Long-snouted seahorse, Hippocampus guttulatus, under global warming

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***Abstract*— The earth's climate system and the global ocean have been warming up, since the mid-twentieth century and it is expected that the global and ocean´s temperature will rise in the next years even more [1,2,13,25,28]. For ectotherms, such as fish, temperature is a determinant cue for several behavioural [29], physiological [24], and metabolic processes [6]. Seahorses' particular life history makes them vulnerable to human or natural disturbances [17]. The long snout seahorse, *Hippocampus guttulatus*, is one of the two species that inhabit the coastal areas of Portugal [23]. The present study aims to assess the effects of ocean warming on growth, feed intake and behavior patterns of adult *H. guttulatus*. Results provide clear evidence of detrimental effects of exposure to warming on this species. Under extreme temperatures (24ºC), the increased food ingestion was not enough for fish to support growth (weighted less), which suggests that fish were spending energy to counterbalance the thermal stress they were exposed to. Fish under both elevated temperature treatments (20 and 24ºC) were also spending more energy in active behaviours. Altogether, these data may indicate that ocean warming will have a drastic effect on seahorse populations currently inhabiting the Sado estuary.**

Keywords*— *Seahorses; Hippocampus guttulatus; climate change; behavior; physiology.

I. Introduction

The earth's climate system and the global ocean have been warming up, since the mid-twentieth century [2,13,25,28]. This warming is mostly due to climate change, induced and accelerated, mainly by anthropogenic activities [18, 5, 27]. It has been proven, with high confidence, that human activities have increased the effect of climate change in the past 30 years, causing a rise in 1.0 ºC of the planet's temperature [1, 22]. Global temperatures are expected to rise in the next years [1] and this increase will have drastic effects on the structure and function of ecosystems globally [26,14,4] including marine ecosystems.

For ectotherms, such as fish, temperature is a determinant cue for several behavioural [29], physiological [24], and metabolic processes [6]. It is thus critical to evaluate how projected ocean warming will act upon individuals and populations. This assumes greater relevance for species that are currently threatened, such as the seahorses.

Seahorses' particular life history makes them vulnerable to human or natural disturbances [17]. Seahorses have low mobility, small home ranges, low fecundity, sparse distribution, mate fidelity and lengthy parental care [12,17]. The main threats for this species are habitat loss and degradation by bottom trawling fishing gear (IUCN, 2022). Seahorses live in shallow coastal areas, where human related disturbances are more severe such as pollution, sedimentation, eutrophication, and habitat disturbance through boating and shipping (IUCN, 2022). The long snout seahorse, *Hippocampus guttulatus*, is one of two species that inhabit the coastal areas of Portugal [23]. The most extensively studied population in Portugal is the Ria Formosa population, with many studies that have already helped in its conservation [3,7,8,9,11,12]. However, there are newly recorded seahorse populations in the Sado estuary which are understudied and in need of attention.

The present study aims to assess the effects of ocean warming on growth, feed intake and behavior patterns of adult *H. guttulatus*. The results of this study will provide information to predict what effect the temperature increase will have on this species and inform policy makers on the implementation of possible mitigation/conservation measures (e.g. marine protected areas).

II. Material and Methods

This study was carried out under approval of Direção-Geral de Alimentação e Veterinária (DGAV, Portuguese Authority for Animal Health, permit 0421/000/000/2020) and according to the University's animal ethics guidelines. Permit to collect fish from the wild was granted by the National Institute of Nature Conservation (ICNF, permits 288 – 290/2022/CAPT). Adult individuals of *Hippocampus guttulatus* were collected by scuba diving in Sado estuary (38.462311,-8.856218), on the west coast of Portugal, on April 8th 2022. We collected 22 individuals, 11 females and 11 males. The temperature at the collection site was 14,5 ºC. The fish were randomly assigned to the treatments, which did not differ in weight and size.

A. Specimen collection and stocking conditions

Seahorses were transported to ISPA fish facilities and placed in 80L tanks (40x40x60 cm), at a density of 2 couples per tank (total of six tanks). With the exception of one of the aquariums, since, during the field trip to collect the animals due to logistical issues, it was not possible to collect 24 seahorses. in order not to expose the seahorses to more weeks of laboratory conditions, the experiment was performed with one pair less than expected. Tanks were equipped with a protein skimmer and biological and mechanical filtration (internal filter, Max 104 F), and maintained at a matching the conditions at the field site (average 15ºC and 35 PSU), and a photoperiod of 14L:10D. To provide environmental enrichment and holdfast, plastic chains were added to each tank. Seahorse were left under these conditions for 15 days, to recover from transport and handling stress, and daily fed with a mix of artemia and mysis. Subsequently, fish were randomly assigned to three temperature treatments: treatment A reflects average temperature conditions (17 ºC), during the reproductive season, at the collection site; treatment B (20 ºC) reflects a warming scenario that fish from this location can still experience in the wild; treatment C (24 ºC) falls outside the natural thermal variability that the species can experience, in their natural habitat. The temperatures in all treatments increased gradually, by about 1 ºC per day, using heaters. As soon as tanks reached the experimental treatment temperature, we considered it day 0 of exposure. At this stage, each individual was measured and weighted. Each treatment had two replicate tanks, each tank with 2 couples (i.e., 4 couples for treatment). Treatment A had only 3 couples (one tank had only 1 couple), since it was only possible to collect 11 females and 11 males. As this is a species with conservation status, and to avoid further disturbance, it was decided to keep only three couples under control conditions. During the 8 weeks trial, fish were fed once per day, with a mix of frozen artemia and mysis. Fish under treatments A and B were provided with 5% of body weight (BW) and fish from treatment C were provided with 7% of BW to allow ad libitum conditions for every fish.

B. Feed intake, behavioural patterns, measuring and weighing

The food was weighed before the feeding and mixed with seawater to defrost. After 24h, the uneaten food was siphoned out of the tank and weighed. The feed intake (FI) was measured by weighing the food content left in the aquarium every day except on the weekends. Feed intake was calculated using the formula: FI (%) = (FS - FU)/FS where FS is the amount of food supplied (g) and FU is the amount of uneaten food (g).

Three times a week, behavioral observations were made, based on an ethogram adapted from [15,16] (Table I). Focal observations were made in the morning (between 7:00 and 10:00 hours), before feeding took place, to avoid pre-prandial bias. Focal observations were made by a motionless observer standing in front of the aquarium. Each focal observation lasted 3 minutes, and this procedure was repeated for every seahorse from each treatment. Focal observations were conducted every third day, with the exception of the weekend, during the 8 weeks of the experiment.

Once a week, individuals were measured for size (cm) and weight (g). Size measurements were performed according to Lourie, 2003, and only measured the height (vertical distance from the median groove at the tip of the coronet, to the tip of the outstretched tail, with the head held at right angles to body [21]). We decided to remove from table 1 the behavioural categories “attached” and “unattached”. These were actually noted on our routine behavioural observations, but they are highly correlated with “activity” and “rest”, and therefore we did not analyse.

TABLE I. Ethogram of hippocampus guttulatus activity patterns and holdfast attachment (adapted from Faleiro et al. 2008, 2015).

Rest	Stationary	The individual does not make any movement
	Head movement	The individual remains motionless but moves his head slightly
Activity	Slow body movement	The individual moves his body slightly but remains in the same place
	Fast body movement	The individual moves his pectoral and dorsal fins and moves the body faster, but remains in the same place.
	Swimming	The individual swims

C. Analysis statistical

The effects of temperature treatment on body weight, feed-intake, general activity and rest was evaluated using a one-way ANOVA, followed by Tukey post hoc tests. Significance level was set at $p < 0.05$.

III. Results

Fish under extreme temperatures (24 ºC) weighted significantly less compared to fish under control [($F_{2,173}$ = 13.9, $p<0.0001$)] conditions, and also compared to fish under high temperature conditions (Tukey) (Fig 1). Despite weighing less, fish under extreme temperature had greater feed intake compared to fish under control temperatures [($F_{2,171}$ = 47.85, $p<0.0001$)] (Fig 1b).

Regarding behaviour, fish under high (20ºC) and extreme (24ºC) temperatures spent significantly less time in motionless activities (Rest) compared to fish under control conditions [($F_{2,459}$ = 35.31, $p<0.0001$)] (Fig. 1c), and, concomitantly, more time active [($F_{2,459}$ = 35.28, $p<0.0001$)] (Fig 1d).

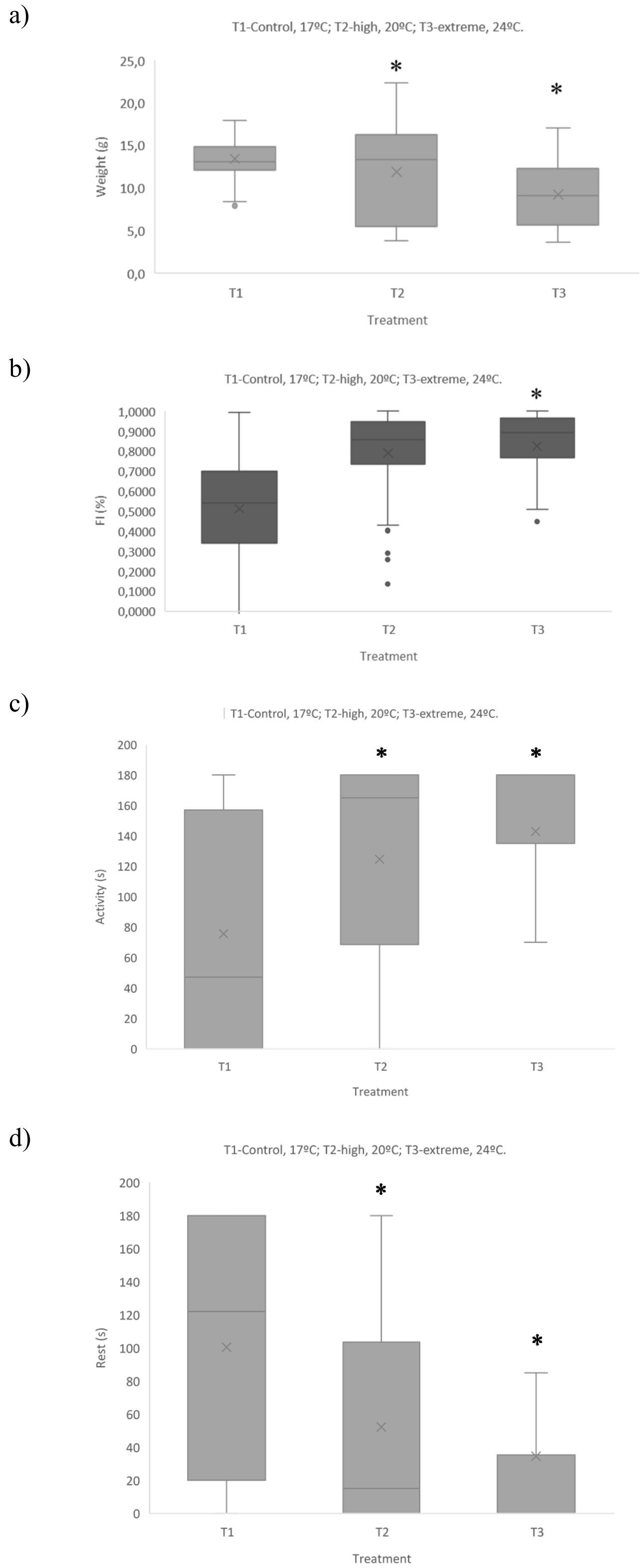

Fig. 1. Effects of temperature increase on *H. guttulatus* a) weight (g); b) FI (%); c) time (s) spent in rest; d) time (s) spent active. Asterisk (*) denotes significant differences from control treatment.

General Remarks

The current study provides clear evidence of warming effects on physiology and behaviour of H. guttulatus. Fish that were kept under extreme temperatures, outside the thermal range they are used to experiencing in their natural habitat, were in poor condition compared to fish under ambient control temperatures, despite the fact they ingested more food. Increased food ingestion under elevated temperature is an expected response to meet the extra energetic demands associated with higher metabolic rates. However, in case of fish under extreme temperatures (24ºC), the increased food ingestion was not enough for fish to support growth (weighted less), which suggests that fish were spending energy to counterbalance the thermal stress they were exposed to. Fish under both elevated temperature treatments (20 and 24ºC) were also spending more energy in active behaviours. Altogether, these data may indicate that ocean warming will have a drastic effect on seahorse populations currently inhabiting the Sado estuary. Due to this influence, it seems urgent to study this population more deeply, monitoring it over a longer period of time, promoting in-situ studies, so that later the population dynamics and resilience can be assessed.

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