperatures may cause significant changes in species' growth, reproduction, and survival. The fish thermal environment is thought to have a greater impact on their distribution, overall condition, and survival than any other aquatic habitat characteristic [46]. Therefore, increasing air temperatures resulting from climate change are expected to increase water temperatures, altering the fish's thermal environment with direct effects on their growth, reproduction, and survival, as well as that of their prey [47–49]. Rising temperatures, within certain limits, can be beneficial. For example, ref. [50] observed that adult *H. reidi* density was positively and significantly correlated with increased water temperatures during El Niño events. Similarly, in aquaculture, higher temperatures can accelerate growth rates and shorten the maturation period of fish. Ref. [51]

reported that juvenile *H. barbouri* can tolerate temperatures between 25 °C and 31 °C, but optimal growth and survival were observed between 25 °C and 28 °C. However, when temperatures exceed the optimal limits for a species, it can negatively impact the health of aquatic animals by causing metabolic stress, increased susceptibility to disease, the proliferation of pathogens, and higher oxygen demand. Ref. [52] found that *H. erectus*, when exposed to acute thermal stress (30 °C for 2–10 h), exhibited a significant increase in basal metabolic rate (breathing rate) and elevated expression of stress response genes (Hsp genes). Chronic exposure to the same temperature (30 °C for four weeks) resulted in a persistently elevated basal metabolic rate, increased stress response gene expression, and higher mortality rates. Ref. [53] noticed that even short-term increases in temperature caused significant changes in the feeding behavior of juvenile *H. kuda*. Overall, these authors suggested a similar pattern where these species were unable to acclimate to chronic thermal stress, potentially leading to mass mortality due to excessively high metabolic rates and stress-induced damage.

Similarly, in the present study, it was also observed that temperature variation had a major impact on the reproduction, growth, and survival of both juvenile and adult long snout seahorses. For adult seahorses, male weight gain was consistently and significantly higher ($p < 0.05$) than that of females, a trait not directly related to the tested temperatures and previously observed in this species [54]. However, male weight gain was significantly lower at 16 °C (the lowest temperature tested) compared to the other three temperatures. This result is expected, as it reflects a decrease in metabolic rate due to the low temperature. Nonetheless, female weight gain did not vary significantly ($p > 0.05$) across the tested temperatures, indicating a different growth response between the sexes. However, this metabolic decrease did not result in any mortality in the tested fish. Conversely, the increased mortality observed at 28 °C is a clear negative effect caused by temperature and a strong indication that this species is unable to cope with prolonged heat periods. At 28 °C, reproduction was impaired as no viable broods were produced. Overall, it was observed that when subjected to a prolonged heat period, the viability of the species is compromised due to the cessation of reproductive activity and, in most cases, death. Similar findings were reported by [55] for the same species, where adult *H. guttulatus* were exposed to temperatures of 17 °C, 20 °C, and 24 °C. The researchers observed that fish were more active and fed more at 24 °C, but the increase in energy intake did not result in a higher growth rate. Ref. [56] made similar observations for the broad-nose pipefish (*Syngnathus typhle*). When exposed to 24 °C, the fish became less active, showed reduced growth, and experienced significantly lower survival rates compared to those kept at 20 °C.

Transposing this evidence to natural circumstances implies that if this species' populations find themselves trapped in prolonged heat periods, their resilience will be affected, leading to a potential reduction in abundance or even the species' disappearance within a short period of time. For example, in the Ria Formosa, according to [15], water temperature varies from 12 °C in winter to 27 °C in summer. These temperature ranges are quite similar to those tested in this study, indicating that the effects of temperature on wild seahorse populations in the lagoon may already be occurring. In a climate change scenario, even a small increase in temperature will negatively impact the species' welfare and their ability to remain healthy and stable [57,58]. Moreover, the Ria Formosa lagoon is a highly dynamic system composed of shallow channels where the water temperature is homogenized and tends to be similar at all depths preferred by this species (3–11 m, personal observation), thus preventing seahorses from moving to find suitable thermal habitats. Therefore, as mentioned above, heat waves, which currently last for 1–2 weeks on the Portuguese coast [13], will become more intense, more frequent, and longer-lasting in warmer climate scenarios [14], leading to extended periods of heat waves with the potential negative impacts described here.

It was observed that fecundity starts to be impaired at 24 °C, as the number of juveniles produced per brood was significantly lower ($p < 0.05$) than at 20 °C. Considering that even under normal circumstances, seahorse fecundity is much lower compared to other fish species [1], this constraint may cause an additional impact on the species' conservation. In this study, this result, combined with the fact that at 24 °C only 10% of the produced juveniles survived to the end of their second month of life—much lower compared to the 62% survival rate at 20 °C—indicates significant thermal sensitivity. Concordantly, refs. [40,59] observed that juvenile *H. guttulatus* have their optimal growth rate at 21 °C, and ref. [60] documented a significant increase in the metabolic rates of newborn juveniles and adult *H. guttulatus* with rising temperatures. According to the authors, the juvenile stages display greater thermal sensitivity and may face greater metabolic challenges with potential cascading consequences for their growth and survival compared to adults. This was confirmed in the present study, where juvenile survival dropped to 10% at 24 °C and reached 100% mortality at 28 °C. Similarly, juvenile *H. reidi* mortality rates can reach 100% at temperatures above 30 °C [61,62]. Ref. [52] also reported increased mortality in juvenile *H. erectus* at 32 °C in laboratory conditions, temperatures that are considered to be at the upper limit of thermal tolerance for these species.

In this study, oxygen consumption was higher during the light phase compared to the dark phase, regardless of the temperature to which the fish were exposed. Since the fish were fed during the light phase, the increased oxygen consumption may be partly explained by the feeding stimulus, which helped to overcome the light–dark cycle. The higher metabolism in the light phase may be due to the metabolic processing of food, a finding similar to that of [42], who observed a similar pattern for juvenile Senegalese sole (*Solea senegalensis*). However, this increase in metabolic activity was also directly related to the temperature increase; irrespective of the feeding effect, oxygen consumption was amplified by temperature. As observed in the first part of the experiment, juvenile growth and survival and adult reproductive success and survival were severely impacted by temperature. In the second part, an overall increase in oxygen consumption was noted, indicating that the observed results are solely a consequence of temperature effects. Metabolic rate is commonly described as scaling with body size following a power function and increasing exponentially with temperature [63], with the mass-scaling exponent decreasing as temperature rises. In the present study, oxygen consumption was significantly higher in the newborn age group (1 DPP) compared to adult seahorses, aligning with previous studies such as [23], who observed a similar trend across 69 other teleost species. It is noteworthy that the oxygen consumption of newly hatched juveniles (1 DPP) was significantly higher ($p < 0.05$) than that observed in 14 and 18 DPP juveniles and in adults. This indicates greater thermal sensitivity in the first days of life, which compromises individual survival. Over time, older juveniles increase their thermal tolerance, reaching levels almost identical to those of adults. Therefore, a clear relationship between allometric variation and thermal sensitivity was observed, consistent with the findings of [23]. Fish sensitivity to cyclic high temperatures during the first life stages is not specific to seahorse species. Ref. [64] reported that when the mean water temperature was increased by just 0.6 °C, there was a reduction in the life history diversity of rainbow trout (*Oncorhynchus mykiss*), and ref. [65] noted that high temperatures (31–35 °C) decreased growth in juvenile Channel catfish (*Ictalurus punctatus*) due to reduced food consumption, feed conversion, and increased activity levels. The prevalence of high-temperature periods above the species' optimal range can, therefore, imply a long-term negative effect that may impact the species beyond its coping point, impairing long-term population health.

In this study, it was observed that adult fish (one year old) had a 34.4% increase in oxygen consumption from 16 °C to 28 °C. Since male seahorses provide paternal care to their broods by maintaining the developing embryos in the brood pouch, oxygen requirement and consumption are hypothesized to be even higher during the breeding season, which occurs during the spring-summer period when longer heat waves also happen. This constraint implies an additional physiological demand on the males and will potentially affect them and their resulting offspring in the same way observed in this study. Environmental factors do not play isolated roles in animal physiology; instead, they interact and combine, causing increased detrimental effects on species' survival. For aquatic species, increased water temperatures lead to a decrease in dissolved oxygen, requiring additional physiological effort to maintain the necessary oxygen levels in their bodies. Seahorses are no exception. For pregnant males, who provide parental care to their broods, this constraint may represent an additional physiological effort, as they need to supply oxygen to the developing embryos in their pouch. This long-term metabolic requirement may help explain the increased male mortality compared to females.

In conclusion, both juvenile and adult *H. guttulatus* are affected by long-term exposure to increased temperatures, with direct implications for their growth, survival, and reproduction. Under natural conditions, this may represent a major constraint for the long-term conservation of this and other seahorse species in coastal areas similar to the Ria Formosa, and ultimately for local aquatic biodiversity as a whole.

Author Contributions: Conceptualization, J.P.; methodology, J.P. and M.C.; formal analysis, J.P.; writing/original draft preparation, J.P.; review and editing, J.P.; F.L.; M.C. and J.P.A.; project administration, J.P.; funding acquisition, J.P. and F.L. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the project HIPPOCAMPUS_RIA "Avaliação e implementação de conservação ecossistémica de cavalos-marinhos" (Fundo Ambiental) and received Portuguese national funds from FCT—Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020.

Institutional Review Board Statement: CCMAR facilities and the research are certified to house and conduct experiments with live animals (Group-C licenses from the Direção Geral de Alimentação e Veterinária, Ministério da Agricultura, Florestas e Desenvolvimento Rural, Portugal). The captive breeding program for *H. guttulatus* (Project HIPPOCAMPUS_RIA) was approved by ICNF—Instituto da Conservação da Natureza e das Florestas under the permit S-009617/2024. Under this approval, the study was performed in compliance with the Guidelines of the European Union Council (86/609/EU) and Portuguese legislation for the use of laboratory animals.

Data Availability Statement: The data used during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors would like to acknowledge António Sykes and João Reis for their valuable contribution and assistance in the respirometry analyses.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Foster, S.J.; Vincent, A.C.J. Life history and ecology of seahorses: Implications for conservation and management. *J. Fish Biol.* **2004**, *65*, 1–61. [CrossRef]
2. Shokri, M.R.; Gladstone, W.; Jelbart, J. The effectiveness of seahorses and pipefish (Pisces: Syngnathidae) as a flagship group to evaluate the conservation value of estuarine seagrass beds. *Aquat. Conserv. Mar. Freshw.* **2009**, *19*, 588–595. [CrossRef]
3. Bell, E.M.; Lockyear, J.F.; McPherson, J.M.; Marsden, A.D.; Vincent, A.C.J. First field studies of an Endangered South African seahorse, *Hippocampus capensis*. *Environ. Biol. Fishes* **2003**, *67*, 35–46. [CrossRef]
4. Caldwell, I.R.; Vincent, A. Revisiting two sympatric European seahorse species: Apparent decline in the absence of exploitation. *Aquat. Conserv. Mar. Freshw.* **2012**, *22*, 427–435. [CrossRef]
5. Correia, M.; Caldwell, I.R.; Koldewey, H.J.; Andrade, J.P.; Palma, J. Seahorse (Hippocampinae) population fluctuations in the Ria Formosa Lagoon, south Portugal. *J. Fish Biol.* **2015**, *87*, 679–690. [CrossRef]
6. Hand, B.K.; Cushman, S.A.; Landguth, E.L.; Lucotch, J. Assessing multi-taxa sensitivity to the human footprint, habitat fragmentation and loss by exploring alternative scenarios of dispersal ability and population size: A simulation approach. *Biodivers. Conserv.* **2014**, *23*, 2761–2779. [CrossRef]
7. Allendorf, F.W.; Luikart, G.; Aitken, S.N. *Conservation and the Genetics of Populations*, 2nd ed.; Wiley-Blackwell: London, UK, 2013.
8. Parry, M.L.; Canziani, O.F.; Palutikof, J.P.; Linden, P.J.V.D.; Hanson, C.E. Technical summary. Climate change 2007: Impacts, adaptation and vulnerability. In *Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*; Parry, M.L., Canziani, O.F., Palutikof, J.P., Linden, P.J.V.D., Hanson, C.E., Eds.; Cambridge University Press: Cambridge, UK, 2007; pp. 23–78.

9. Hiscock, K.; Southward, A.; Tittley, I.; Hawking, S. Effects of changing temperature on benthic marine life in Britain and Ireland. *Aquat. Conserv. Mar. Freshw.* **2004**, *14*, 333–362. [CrossRef]
10. Lenton, T.M.; Held, H.; Kriegler, E.; Hall, J.W.; Lucht, W.; Rahmstorf, S.; Schellnhuber, H.J. Tipping elements in the Earth's climate system. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 1786–1793. [CrossRef]
11. Curtis, J.M.R.; Ribeiro, J.; Erzini, K.; Vincent, A.C.J. A conservation trade-off? Interspecific differences in seahorse responses to experimental changes in fishing effort. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2007**, *17*, 468–484. [CrossRef]
12. Correia, M. Monitoring of Seahorse Populations, in the Ria Formosa Lagoon (Portugal), Reveals Steep Fluctuations: Potential Causes and Future Mitigations. *Proc. Zool. Soc.* **2022**, *75*, 190–199. [CrossRef]
13. Miranda, P.M.A.; Coelho, F.E.S.; Tomé, A.R.; Valente, M.A. 20th Century Portuguese Climate and Climate Scenarios. In *Climate Change in Portugal. Scenarios, Impacts and Adaptation Measures-SIAM Project*; Santos, F.D., Forbes, K., Moita, R., Eds.; Gradiva: Lisboa, Portugal, 2002; pp. 27–83.
14. Solomon, S.; Qin, D.; Manning, M.; Chen, Z.; Marquis, M.; Averyt, K.B.; Tignor, M.; Miller, H.L. *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2007; p. 996.
15. Newton, A.; Mudge, S.M. Temperature and salinity regimes in a shallow, mesotidal lagoon, the Ria Formosa, Portugal. *Estuar. Coast. Shelf. Sci.* **2003**, *57*, 73–85. [CrossRef]
16. Machado, L.M. A Incidência de Radiação UV-B na Ria Formosa: Incidência e Impactes Biológicos. Ph.D. Thesis, University of Algarve, Faro, Portugal, 2010.
17. Correia, M. Trends in Seahorse Abundance in the Ria Formosa, South Portugal: Recent Scenarios and Future Prospects. Ph.D. Thesis, University of Algarve, Faro, Portugal, 2015.
18. Schulte, P.M. TEMPERATURE | Effects of Temperature: An Introduction. In *Encyclopedia of Fish Physiology*; Farrell, A.P., Ed.; Academic Press: San Diego, CA, USA, 2011; pp. 1688–1694. [CrossRef]
19. Cossins, A.; Fraser, J.; Hughes, M.; Gracey, A. PostGenomic Approaches to Understanding the Mechanisms of Environmentally Induced Phenotypic Plasticity. *J. Exp. Biol.* **2006**, *209*, 2328–2336. [CrossRef]
20. Gollock, M.J.; Currie, S.; Petersen, L.H.; Gamperl, A.K. Cardiovascular and Haematological Responses of Atlantic Cod (*Gadus morhua*) to Acute Temperature Increase. *J. Exp. Biol.* **2006**, *209*, 2961–2970. [CrossRef]
21. Dillon, M.E.; Wang, G.; Huey, R.B. Global metabolic impacts of recent climate warming. *Nature* **2010**, *467*, 704–706. [CrossRef]
22. Portner, H.O. Oxygen- and capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. *J. Exp. Biol.* **2010**, *213*, 881–893. [CrossRef]
23. Clarke, A.; Johnston, N.M. Scaling of metabolic rate with body mass and temperature in teleost fish. *J. Anim. Ecol.* **1999**, *68*, 893–905. [CrossRef]
24. Whitfield, J. Ecology's Big, Hot Idea. *PLoS Biol.* **2004**, *2*, e440. [CrossRef]
25. Price, C.A.; Weitz, J.S.; Savage, V.M.; Stegen, J.; Clarke, A.; Coomes, D.A.; Dodds, P.S.; Etienne, R.S.; Kerkhoff, A.J.; McCulloh, K.; et al. Testing the metabolic theory of ecology. *Ecol. Lett.* **2012**, *15*, 1465–1474. [CrossRef]
26. Donelson, J.M.; Munday, P.L.; McCormick, M.I.; Pitcher, C.R. Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nat. Clim. Chang.* **2012**, *2*, 30–32. [CrossRef]
27. Munday, P.L.; McCormick, M.I.; Nilsson, G.E. Impact of global warming and rising CO_2 levels on coral reef fishes: What hope for the future? *J. Exp. Biol.* **2012**, *215*, 3865–3873. [CrossRef]
28. Pörtner, H.O. Integrating climate-related stressor effects on marine organisms: Unifying principles linking molecule to ecosystem-level changes. *Mar. Ecol. Prog. Ser.* **2012**, *470*, 273–290. [CrossRef]
29. Clark, T.D.; Sandblom, E.; Jutfelt, F. Aerobic scope measurements of fishes in an era of climate change: Respirometry, relevance and recommendations. *J. Exp. Biol.* **2013**, *216*, 2771–2782. [CrossRef]
30. Brett, J.R. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerkd*). *Am. Zool.* **1971**, *11*, 99–113. [CrossRef]
31. Brett, J.R.; Groves, T.D.D. Physiological energetics. In *Fish Physiology*; Hoar, W.S., Randall, D.J., Brett, J.R., Eds.; Academic Press: New York, NY, USA, 1979; pp. 279–352, ISBN 978-0-12-350408-1.
32. Fry, F.E.J. The effect of environmental factors on the physiology of fish. In *Fish Physiology*; Hoar, W.S., Randall, D.J., Eds.; Academic Press: Cambridge, MA, USA, 1971; pp. 1–98, ISBN 978-0-12-350408-1.
33. Cunha, I.; Conceição, L.E.C.; Planas, M. Energy allocation and metabolic scope in early turbot, *Scophthalmus maximus*, larvae. *Mar. Biol.* **2007**, *151*, 1397–1405. [CrossRef]
34. Lucas, M.C.; Priede, I.G. Utilization of metabolic scope in relation to feeding and activity by individual and grouped zebrafish, *Brachydanio rerio* (HamiltonBuchanan). *J. Fish Biol.* **1992**, *41*, 175–190. [CrossRef]
35. Bihun, C.J.; Murphy, M.K.; Johnson, T.B.; Fisk, A.T.; Guzzo, M.M.; Madenjian, C.P.; Raby, G.D. Effects of temperature, body size, and sex on the standard metabolic rates of a sexually dimorphic freshwater piscivore, walleye (*Sander vitreus*). *Can. J. Fish. Aquat. Sci.* **2024**, *81*, 879–886. [CrossRef]
36. Schulte, P.M. The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J. Exp. Biol.* **2015**, *218*, 1856–1866. [CrossRef]

37. Glazier, D.S. Beyond the '3/4-power law': Variation in the intra-and interspecific scaling of metabolic rate in animals. *Biol. Rev.* **2005**, *80*, 611–662. [CrossRef]
38. Sandblom, E.; Gräns, A.; Axelsson, M.; Seth, H. Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: Implications in a thermally extreme future. *Proc. R. Soc. B* **2014**, *281*, 20141490. [CrossRef]
39. Woods, C.M.C. Improving initial survival in cultured seahorses, *Hippocampus abdominalis* Leeson, 1827 (Teleostei: Syngnathidae). *Aquaculture* **2000**, *190*, 377–388. [CrossRef]
40. Palma, J.; Bureau, D.P.; Andrade, J.P. Effect of different *Artemia* enrichments and feeding protocol for rearing juvenile long snout seahorse, (*Hippocampus guttulatus*). *Aquaculture* **2011**, *318*, 439–443. [CrossRef]
41. Lourie, S.A.; Pritchard, J.C.; Casey, S.P.; Truong, S.K.; Hall, H.J.; Vincent, A.C.J. The taxonomy of Vietnam's exploited seahorses (family Syngnathidae). *Biol. J. Linn. Soc. Lond.* **1999**, *66*, 231–256. [CrossRef]
42. Castanheira, M.F.; Martins, C.I.M.; Engrola, S.; Conceição, L.E.C. Daily oxygen consumption rhythms of Senegalese sole *Solea senegalensis* (Kaup, 1858) juveniles. *J. Exp. Mar. Bio. Ecol.* **2011**, *407*, 1–5. [CrossRef]
43. Careau, V.; Thomas, D.; Humphries, M.M.; Réale, D. Energy metabolism and animal personality. *Oikos* **2008**, *117*, 641–653. [CrossRef]
44. Martins, C.I.M.; Castanheira, M.F.; Engrola, S.; Costas, B.; Conceição, L.E.C. Individual differences in metabolism predict coping styles in fish. *Appl. Anim. Behav. Sci.* **2011**, *130*, 135–143. [CrossRef]
45. Sunday, J.M.; Bates, A.E.; Dulvy, N.K. Thermal tolerance and the global redistribution of animals. *Nat. Clim. Chang.* **2012**, *2*, 686–690. [CrossRef]
46. Wootton, R.J. *The Ecology of Teleost Fishes*, 2nd ed.; Fish and Fisheries Series 24; Kluwer: Dordrecht, The Netherlands, 1998.
47. Woodward, G.; Perkins, D.M.; Brown, L.E. Climate change and freshwater ecosystems: Impacts across multiple levels of organization. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2010**, *365*, 2093–2106. [CrossRef]
48. Hershkovitz, Y.; Dahm, V.; Lorenz, A.W.; Hering, D. A multi-trait approach for the identification and protection of European freshwater species that are potentially vulnerable to the impacts of climate change. *Ecol. Indic.* **2015**, *50*, 150–160. [CrossRef]
49. Kanno, Y.; Letcher, B.H.; Hitt, N.P.; Boughton, D.A.; Wofford, J.E.B.; Zipkin, E. Seasonal weather patterns drive population vital rates and persistence in a stream fish. *Glob. Chang. Biol.* **2015**, *21*, 1856–1870. [CrossRef]
50. Freret-Meurer, N.V.; Fernández, T.C.; Vaccani, A.C. Influence of the Atlantic Ocean thermal anomaly on the longsnout seahorse *Hippocampus reidi* in a Brazilian estuary. *J. Fish Biol.* **2022**, *101*, 960–971. [CrossRef]
51. Er, W.C.V.; Christianus, A.; Muta, H.Z.; Chong, C.M. Influence of stocking density and temperature on the growth and survival of Seahorse Juveniles, *Hippocampus barbouri* (Jordan and Richardson, 1908). *J. Surv. Fish. Sci.* **2020**, *6*, 27–38. [CrossRef]
52. Qin, G.; Johnson, C.; Zhang, Y.; Zhang, H.; Yin, J.; Miller, G.; Turingan, R.G.; Guisbert, E.; Lin, Q. Temperature-induced physiological stress and reproductive characteristics of the migratory seahorse *Hippocampus erectus* during a thermal stress simulation. *Biol. Open* **2018**, *7*, bio032888. [CrossRef]
53. Xia, P.; Xiquan, S.; Wenxin, Z.; Yongjian, X. Effects of short-term temperature changes on the physiology, biochemistry and feeding behaviour of the juvenile yellow seahorse—*Hippopotamus kuda* Bleeker. *Aquacult. Res.* **2022**, *53*, 1084–1097. [CrossRef]
54. Palma, J.; Andrade, J.P.; Bureau, D.P. The impact of dietary supplementation with astaxanthin on egg quality and growth of long snout seahorse (*Hippocampus guttulatus*) juveniles. *Aquacult. Nut.* **2017**, *23*, 304–312. [CrossRef]
55. Costa, A.B.; Correia, M.; Silva, G.; Lopes, A.F.; Faria, A.M. Performance of the long-snouted seahorse, *Hippocampus guttulatus*, under warming conditions. *Front. Mar. Sci.* **2023**, *10*, 1136748. [CrossRef]
56. Miranda, L.M.; Correia, M.; Barraca, C.; Baylina, N.; Faria, A.M. Prolonged exposure to elevated temperature leads to severe thermal stress in the broad-nosed pipefish, *Syngnathus typhle*. *Mar. Freshw. Behav. Physiol.* **2024**, *57*, 1–16. [CrossRef]
57. Shokri, M.; Cozzoli, F.; Vignes, F.; Bertoli, M.; Pizzul, E.; Basset, A. Metabolic rate and climate change across latitudes: Evidence of mass-dependent responses in aquatic amphipods. *J. Exp. Biol.* **2022**, *225*, jeb244842. [CrossRef]
58. Shokri, M.; Lezzi, L.; Basset, A. The seasonal response of metabolic rate to projected climate change scenarios in aquatic amphipods. *J. Therm. Biol.* **2024**, *124*, 103941. [CrossRef]
59. Valladares, S.; Planas, M. Application of Effective Day Degrees in the Assessment of Stable Isotope Patterns in Developing Seahorses under Different Temperatures. *Animals* **2020**, *10*, 1571. [CrossRef]
60. Aurélio, M.; Faleiro, F.; Lopes, V.M.; Pires, V.; Lopes, A.R.; Pimentel, M.S.; Repolho, T.; Baptista, M.; Narciso, L.; Rosa, R. Physiological and behavioral responses of temperate seahorses (*Hippocampus guttulatus*) to environmental warming. *Mar. Biol.* **2013**, *160*, 2663–2670. [CrossRef]
61. Hora, M.S.C.; Joyeux, J. Closing the reproductive cycle: Growth of the seahorse *Hippocampus reidi* (Teleostei, Syngnathidae) from birth to adulthood under experimental conditions. *Aquaculture* **2009**, *292*, 37–41. [CrossRef]
62. Tseng, C.C.; Chien, J.H.; Chu, T.W.; Cheng, A.C.; Shiu, Y.L.; Han, T.W.; Liu, C.H. Effects of food type, temperature and salinity on the growth performance and antioxidant status of the longsnout seahorse, *Hippocampus reidi*. *Aquacult. Res.* **2020**, *51*, 4793–4804. [CrossRef]
63. Ohlberger, J.; Mehner, T.; Staaks, G.; Hölker, F. Intraspecific temperature dependence of the scaling of metabolic rate with body mass in fishes and its ecological implications. *Oikos* **2012**, *121*, 245–251. [CrossRef]

64. Benjamin, J.R.; Connolly, P.J.; Romine, J.G.; Perry, R.W. Potential Effects of Changes in Temperature and Food Resources on Life History Trajectories of Juvenile *Oncorhynchus mykiss*. *Trans. Am. Fish. Soc.* **2013**, *142*, 208–220. [CrossRef]
65. Arnold, M.B.; Torrans, E.L.; Allen, P.J. Influences of Cyclic, High Temperatures on Juvenile Channel Catfish Growth and Feeding. *N. Am. J. Aquac.* **2013**, *75*, 77–84. [CrossRef]