Impacts of acute hypoxia on the short-snouted seahorse metabolism and behaviour

Matilde Gomes (mcrsilva@fc.ul.pt)

Vanessa M. Lopes

Monica G. Mai

José R. Paula

Regina Bispo
Center for Mathematics and Applications (NovaMath), FCT NOVA and Department of Mathematics, FCT NOVA, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal.

Hugo Batista
Oceanário de Lisboa, Esplanada D. Carlos I, 1900-005, Lisbon, Portugal.

Catarina Barraca
Oceanário de Lisboa, Esplanada D. Carlos I, 1900-005, Lisbon, Portugal.

Núria Baylina
Oceanário de Lisboa, Esplanada D. Carlos I, 1900-005, Lisbon, Portugal.

Rui Rosa

Marta S. Pimentel
Research Article

Keywords: seahorse, Hippocampus hippocampus, hypoxia, behaviour, metabolism.

Posted Date: May 8th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2899092/v1

License: ☛ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Seahorses are one of the most unique and enigmatic animals, recognized as flagship species for several conservation issues. Unfortunately, seahorses’ populations have been declining worldwide and their unique lifestyle may constrain the ability of these animals to evolve in the future climate scenarios. They inhabit shallow coastal waters that display daily or seasonal environmental fluctuations, Yet, few studies have scrutinized the impacts of climate changes on these iconic species. Within this context, the objective of this work was to test the effects of an extreme hypoxia exposure (~ 27% dissolved oxygen) on the metabolism, behaviour and food intake of the temperate seahorse *Hippocampus hippocampus*. Regarding metabolism, hypoxia exposure led to a significant reduction in metabolic and ventilation rates. Seahorses showed signs of movement lethargy under oxygen depletion. The results show that hypoxia induces metabolic and behavioural changes that may jeopardize the development and survival of these iconic organisms.

Introduction

Since the industrial revolution, the anthropogenic emissions of greenhouse gases led to unnatural changes in the Earth's thermal balance and, consequently, to atmospheric and ocean warming (Duarte, 2014; Harley et al., 2006; IPCC, 2022). These environmental changes may impact animals’ adaptation and survival, even more when considering extreme climate events, such as marine heatwaves and extreme hypoxia, which occur rapidly and may not give the animals enough time to react (Otto, 2018; Somero, 2010). Oxygen depletion represents one of the greatest environmental challenges for marine organisms (Earle et al., 2018; Huang et al., 2018; Sampaio et al. 2021; Borges et al. 2022).

In marine and estuarine systems, extreme hypoxia events are characterized by a low level of dissolved oxygen (DO), normally ≤ 2 mg of O₂ L⁻¹ (the exact concentration is organism-specific; Breitburg et al., 2018; Diaz & Breitburg, 2009). In coastal and shallow waters, these events occur naturally, due to 1) the strong vertical stratification that limits the exchange of oxygen (O₂) between the different layers of water, 2) imbalance between respiration and photosynthesis, especially during the night, 3) the low tide, which forms tide pools in the intertidal zones and 4) other specific circumstances like rain, storms, cloud cover, snow and ice, since they change the water flows and/or block water-air oxygen exchange (Altieri & Gedan, 2014; Breitburg et al., 2018; Diaz & Breitburg, 2009). These conditions are frequently accentuated by eutrophication, often caused by anthropogenic processes (Diaz & Rosenberg, 2008). Oxygen (O₂) availability is known to be strongly related to metabolic processes (Willmer et al., 2005), in normoxic conditions, more than 95% of the oxygen consumed by a fish is used in aerobic processes to produce the energy needed by the organisms' physiological processes. Upon exposure to hypoxia, fish survival depends on the: 1) fast reorganization of the biochemical and physiological system to maximize the oxygen uptake rates and sustain the routine metabolic rate or 2) cellular modifications to produce energy in O₂-limiting conditions, through anaerobic processes (Richards, 2009). This can result in certain
changes in fish swimming, feeding and reproduction since the energy is directed to more $O_2$-sensitive tissues, as reviewed by Farrell and Richards (2009).

Marine ectotherms, especially those that inhabit coastal shallow areas, have developed physiological and behavioural adaptation mechanisms to survive in these habitats that undergo seasonal and daily environmental changes (Coelho et al., 2023; Pigliucci, 2003, Mascaró et al., 2016). Yet, when these mechanisms do not exist or are not sufficient, many animals migrate to more environmentally suitable habitats. In the temperate seahorse *Hippocampus hippocampus* case, due to their specific lifestyle, this can be difficult or even impossible. They inhabit specific biogenic environments, usually close to seagrass areas and/or soft bottoms between rocks and algae, in European shallow coastal areas like estuaries (Curtis et al., 2017; Curtis & Vincent, 2005; Lourie et al., 2004). These structurally complex habitats are very important for seahorses, since they spend most of their time resting, attached by their tail to holdfasts such as seagrass (Lourie et al., 2004). These structures also allow them to camouflage, as they wait motionless for the prey to come close to their snout (Foster & Vincent, 2004). These characteristics, coupled with their poor swimming ability, small home range sizes, small population densities and high site fidelity, decrease the chances of these animals finding suitable habitats. Furthermore, their reproductive characteristics, namely their short generation period, low batch fecundity, male parental and monogamy can also be challenging (Foster & Vincent, 2004), since migration can jeopardize the continuity of generations due to the possible breaking of the pair bond (Faleiro et al., 2015; Foster & Vincent, 2004). The search for a new pair, coupled with the dispersion of populations and the possible lower efficiency of reproduction with the new partner, makes this strategy even more difficult (Faleiro et al., 2015; Foster and Vincent, 2004). Thus, seahorses may have to depend on their physiological capacity to deal with climate change, especially with short-term events, since the adaptation cannot be as gradual as long-term changes (Altieri & Gedan, 2014; Somero, 2010), further minimizing the possibility of migration.

Although several fish species have been shown to be affected by oxygen depletion (e.g. Richards, 2009; Ekau et al., 2010; Wang et al., 2016; Pau et al., 2017), few studies have analyzed the effects of hypoxia on seahorses and pipefishes (Ripley and Foran, 2007; Negreiros et al. 2011). Within this context, the aim of this study was to understand effects of an extreme hypoxia event (~27% DO) on the physiology and behaviour of the seahorse *Hippocampus hippocampus*. More specifically, we aimed to scrutinize the effects of hypoxia on metabolic rates, ventilation rates, behavioural patterns (namely resting, individual and social activities) and food intake.

**Materials and methods**

**2.1 Species collection**

Adult *Hippocampus hippocampus* were laboratory-raised and provided by Oceanário de Lisboa (Lisbon, Portugal). The previous generations of these animals are related to the Sado Estuary (Portugal). From their captive facilities, 7-month-old seahorses (ranging from 0.70 to 3.69 g) born at the Oceanário de
Lisboa were transported under controlled conditions to the recirculating aquaculture systems (RAS) in the Laboratório Marítimo da Guia (LMG, Cascais, Portugal), where the experiment took place.

2.1.1 Ethical statement

The present experiments and analysis were reviewed and authorized by the Portuguese Foundation for Science and Technology (FCT) and the Faculty of Science of the University of Lisbon animal welfare body (ORBEA) in accordance with the requirements imposed by the national (Decreto-Lei 113/2013) and EU legislation (Directive 2010/63/EU) on animal protection used for scientific purposes.

2.2 Experimental setup and acclimatization

Upon arrival at LMG, the animals were first acclimatized to the new captive conditions for one month, during which no experimental trials were carried out. Seahorses were kept in similar conditions to the ones described in previous studies (Aurélio et al., 2013; Faleiro et al., 2015) and those found in their natural environment: temperature 17.0 ± 0.3°C, DO approximately 100% (7.6 ± 0.1 mg of O₂ L⁻¹), salinity 33 ± 1 and pH 8.0 ± 0.1. Ammonia, nitrites, and nitrates concentrations were kept below detected values.

Seahorses were randomly distributed into semi-opened aquaria systems, each composed of three 70-L acrylic aquaria (30 × 41 × 60 cm) and a common water outflow tank (sump). Each system functioned as a treatment, and each aquarium as a replicate. Natural seawater (salinity 35) was pumped directly from the sea, filtered and UV-sterilized, being then pumped from the sump to each aquarium. To adapt the salinity of the water to the needs of these fish, fresh water purified by activated carbon was added daily to the systems. Each sump had a filtration system and was continuously renewed by a water drip system also filtered and UV-sterilized. This type of system design, along with daily 20 to 30% water changes, allowed to maintain water quality. Water temperature was regulated through room temperature and a chiller connected to the sump that allowed the temperature to never rise above the intended value.

Oxygen levels were controlled by an air compressor connected to air diffusers, three in each sump and one in each aquarium (but with a lower diffusion rate). Aquaria illumination was provided through overhead fluorescent lighting, with a photoperiod of 14h of light:10h of dark cycles. Environmental enrichment structures, e.g., artificial plants, plastic chains and nets, were also added to all aquariums to provide holdfast for seahorses’ attachment and to improve the welfare of the animals during the experiments. The fish were fed *ad libitum*, three to five times a day, except the day before the experimental tests. Their diet consisted mainly of frozen food: more frequently and in greater quantity *Mysis*, and less frequently and in smaller quantities enriched adult *Artemia*, copepods and cyclops. Live *Mysis* were introduced as much as possible.

Seahorses were then exposed to two different oxygen level conditions:

1) control, representing the current annual mean environmental conditions of the Sado estuary (n=9, ~100% DO ~ 7.6 mg O₂ L⁻¹); and
2) hypoxia, simulating an extreme decrease in DO in the water; (n=5, ~ 27% DO ~ 2.1 mg O2 L⁻¹).

To apply refinement and reduction of the 3 R’s rules of the ethics of animal experimentation, first introduced by Russell and Burch (1959), individuals from hypoxia treatments were also used under control conditions.

2.2.1 Hypoxia exposure

A nitrogen (N₂) gas injection was used to regulate and maintain the DO around 27% (2.1 mg O₂ L⁻¹). To make sure that the N₂ was sufficient to maintain the desired levels of O₂ during exposure, an Ardoxy controlling system connected to solenoid valves automatically controlled the N₂ flow injected into the water, by injecting N₂ when O₂ levels rise above 27% (2.1 mg L⁻¹) and stopping injecting when the reverse occurs. The hypoxic exposure had a total duration of approximately seven hours, divided into two parts: 1) 5h30 of exposure occurred in the chambers during the oxygen uptake rates (MO₂) measurement and 2) approximately one hour in the aquaria, after the MO₂ measurement. At the beginning of the respirometry trial, after an acclimatization period to the chamber, a slow decrease in O₂ concentration was implemented in the water that filled the chambers until the desired O₂ concentration inside the chambers was obtained. After 5h30 of MO₂ measurement, the fish were again transferred to their aquarium tanks where exposure to hypoxia conditions continued for approximately an hour. To achieve desired dissolved O₂ levels in the aquariums tanks, N₂ was injected in a cylindrical column tank, added to the overall system design, which altered the so far path of the water in the recirculating system. The water was pumped from the sump to the cylinder column and only then to the aquarium.

2.3 Metabolic rates and ventilation rates

Following previous methods (Clark et al., 2013; Paula et al., 2022; Rummer et al., 2016), an intermittent flow respirometry system was used for the measurement of the oxygen uptake rates (MO₂), applied to estimate the standard metabolic rates (SMR). Seahorses were individually placed in 606 ml (including tygon chemical tubing) respirometry chambers which were then completely closed so that there was no external infiltration of O₂. The chambers were submerged in a recirculating system, in a water bath with the same temperature conditions as the respective treatment, ensured by a digital heater and a chiller, regulated by a Profilux controlling system with a temperature probe. Small holdfasts were provided to the seahorse's attachment. Before each trial, a 24-hour period of starvation was implemented in the experimental aquariums, to guarantee a postabsorptive metabolic state (Niimi & Beamish, 1974). During the entire process, the animals were continuously and carefully observed to ensure their well-being.

The MO₂ were measured through seven cycles of 30 minutes, each consisting of a measurement period (25 minutes), a waiting period (1 minute) and a flush period (4 minutes). Each respirometry trial would start around 11:00am and had a duration of 5h30, of which 2h of acclimatization and 3h30 of O₂ measurements, as described in Aurélio et al. (2013). The initial period of 2 hours allowed acclimatization to the new environment and to the desired oxygen concentration in the hypoxia treatments. The total
duration of the measurement period ensures that the O$_2$ levels inside the chambers never went below 80% air saturation (Paula et al., 2022) in the control treatment, ensuring that the measurements of MO$_2$ are not influenced with any sharp metabolic decrease which may affect animal welfare. Between measurement periods, an automated flush pump submerged in a tank with treatment water supplied and renewed the chambers with clean seawater. These pumps were regulated via Profilux controlling system with a programmed timing sequence. The flush period was long enough to allow the oxygen levels in the chambers to be completely renewed with new treatment water.

To ensure the water mixture inside each chamber during the measurement periods, each chamber was connected to an individual respirometry pump that boosted the water through the chamber in an external close loop of gas-tight tubing (flowrate: 100 ml/min). Connected to this tubing, each chamber had a flow-through cell with an integrated optical oxygen sensor linked with fiber-optic cables to a Firesting Optical Oxygen Meter. These sensors recorded the temperature-compensated oxygen concentration (mg L$^{-1}$) of the water every two seconds, saving data on a connected portable computer.

Before each respirometry trial, the temperature and the O$_2$ sensors of the setup were calibrated. To eliminate the influence of possible bacteria and microorganisms’ activity, before and after each respirometry trial, the entire setup was disinfected with hydrogen peroxide, cleaned with fresh water and then refilled with clean and filtered seawater of each treatment. To further minimize the microorganisms’ influence in the MO$_2$ measurements, a background respiration was performed in each chamber, before and after each run, without the respective seahorse inside. Since it was assumed that background MO$_2$ increased linearly (from start to end of each run), the results of the background respiration were then subtracted from the respective seahorse respiration. The whole analysis was made with R software package “respR” (Harianto et al., 2019), where the O$_2$ concentration data was corrected for fish mass [mgO$_2$ g$_{fish}^{-1}$ hour$^{-1}$].

After the respirometry trials, seahorses were transferred to their respective aquaria where the sampling continued. After an acclimatization period of 30 minutes, individual ventilation rates were measured by counting the number of opercular beats per minute. This procedure was repeated three times per individual.

### 2.4 Behavioural patterns

A careful observation during the initial month of acclimatization to captive conditions allowed the formulation of a behavioural ethogram to *H. hippocampus* (Table 1). During the experimental trial, 30-minute videos were recorded to assess seahorses’ activity patterns and their food intake in their original aquarium tanks. The time spent by each seahorse in each category and behaviour was measured and converted into a percentage of total time. Regarding the feeding behaviour, the frequencies of attack, capture and miss were also recorded. Capture and miss variables were then calculated as the percentage of total attack events (based on Drost, 1987; Pimentel et al., 2016). At the beginning of the recordings, 20 live *Mysis* were placed in the aquarium and at the 20-minute mark, 10 frozen ones were added. Food
intake, both live and frozen *Mysis*, was visually counted in the recordings and transformed into percentages of the total *Mysis* amount. Previous studies show that the pacific and careful observation of seahorses does not alter the normal behaviours that these fish exhibit (e.g. Aurélio et al., 2013; Faleiro et al., 2008).

**Table 1**: Ethogram of *Hippocampus hippocampus* activity patterns. The visual observations made during the initial month of acclimatization were complemented with terminologies from Anderson et al., 2011; Faleiro et al., 2008; Felício et al., 2006; Naud et al., 2008; Pimentel et al., 2016; Vincent, 1994.
<table>
<thead>
<tr>
<th>Category</th>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>Stationary</td>
<td>Seahorse remains completely still.</td>
</tr>
<tr>
<td></td>
<td>Swinging</td>
<td>Light head and/or body movements while the seahorse is attached to a holdfast.</td>
</tr>
<tr>
<td>Individual activity</td>
<td>Adjustment</td>
<td>Seahorse often adjusts the tail on the holdfast, rotating or moving vertically along it.</td>
</tr>
<tr>
<td></td>
<td>Slow body movement</td>
<td>Seahorse moves slowly, mainly using the tail and not the dorsal and/or pectoral fins or using them lightly to propel themselves.</td>
</tr>
<tr>
<td></td>
<td>Swimming</td>
<td>Seahorse swims actively, constantly moving the dorsal and pectoral fins.</td>
</tr>
<tr>
<td></td>
<td>Feeding</td>
<td>Seahorse visualizes and approaches the prey, stretching his body while attached to a holdfast or swimming toward it. It directs the snout to the prey and attacks, capturing it or not.</td>
</tr>
<tr>
<td></td>
<td>hg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Capture</td>
<td>When the seahorse catches and ingests the prey.</td>
</tr>
<tr>
<td></td>
<td>Miss</td>
<td>When the seahorse attacks but does not catch the prey.</td>
</tr>
<tr>
<td></td>
<td>Attack</td>
<td>Sum of Capture and Miss behaviours.</td>
</tr>
<tr>
<td>Social activity</td>
<td>Interaction with aggression</td>
<td>Seahorses interact via tail wrestling (especially when one of them is trying to free itself from the tail grasp of the other seahorse), snapping (using the snout or other part of the body) and /or chasing.</td>
</tr>
<tr>
<td></td>
<td>Interaction without</td>
<td>Seahorses from the same sex follow each other;</td>
</tr>
</tbody>
</table>
aggression

swim together around a holdfast, at the bottom of the aquarium tank or in the water column; grab each other's tails or inflated the pouch with water (in males only).

Courtship

Seahorses from different sex approach, bright their colors, hold each other and promenade, raise in the water column (tilting and quivering), copulate, and transfer the oocyte (or attempt to).

2.5 Data analysis

Statistical analysis of all variables was performed in R (version 4.0.2, R Core Team, 2020), via Generalized Linear Mixed Models (GLMM, Zuur et al. 2009). Treatment was used as a fixed factor and individuals as a random factor, to consider the experimental design and account for dependence between observations in the same individual. The random effect was kept in all models regardless of the amount of variation it explained, as recommended by Barr et al., (2013). All models were tested using the “glmmTMB” function from package “glmmTMB” (Brooks et al., 2017). Function “Anova” from the package “car” (Fox & Weisberg, 2011) was used to perform Type II Wald chi-squared tests of each model, to test the significance of each explanatory variable over the response variable. Post-hoc multiple comparisons between treatments were also performed, using the “emmeans” package (Searle et al., 1980), with Tukey corrections to minimize type I error (Lenth, 2022). The package “performance” (Lüdecke et al., 2021) was used to validate the models' performance and assumptions.

Results

2.1 Metabolic rates and ventilation rates

Regarding standard metabolic rates (SMR, Fig. 1a), hypoxia (H) elicited a significant decrease in the SMR of the adult seahorses (p < 0.0001). Hypoxia promoted a significant increase in the ventilation rates (Fig. 1b, p < 0.0001), reaching a value of 55.3 ± 2.6 beats min⁻¹, which represents an increase of 129.5% in comparison to control conditions.

2.2 Behavioural patterns and food intake

The results of the behavioural analysis and food intake are shown in Fig. 2, 3, 4, 5 and 6.

2.2.1 Rest

Seahorses under control conditions spent an average of 80.4 ± 2.5% of their time resting (Fig. 2a), on the other hand, the time they spent resting increased to 98.8 ± 0.5% under H (p = 0.0001), which represented an increase of 22.9%.
Regarding the stationary behaviour (Fig. 2b), exposure to H prompted significant changes in this behaviour (p < 0.0001). The time that the seahorses remained stationary increased from 2.6 ± 1.2% under control conditions to 80.1 ± 4.4% in the hypoxia treatment. Alongside, the percentage of time spent by seahorses swinging (Fig. 2c) was 77.1 ± 3.5 under control conditions, which decreased significantly with hypoxia treatment to 17.9 ± 3.4% under hypoxia (H) (p < 0.0001).

### 2.2.2 Individual activity

Hypoxia treatment did cause a significant change in seahorses’ individual activity (p < 0.0001), from an average of 15.1 ± 1.8% under control conditions to 0.8 ± 0.4% under hypoxia. Following the same trend, the reduction of O\(_2\) led to significant impacts on the slow body movement behaviour (Fig. 3b). The control group of seahorses spent an average of 12.3 ± 1.6% of their time in this behaviour, which decreased significantly to 0.8 ± 0.3% under H (p < 0.0001). No significant differences were found between treatments regarding swimming (Fig. 3c, p > 0.05) and adjustment behaviours (Fig. 3d, p > 0.05). Seahorses feeding duration (Fig. 3e), which lasted an average of 1.9 ± 0.5% under control conditions, decreased significantly to 0.2 ± 0.1% under hypoxia (p = 0.0115).

The number of attacks (Fig. 4a), which includes total live and frozen *Mysis*, did not vary significantly with treatments (p > 0.05). Regarding the capture success (Fig. 4b), hypoxia caused a significant decrease to 0.5 ± 0.4 prey ingested (p = 0.0117). The miss percentage (Fig. 4c) did not follow the same trend since it did not show any significant changes with hypoxia exposure (p > 0.05). Regarding live and frozen food intake (Fig. 5a and Fig. 5b, respectively), there was no significant effect on the live *Mysis* consumption between control conditions and hypoxia treatments (p > 0.05).

### 2.2.3 Social activity

Regarding the social activity of seahorses (Fig. 6a), there was a significant decrease between the control (3.7 ± 1.5%) and hypoxia treatment (1.3 ± 0.6%, p > 0.05). Moreover, there were no significant changes in relation to interaction without aggression and courtship behaviours among treatments (Fig. 6b and 6c, p > 0.05).

### Discussion

#### 3.1 Metabolic rates and ventilation rates

The hypoxia exposure (~27% DO) resulted in a significant decrease in metabolic rates, associated with an increase in opercular beats. Increased ventilation and heart rates and changes in the oxygen binding capacity of hemoglobin combined with an increase of red blood cells, are some of the first physiological responses of some species during periods of oxygen depletion (Wu, 2002). This allows to maximize the O\(_2\) extraction from the environment and maintain the oxygen delivery (Wu, 2002). Negreiros et al. (2011) even detected an elongation of the lamellae on *Hippocampus reidi* exposed to hypoxia, probably associated with a higher blood circulation in the gills, that allows a greater gas exchange efficiency.
However, the results of our study indicate that this primary physiological change observed was not sufficient, so *H. hippocampus* entered a more hypometabolic stage. The metabolic depression, together with a reduction of protein synthesis and of some regulatory enzymes such as the phosphofructokinase of the glycolysis pathway, are some secondary responses and strategies used when O\textsubscript{2} delivery is no longer achieved (Wu, 2002). This allows them to maintain aerobic metabolism with a lower energy demand, which is allocated from non-essential processes to essential maintenance costs (Pörtner and Peck 2010; Rosa & Seibel, 2010). This extends the survival of the organisms during a certain period, but, when the animals are no longer able to tolerate these conditions, changes in the fitness of the animals can occur, which jeopardizes the organism and population development and survival (Vaquer-Sunyer & Duarte, 2008; Sampaio et al., 2021).

### 3.2 Behavioural patterns and food intake

Seahorses have reduced swimming abilities, to what, they spend most of their time resting attached to a holdfast (Foster and Vincent, 2004). This fact was also verified in this study, where control seahorses spend 80% of their time resting. Despite that, this frequency of time changed throughout the experimental exposures. When exposed to hypoxia, seahorses showed signs of movement lethargy, spending about 98% of the time resting. Furthermore, the time seahorses spent resting was divided and analyzed into two different behaviour categories, as they stayed completely stationary (not active at all) or slightly moved their heads, the so-called swinging behaviour (some activity). Both the stationary and swinging behaviours showed a significant increase in hypoxic conditions, which is consistent with the lower activity observed in these animals.

Despite being less frequent, seahorses also have individual activities that involve their movement, from simple adjustments or small movements between holdfasts to active swimming and feeding. Regarding this individual activity, except for adjustment and swimming behaviour, there was only a considerable impact of the hypoxia event. Regarding seahorses feeding behaviours, in situations of oxygen depletion, the animals were significantly less active, which resulted in a decrease in the capture of their prey.

In addition to individual activities, *H. hippocampus* also presents social activities, between the same sex or between different sexes, in this case considering reproduction behaviours. In this study, there was only a decrease in the social activity category, but not in the behaviours associated with it, which may have occurred due to the reduced number of observations and/or the short period of time used for the analysis. Oxygen concentration have already been shown to affect the social behaviours of Syngnathidae (e.g. Lin et al., 2006; Qin et al., 2017, 2018; Sundin et al., 2015), as seen for example in the pipefish *Syngnathus typhle*. An acute exposure to hypoxia (40% dissolved oxygen) prolongs the latency period to courting and copulating of this fish, but it does not alter the probability of mating, the time spent doing it or the different characteristic reproductive behaviours (Sundin et al., 2015).

**Conclusion**
Summing up, as shown for other seahorses’ species, a short but extreme period of oxygen depletion, although tolerable, resulted in a metabolic suppression, coupled with a reduction in the seahorse *H. hippocampus* activity. In the long run, this may imply a cascade of consequences due to changes in the fitness and development of these animals and due to the larger susceptibility to other factors such as predation, starvation and diseases (Portner & Knust, 2007; Wang & Overgaard, 2007). Knowledge of the effects of these events on seahorses are still scares, but are extremely important as the frequency, strength and duration of these events is increasing and is expected to worsen if current climatic conditions continue (Altieri & Gedan, 2014; IPCC, 2022; Oliver et al., 2018). More studies are still needed to elucidate the effects of extreme environmental conditions and how animals may endure under such conditions. Thus, future studies should focus on the combined impacts of climate change on the various species of seahorses, considering both gradual environmental changes such as warming, acidification and deoxygenation, as well as short-term extreme temperature, acidification and hypoxic events. In addition, it will be relevant to understand how the different life stages of these animals will react to climate changes in order to understand the possibility of acclimatization and adaptation of the different generations.

**Declarations**

**CRediT authorship contribution statement**

**Matilde Gomes:** Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - Original Draft, Visualization. **Vanessa Madeira Lopes:** Methodology, Software, Recourses, Investigation, Writing - Review & Editing. **Monica Giacometti Mai:** Methodology, Investigation, Writing - Review & Editing. **José Ricardo Paula:** Methodology, Formal analysis, Software, Resources, Writing - Review & Editing. **Regina Bispo:** Methodology, Formal analysis, Software, Writing - Review & Editing. **Hugo Batista:** Resources, Writing - Review & Editing. **Catarina Barraca:** Resources, Writing - Review & Editing. **Núria Baylina:** Resources, Writing - Review & Editing. **Rui Rosa:** Conceptualization, Validation, Formal analysis, Resources, Investigation, Writing - Review & Editing, Supervision, Project administration, Visualization. **Marta Silva Pimentel:** Conceptualization, Methodology, Validation, Software, Formal analysis, Investigation, Resources, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

**Declaration of competing interest**

The authors have no competing interests to declare.

**Acknowledgements**

The authors would also like to express their gratitude to Oceanário de Lisboa and Aquário Vasco da Gama, and to Filipa Faleiro, António Gomes and José Pereira for the availability to support this work. This study was supported by the FCT - Fundação para a Ciência e a Tecnologia, I.P., through CEEC Individual call (2020.00174.CEECIND to M.S.P, and 2021.01030.CEECIND to J.R.P) and a project grant to M.S.P.
(TRANSFISH – PTCD/BIA-BMA/28647/2017, LISBOA-01-0145-FEDER-028647), and also through the strategic project UIDB/04292/2020 awarded to MARE and project LA/P/0069/2020 granted to the Associate Laboratory ARNET. R.B. work is funded by national funds through the FCT - Fundação para a Ciência e a Tecnologia, I.P., under the scope of the projects UIDB/00297/2020 and UIDP/00297/2020 (Center for Mathematics and Applications).

References


**Figures**
Figure 1

**a)** Oxygen consumption rates (MO$_2$) and **b)** ventilation rates of seahorses Hippocampus hippocampus exposed to the control (C, n=8) and (H, n=7) treatments. Solid black points correspond to predicted means ± 95% confidence intervals. The dots with different colors represent the observations for each treatment (blue for control and green for hypoxia). Significant differences (p-value < $\alpha$, $\alpha = 0.05$) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.
Figure 2

Behavioural patterns of seahorses Hippocampus hippocampus exposed to the control (C, n=9) and hypoxia (H, n=8) treatments. **a)** Rest category, which includes **b)** stationary and **c)** swinging behaviours. Solid black points correspond to predicted means ± 95% confidence intervals. The dots with different colors represent the observations for each treatment (blue for control and green for hypoxia). Significant differences (p-value < \( \alpha \), \( \alpha = 0.05 \)) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.
Detailed behavioural patterns of seahorses Hippocampus hippocampus exposed to the control (C, n=9) and hypoxia (H, n=8) treatments. **a)** Individual activity category, which includes **b)** slow body movement, **c)** swimming, **d)** adjustment and **e)** feeding behaviours. Solid black points correspond to predicted means ± 95% confidence intervals. The dots with different colors represent the observations for each treatment.
(blue for control and green for hypoxia). Significant differences (p-value < α, α = 0.05) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.

Figure 4

Feeding behaviour patterns of seahorses Hippocampus hippocampus exposed to the control (C, n=9) and hypoxia (H, n=8 treatments. a) Percentage of attacks, b) capture and c) miss behaviours. Solid black points correspond to predicted means ± 95% confidence intervals. The dots with different colors represent the observations for each treatment (blue for control and green for hypoxia). Significant differences (p-value < α, α = 0.05) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.
Figure 5

Food intake of seahorses Hippocampus hippocampus exposed to the control (C, n=9), and hypoxia (H, n=8) treatments. **a** Percentage of live Mysis ingested. **b** Percentage of frozen Mysis ingested. Solid black points correspond to predicted means ± 95% confidence intervals. The dots with different colors represent the observations for each treatment (blue for control and green for hypoxia). Significant differences (p-value < \( \alpha \), \( \alpha = 0.05 \)) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.
Figure 6

Behavioural patterns of seahorses Hippocampus hippocampus exposed to the control (C, n=9) and hypoxia (H, n=8) treatments. **a)** Social activity category, which includes **b)** interaction without aggression and **c)** courtship. Solid black points correspond to predicted means ± 95% confidence intervals. The dots with different colors represent the observations for each treatment (blue for control and green for hypoxia). Significant differences (p-value < α, α = 0.05) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.