UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL

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

Matilde Juliana Duarte Gomes

Mestrado em Ecologia Marinha

Dissertação orientada por:
Doutora Marta Pimentel
Professor Doutor Rui Rosa

2022

Dedico-a a ti, por me teres acompanhado. Ahoy!

Agradecimentos

É incrível como tudo isto aconteceu. Um sonho que começou com 10 anos de idade, tornou-se real neste momento. Não foi um caminho totalmente fácil e como em tudo houve vários desafios a superar, mas chegou a um resultado que fico bastante feliz de mostrar. Mas não cheguei a ele sozinha, e é a essas pessoas que tenho a agradecer. Desde quem esteve comigo na primeira linha de combate, a quem me ajudou quando não sabia que caminho seguir, a quem simplesmente sorriu e me cumprimentou na rua em dias menos bons.

Gostaria de agradecer aos meus orientadores, porque sem vocês esta experiência nunca teria acontecido. Obrigado Marta e Rui, por me aceitarem e apoiarem, ensinarem todo o vosso conhecimento e por me proporcionarem a oportunidade de trabalhar ao vosso lado na Guia. E um especial obrigado por este bichinho da ciência, que conto continuar a manter na minha vida, tendo-vos a vocês como exemplo.

À Kuka, pela ajuda permanente no projeto. Obrigado por todos os momentos, todos os conselhos, a paciência e motivação nos momentos de desespero. Obrigado, amiga, por me incluíres na tua vida.

Agradeço à Mônica, por garantir que este projeto se concretizava. Obrigado pela tua boa disposição, por estares sempre lá e arranjares solução, e pelo teu saber, que sem dúvida vou levar para a vida.

À restante equipa do Laboratório Marítimo da Guia, Sandra, Maria Rita, Zé, Eve, Tiago, Bea e Mélanie, por me receberem tão bem, me ajudarem sempre que necessário e por me incluírem nos vossos próprios projetos. Obrigado por tornarem a experiência ainda melhor.

Agradeço aos amigos que conheci e me acompanharam nestes dois anos, especialmente à Samra, Andreia, Tiago e Vasco. Partilharam a vossa experiência e estiveram ao meu lado na minha, e por isso obrigado.

Obrigado a CMA, por me ouvirem e me aconselharem, mas também por me distraírem quando necessário. E obrigado a todos os meus amigos bracarenses, pelo orgulho que mostram em mim, o qual só tenho a dizer que é recíproco.

Já à minha família, o maior dos obrigados. Em especial, obrigados pais e Miguel. Foram momentos difíceis, onde nem toda a gente, incluindo eu, acreditou. Mas vocês sempre o fizeram, sem qualquer questão. Obrigado pelo amor, por me darem o vosso todo, por simplesmente existirem. É por vocês e com vocês que sigo este caminho.

Por último gostava de agradecer à FCT por financiar este projeto (PTDC/BIA-BMA/28647/2017), ao Oceanário de Lisboa em especial à Catarina Barraca, Hugo Batista e Núria Baylina, e também à Filipa Faleiro, António Gomes e ao José Pereira, por proporcionarem o suporte necessário para a realização deste projeto.

Abstract

Seahorses are teleost fish with unusual anatomical and behavioural characteristics, that make them one of the most unique and enigmatic animals. They are recognized as flagship species for several conservation issues. Unfortunately, seahorses' populations have been declining worldwide, mainly due to anthropogenic pressure. Their unique lifestyle, namely, their reduced swimming ability, small distribution, high site fidelity and monogamy, constrains their ability to migrate, adapt and evolve in future climate scenarios, especially when exposed to short-term extreme events such as marine heatwaves and acute hypoxia. Yet, seahorses inhabit shallow coastal waters that display daily or seasonal fluctuations of temperature and oxygen, which suggests that these animals may show some phenotypic plasticity to these environmental changes. Within this context, the objective of this dissertation was to test the individual and combined effects of a category II (strong) marine heatwave (21.5°C) and an extreme hypoxia exposure (~27% dissolved oxygen) on the metabolism, behaviour and food intake of the temperate seahorse *Hippocampus hippocampus*. Concomitantly, the impact of the marine heatwave on the number and size of seahorses oocytes was also examined. Regarding metabolism, hypoxia exposure, alone or accompanied by the marine heatwave, led to a significant reduction in metabolic and ventilation rates. The metabolic rates under the combined treatment were slightly higher than that of those exposed to hypoxia, which may indicate a temperature compensation in relation to oxygen depletion. The marine heatwave treatment led to a significant increase in ventilation rates and food intake, but did not change seahorse behavioural patterns. In contrast, seahorses showed signs of movement lethargy whenever there was oxygen depletion. The number and size of the oocytes increased significantly with increasing temperature. The results show that, although seahorses may have some adaptation elasticity to heat stress, an extreme decrease in dissolved oxygen results in metabolic and behavioural changes that may jeopardize the development and survival of these iconic organisms. The two stressors together have similar impacts to those observed in the hypoxia treatment, which suggests that oxygen depletion elicits greater biological effects than strong marine heatwaves.

Keywords: *Hippocampus hippocampus*, marine heatwave, hypoxia, behaviour, metabolism.

Resumo alargado

Ao longo dos últimos séculos, sobretudo a partir da revolução industrial, a concentração de gases com efeito de estufa, sobretudo dióxido de carbono (CO_2), aumentou consideravelmente devido ao aumento da pressão antropogénica. Este aumento resultou em alterações no equilíbrio térmico da Terra, essencial para existência de vida, o que levou à acumulação de calor na atmosfera e consequentemente ao aquecimento global. O oceano global, estando em contacto permanente com a atmosfera, absorve uma grande quantidade desse CO_2 e excesso de calor. Deste modo, tem-se verificado alterações ambientais significativas no oceano global, tais como o aquecimento e desoxigenação, e um aumento da frequência, intensidade e extensão dos eventos extremos, como ondas de calor e eventos extremos de hipóxia. As ondas de calor marinhas são anomalias discretas de temperatura acima do limiar do percentil 90 de um período climatológico de 30 anos, que persistem por pelo menos cinco dias consecutivos. Os eventos extremos de hipóxia caracterizam-se por níveis baixos de oxigénio (O_2) dissolvido que, em ambientes marinhos e estuarinos, é normalmente inferior a 2 ml de O_2 L^{-1}. Nas águas costeiras, estes eventos ocorrem naturalmente devido, sobretudo, à forte estratificação vertical que limita as trocas de O_2 entre as diferentes camadas de água.

A temperatura e o O_2 são bastante importantes para o desenvolvimento dos peixes. Por um lado, a temperatura está relacionada à energia cinética das moléculas ao que, um aumento da temperatura leva ao aceleramento das reações biogeoquímicas. Assim os animais apresentam uma temperatura ótima na qual a produção de energia e todos os restantes processos biológicos estão na sua taxa máxima. Para além desta temperatura, e atingidos os limites térmicos críticos, o metabolismo, crescimento, alimentação, reprodução e comportamentos podem ser negativamente afetados. Por outro lado, a maior parte do O_2 captado pelos peixes é utilizado também para a produção de energia. Em momento de depleção de oxigénio, o organismo tem de se reorganizar de forma a maximizar a captação do mesmo, alterando por exemplo as taxas de ventilação e os seus comportamentos. No entanto, a temperatura e o O_2 podem ainda apresentar efeitos sinergísticos entre eles, uma vez que a solubilidade do O_2 na água é inversamente proporcional à temperatura da mesma.

Os cavalos-marinhos (família Syngnathidae) são peixes teleósteos com características anatómicas e comportamentais incomuns, o que os torna animais únicos e enigmáticos, reconhecidos como espécies bandeira em diversas questões de conservação. Infelizmente, as populações de cavalos-marinhos têm diminuído em todo o mundo, principalmente devido à pressão antropogénica, levando à inserção de várias espécies na Lista Vermelha da União Internacional para a Conservação da Natureza, bem como em outras listas de espécies ameaçadas. O seu estilo de vida único, nomeadamente, a sua reduzida capacidade natatória, pequena distribuição, elevada fidelidade ao seu habitat e monogamia, limita a capacidade destes animais de migrar, adaptar-se e evoluir se as condições climáticas atuais se mantiverem ou agravarem, especialmente eventos extremos de curta duração como as ondas de calor marinhas e hipóxia. No entanto, a distribuição destes animais ectotérmicos por águas costeiras rasas, que exibem flutuações diárias ou sazonais de temperatura e O_2, leva à suposição de que os cavalos-marinhos podem apresentar alguma plasticidade fenotípica a essas mudanças ambientais.

O presente estudo tem como objetivo perceber os efeitos individuais e combinados de uma onda de calor marinha de categoria II (21,5°C) e um evento extremo de hipóxia (~27% de oxigénio dissolvido) no metabolismo, padrões comportamentais e ingestão de alimento do cavalo-marinho temperado *Hippocampus hippocampus*. Mais especificamente, foram analisadas as taxas metabólicas e de ventilação, bem como a sensibilidade térmica (Q_{10}), calculada a partir das taxas metabólicas. Relativamente aos padrões comportamentais, foram analisados três categorias: 1) descanso, onde os animais permanecem completamente imóveis ou com pequenas movimentações da cabeça; 2) atividades individuais, como pequenos ajustes nas suas estruturas de ancoragem e suporte, comportamentos natatórios e comportamentos associados à alimentação e 3) atividades sociais, que inclui comportamentos de interação entre animais, com recurso ou não a agressão, entre animais do mesmo sexo ou de sexos diferentes (comportamentos associados à reprodução). Adicionalmente, foi analisado o impacto da onda de calor marinha no número e na dimensão dos oócitos destes mesmos animais.

O aumento da temperatura, apesar de ter resultado num aumento significativo das taxas de ventilação, não afetou significativamente as taxas metabólicas destes animais. No geral, os padrões comportamentais também não sofreram alterações, apenas se verificando um aumento significativo da ingestão de alimentos devido provavelmente a um ligeiro aumento na demanda energética. O número e tamanho dos ovos aumentaram significativamente com o aumento da temperatura. Assim, supõe-se que a temperatura em questão é tolerável pelos cavalos-marinhos, podendo até estar perto de ser uma temperatura ótima para o seu desenvolvimento. O evento de hipóxia, por outro lado, levou a uma supressão metabólica acentuada, verificando-se uma diminuição significativa das taxas metabólicas e de ventilação. Para além disso, os diferentes comportamentos foram negativamente afetados por esta condição ambiental, levando os cavalos-marinhos a apresentaram sinais de letargia de movimento, com atividades muito reduzidas. A alimentação não sofreu alterações com a hipóxia em relação ao controlo. O número e o tamanho dos oócitos aumentaram significativamente com o aumento da temperatura.

Os resultados mostram que, embora os cavalos-marinhos possam ter alguma elasticidade de adaptação ao stress térmico provocado, uma diminuição extrema do oxigénio dissolvido pode resultar em alterações metabólicas e comportamentais que comprometem o desenvolvimento e a sobrevivência destes organismos. Os efeitos combinados destes dois fatores ambientais foram bastante semelhantes aos observados no tratamento com hipóxia, o que sugere que a hipóxia é o principal condutor das alterações biológicas verificadas.

Palavras-chave: *Hippocampus hippocampus*, onda de calor marinha, hipóxia, comportamento, metabolismo.

List of abbreviations, acronyms and symbols

C	Control
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CO_2	Carbon dioxide
DO	Dissolved oxygen
ENSO	El Niño-Southern Oscillation
GLMM	Generalized Linear Mixed Models
GHG	Greenhouse gases
H	Hypoxia
Ht	Total height
IUCN	International Union for Conservation of Nature
LMG	Laboratório Marítimo da Guia
MHW	Marine heatwave
MHW+H	Marine heatwave combined with hypoxia
MO_2	Oxygen uptake rates
N_2	Nitrogen
NAO	North Atlantic Oscillation
$NH4^+$	Ammonia
NO^-_2	Nitrate
NO^-_3	Nitrite
NOAA	National Oceanic and Atmospheric Administration
O_2	Oxygen
OMZ	Ocean oxygen minimum zones
OSPAR Convention	Convention for the Protection of the Marine Environment of the North-East Atlantic
Q_{10}	Thermal coefficient
RAS	Recirculating aquaculture systems
SMR	Standard metabolic rates
SST	Sea surface temperature
TCM	Traditional Chinese Medicine

Table of contents

List of figures and tables

1. Introduction

Earth has the ideal climate for the existence of life, owing to its exact position in the solar system and due to the natural greenhouse gases (GHG) in the atmosphere, which help to maintain the global average temperature in a suitable range (Karl & Trenberth, 2003; Kweku et al., 2018). Nevertheless, climate changes still occur naturally due to, e.g., solar or astronomical forces and processes, volcanic eruptions and earthquakes (Duarte, 2014) or ocean-atmospheric oscillations such as seasonal cycles, inter-annual patterns like the El Niño-Southern Oscillation (ENSO), inter-decadal phenomena including the North Atlantic Oscillation (NAO), and multi-millennial alterations such as glacial and interglacial periods (Duarte, 2014; Harley et al., 2006). However, over the past few centuries, human activities have become a significant component of this system, causing a cascade of physical and biogeochemical modifications in the seas and oceans (Doney, 2010; Duarte, 2014; Harley et al., 2006). This climatic change has become more evident since the industrial revolution in the 19th century, mainly due to GHG emissions, especially carbon dioxide (CO_2). Since then, the total anthropogenic GHG emissions have increased every year to what, in 2020, the annual average concentration of CO_2 in the atmosphere was 413 ppm (WMO, 2022), the highest recorded value in at least two million years (IPCC, 2021).

The ocean acts as a shield for the planet, absorbing the large amount of CO_2 produced by human activities, as well as the excess heat trapped in the atmosphere. As a result, three major challenges have emerged globally for marine ecosystems: warming, acidification and loss of oxygen (Altieri & Gedan, 2014; IPCC, 2022). If the GHC emission maintains the same trend until 2100, the sea surface temperature (SST) could increase by 3 to 4°C, the pH could decrease by about 0.4 units and the dissolved oxygen (DO) could decrease by approximately 11% (IPCC, 2022). In addition to the gradual impacts of the three challenges mentioned, the frequency, strength and extent of extreme events will also increase, with short-term warming, acidification and extreme hypoxia events occurring (Altieri & Gedan, 2014; Burguer et al., 2020; IPCC, 2022; Oliver et al., 2018). These factors are likely to have a greater impact on species than gradual changes alone (Oliver et al., 2018).

1.1. Marine heatwaves

Heatwaves, an extreme temperature events characterized by periods of excessively hot weather, occur frequently in the atmosphere (Perkins & Alexander, 2013) but also in marine environments, in this case known as marine heatwaves (MHWs; Hobday et al., 2016). Despite being similar, they present small differences among them, as processes occurring in the ocean have naturally longer time scales of variability than in the atmosphere (Hobday et al., 2016). Thus, following the qualitative definition of Hobday et al. (2016), MHWs are defined as discrete temperature anomalies above the 90th percentile threshold of a 30-year climatological period that persist for at least five consecutive days. MHWs with intervals of two days or less are considered part of the same event. The use of percentiles thresholds allows temperature anomalies to be quantified based on local variability, time of the year and the temperature regime of the marine environment (Agusti et al., 2020; Hobday et al., 2016). These anomalies, as the definition implies, can occur in various marine environments, which can include subsurface waters, estuarine areas, enclosed seas or, depending on the case study, intertidal zones (Hobday et al., 2016). Vertically, these events can spread through hundreds of meters in depth (Benthuysen et al., 2018).

Quantitatively, each MWH can be characterized by four features: its intensity (or magnitude), duration, rate of evolution and spatial extent (Figure 1). The duration represents the time from the start to the end date of the MHW, that is, the period during which the temperature remains above the seasonally varying threshold value. Intensity refers to the temperature anomaly above the climatology throughout the event duration, expressed as a maximum, a variation, a mean or a cumulative value. The rate of evolution evaluates the rate of temperature change from the onset of the MHW to the maximum intensity (rate of onset) and from this to the end of the MHW (rate of decline; Hobday et al., 2016; Spillman et al., 2021). The spatial extent can be described as the ocean area where the event occurred or the length of the coastline (Hobday et al., 2016). Furthermore, it is possible to analyze a certain period of interest through the frequency of MHWs or the total number of MHW days in that specific period (Hobday et al., 2016; Oliver et al., 2018).

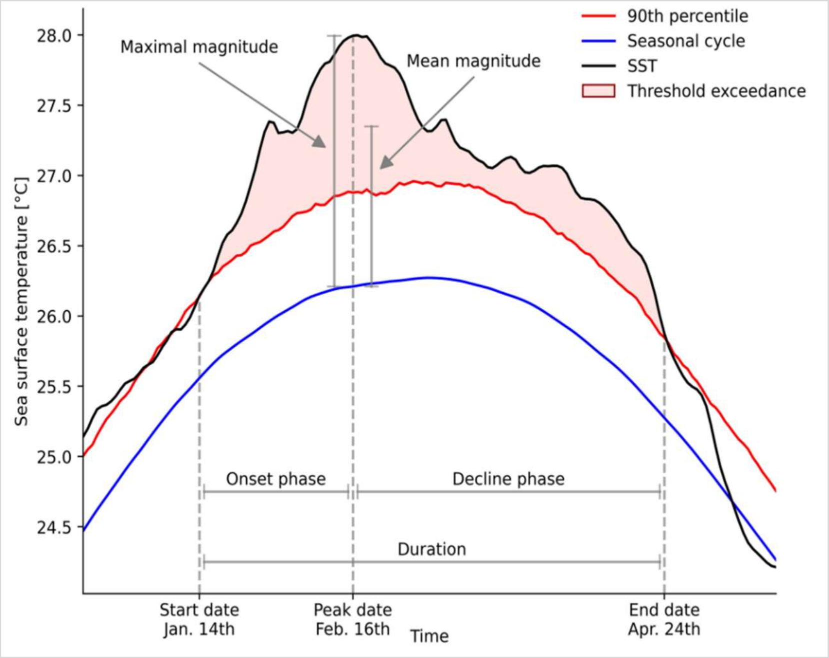

Figure 1: Schematic figure demonstrating the different metrics used in the definition of a MHW. The red-shaded area above the 90th percentile threshold (red line) of the climatological mean (blue line) indicates the MHW. The period between the star date and the end date represents the duration of the MHW, in which the onset phase (since the star date until de peak date) and decline phase (since the peak date until the end date) were identified. Also shown is the maximum magnitude (maximum sea surface temperature) and the mean magnitude (averaged sea surface temperature over the duration of the MHW; source: Vogt et al., 2022).

To further classify MHWs, a scheme was established based on multiples of the difference between the climatological mean and the climatological 90th percentile (Figure 2). From that, four categories were created based on their maximum intensity measure: Category I, defined as moderate (1-2x the 90th percentile difference); Category II, defined as strong (2-3×); Category III as severe (3-4×) and Category IV as extreme (4-5×; Hobday et al., 2018). MHWs have several significant, devastating and long-lasting ecological and economic impacts on marine ecosystems and the goods and services they provide (Frölicher & Laufkötter, 2018; Oliver et al., 2018). Some of the impacts include altered primary productivity and changes in nutrient transportation due to the increased ocean stratification; displacement, loss and replacement of ocean habitats; changes in the habitat ranges and structure of populations of different marine groups; mass mortality and fishery and aquaculture disasters (Bond et al., 2015; Cavole et al., 2016; Oliver et al., 2017; Wernberg et al., 2016).

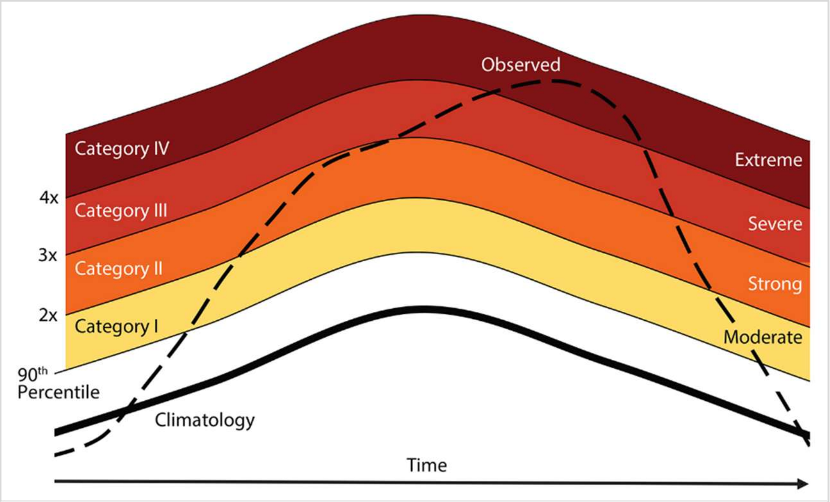

Figure 2: Scheme for marine heatwaves (MHWs) categorization. Multiples of the difference between the mean climatological value and the 90th percentile (2× twice, 3× three times, 4× four times) define the MHW categories. The climatological mean (bold line) and the 90th percentile threshold (thin line) can be observed. The observed temperature time series (as an example; dashed line) shows that it is a category IV MHW (source: Hobday et al., 2018).

1.2. Hypoxia

Since the 20th century, there has been a continuous decrease in DO of both coastal areas and open ocean (Breitburg et al., 2018; Diaz & Rosenberg, 2008). This results in open-ocean oxygen minimum zones (OMZs; Breitburg et al., 2018; Diaz & Breitburg, 2009; Stramma et al., 2008) and coastal dead zones (Figure 3; Altieri & Gedan, 2014; Breitburg et al., 2018; Diaz & Breitburg, 2009). In coastal waters, hypoxia events occur when the oxygen (O_2) supply is not enough to sustain the life of the ecosystem, i.e., there is a negative difference between the physical transport, air-sea fluxes and photosynthesis processes that provide O_2, and the biogeochemical consumption of it (Breitburg et al., 2018; Diaz & Breitburg, 2009). In marine and estuarine systems, these events are characterized by a low level of DO, normally ≤ 2 mg of O_2 L^{-1} (the exact concentration is organism specific; Diaz & Breitburg, 2009).

The concentration of O_2 in water bodies varies over time (Stramma et al., 2008), due to a variety of factors. Compared to the atmosphere, the O_2 in the water has a relatively low diffusion and solubility, which is highly dependent on three characteristics: salinity and temperature, both inversely proportional to the solubility, and pressure, which increases side by side with the solubility (Xing et al., 2014). In coastal areas, low O_2 is mainly a physically driven process due to the presence of a strong vertical stratification which limits the mixing of oxygen-deprived deep waters with oxygen-rich surface waters. This condition is often accentuated by eutrophication, which leads to a mass overgrowth and death of algae in the surface water, which then sinks and joins the flow of organic matter in the seabed (Diaz & Rosenberg, 2008). In shallow waters and coral reefs, natural events of nocturnal hypoxia can occur, due to the balance between photosynthesis production of O_2 and respiration, since production is higher during the day but lower at night (Diaz & Breitburg, 2009; Nilsson et al., 2006). Additionally, organisms that inhabit the intertidal zones may experience moments of hypoxia during low tide in tide pools (Burnett, 1997). Other specific circumstances that can influence the DO concentrations are the daily or seasonal climate conditions like rain, storms, cloud cover, snow and ice, by changing water flows and/or blocking water-air oxygen exchange (Altieri & Gedan, 2014; Diaz & Breitburg, 2009). These factors, in combination with each other, can lead to hypoxia events and their persistence (Diaz & Breitburg, 2009).

Anthropogenic pressure has made coastal and open ocean ecosystems even more vulnerable to the development of hypoxic zones (Diaz & Breitburg, 2009). In 1960, the number of systems with hypoxia conditions was lower than 50, a value that has increased to 400 nowadays, strongly associated with human activities (Diaz & Rosenberg, 2008). Furthermore, Altieri and Gedan (2014) predicted that 94% of these areas will likely experience an increase of about 2ºC by the end of 2100, that will result in a direct decrease in O_2 solubility and in an increase in the vertical stratification of the water bodies (Diaz & Breitburg, 2009). Thus, temperature is the climate-related factor that may have the greatest impact on the expansion of the duration and spatial extent of hypoxia events (Altieri & Gedan, 2014). Yet, the land use changes will also affect the future status of hypoxia. In a lot of coastal dead zones, one of the biggest causes of hypoxia seems to be eutrophication (Diaz & Rosenberg, 2008), since nutrient discharges from agriculture and sewage increased by 43% from 1970 to 2000 (Seitzinger et al., 2010).

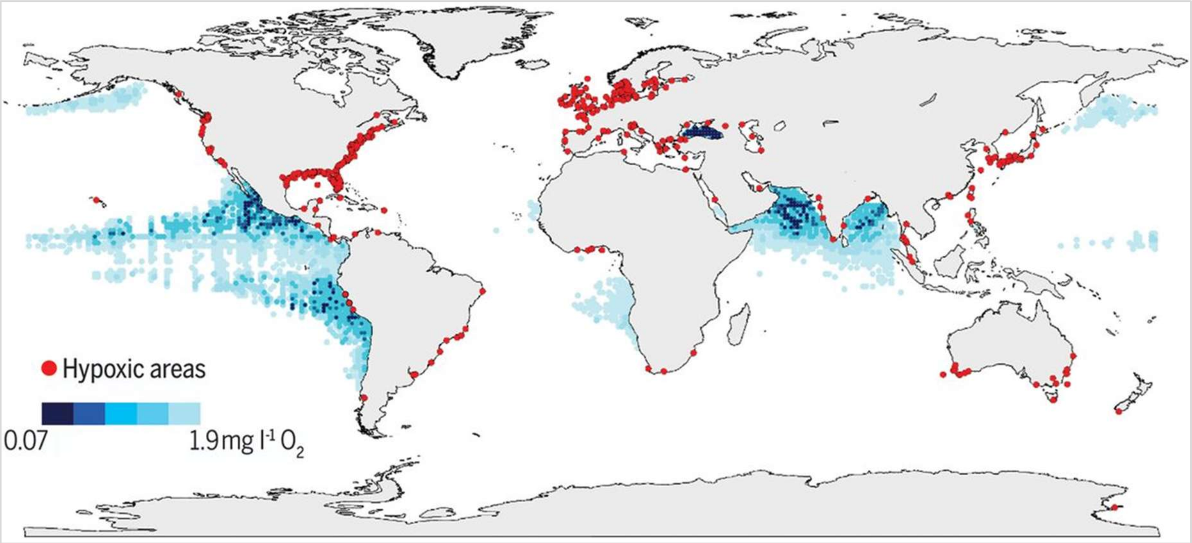

Figure 3: Map with the global distribution of oxygen-depleted zones. The red dots represent the coastal areas where the oxygen concentrations reached values <2 mg L^{-1} and the shaded blue regions represent the OMZs at 300 meters of depth (source: Breitburg et al., 2018).

Coastal dead zones have significant consequences for marine ecosystems' resilience, affecting their biodiversity and the services they provide (Altieri & Gedan, 2014; Diaz & Rosenberg, 2008). The most severe negative effects include habitat compression and the loss of fauna, especially of bottom-dwelling and benthic organisms, low primary productivity and changes in the nutrient cycling. From there, many other consequences arise, one of the most important being the negative effect on fisheries (Farrell & Richards, 2009). Although the impacts of low oxygen are crucial on marine life, its interaction with other environmental stressors (e.g., warming and acidification) are often neglected (Borges et al. 2022; Sampaio et al. 2019, 2021).

1.3. Impacts of temperature and hypoxia on fish physiology

Prior to the industrial revolution, when the chemical flow between the ocean and the atmosphere was controlled by long non-anthropogenic processes, most animals were able to evolve and adapt (Otto, 2018). The current anthropogenic pressure may impact animals' environmental adaptation, even more when considering extreme climate events, which occur rapidly and may not give the animals enough time to react (Otto, 2018; Somero, 2010).

Within the cells of each organism there are biochemical reactions that catabolize all the macromolecules (carbohydrates, lipids and proteins) to produce the energy essencial for organisms' physiological processes, essential to their development and survival. To compensate, other macromolecules are being produced at the same time by anabolism, which leads us to the so-called metabolic rate, i.e., 'a measure of the total energy metabolized by an animal in unit time' (Willmer et al., 2005). This rate depends on several factors such as activity and resting patterns, body weight, developmental stage, age, diet, but temperature and O_2 availability are the focus of this case study (Pörtner and Peck 2010; Richards, 2009; Willmer et al., 2005).

Temperature is directly related to the kinetic energy of the molecules so, up to a certain point, an increase in temperature results in an increase in molecular vibration, which increases the biochemical reactions velocity (Willmer et al., 2005). Thus, within their thermal tolerance range (between minimal and maximal critical temperatures), fish can normally adapt and maintain their biological processes functioning at an acceptable rate, due to reversible behavioural and physiological acclimation responses (Figure 4; Pörtner & Farrell, 2008; Willmer et al., 2005). Inside this range, their performance is maximum at an optimal temperature. But as the temperature approaches increasingly the critical limits, fish metabolism, growth, feeding, reproduction, and behaviour can be negatively affected. Once the lower and upper critical temperatures are reached, the probability of organisms' survival decreases (Roessig et al., 2004; Somero, 2010). Thermal sensitivity varies with species and populations, being associated with their geographical distribution (Frederich et al., 2000; Roessig et al., 2004). In some cases, even a slight increase of 1°C can result in have severe damages (Brierley & Kingsford, 2009).

Besides temperature, there is a strong relationship between O_2 availability and the metabolism processes, with the measure of the oxygen uptake rates (MO_2) being the most used way to measure the metabolic rate (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 MO_2 and sustain the routine metabolic rate (e.g., changes in behaviour, ventilation rates, bonds between haemoglobin and oxygen and cardiovascular function) or 2) cellular modifications to produce energy in O_2-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).

Furthermore, O_2 solubility is dependent on the water temperature (Xing et al., 2014) which can result in synergistic effects between the two climatic variables (McBryan et al., 2013; Pörtner et al., 2004). Many species can resist and overcome these effects unless a certain temperature, the *pejus* temperature, is exceeded (Figure 4). At this point, O_2 transport is at its maximum and cannot keep up with the progressive increase in metabolism (Frederich et al., 2000; Pörtner et al., 2004).

Thus, warming in combination with DO decrease in the surrounding environment can result in several effects on fish physiology and behaviour (on the organism level), and on their geographical distribution (on the population level) due to changes in their biochemical reaction rates (Roessig et al., 2004; Clarke & Johnston, 1999).

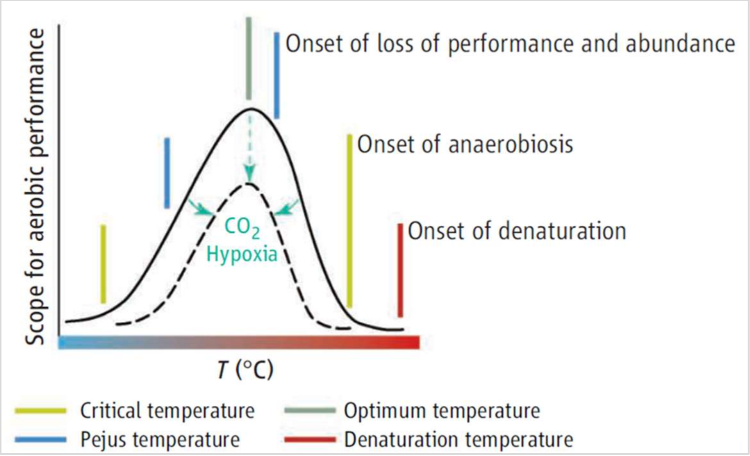

Figure 4: Schematic representation of the thermal windows of aerobic performance of an ectotherm. At an optimal temperature the performance is maximum. The minimal and maximal critical temperatures define an organism's thermal tolerance. At the denaturation temperature, tolerance becomes difficult and depends on the exposure time (source: Pörtner & Farrell, 2008).

1.4. Seahorses

Seahorses (*Hippocampus* spp.) belong to the enigmatic and recognizable Syngnathidae family, along with pipefishes, pipehorses and seadragons (Teske et al., 2004). They stand out from other teleost fish mainly due to their unusual anatomical and physiological features (Curtis, 2006; Foster & Vincent, 2004).

Regarding their morphology, seahorse species can reach about 20 to 300 mm in total height (Ht, from the coronet to the tip of the straightened tail; Figure 5), with no considerable differences between males and females. These animals present some peculiar features, from horse-shaped heads with a tubular snout, eyes that rotate independently, a brood pouch (only in males), absence of scales but the presence of bony plates and prehensile tails (Figure 5; Curtis, 2006; Foster & Vincent, 2004; Teske et al., 2004). Still different from the general morphology of other teleost fish, seahorses have only two small pectoral fins in their head, for stabilization and steering, one dorsal fin for propulsion and a small anal fin (Figure 5; Foster & Vincent, 2004). In addition, they have a great ability to camouflage due to their variability in color and the presence of skin filaments that extend from the spines, which facilitates both predation and predator avoidance (Curtis, 2006; Foster & Vincent, 2004).

Seahorses have a circumglobal distribution (Žalohar et al., 2009), inhabiting the temperate and tropical shallow coastal waters (Foster & Vincent, 2004). Overall, the most common habitats are seagrass and algae meadows for temperate species or coral reefs for tropical species (Foster & Vincent, 2004). All these structures serve as possible holdfasts for seahorses' attachment with their prehensile tails (Foster & Vincent, 2004). Their lifestyle is completely dependent on this behaviour (Correia et al., 2015). Some species can also be found in mangroves, estuaries, and lagoons. Most seahorses tend to have small home range sizes, low population densities and high site-fidelity (Lourie et al., 2004).

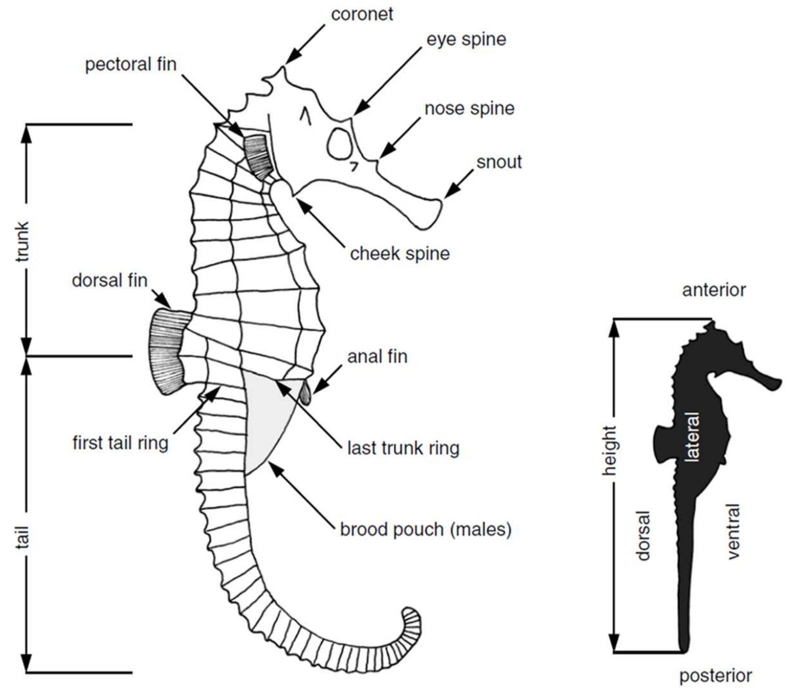

Figure 5: Illustration of the external morphology of a seahorse and the morphometric measurement height (source: Lourie et al., 2004).

Seahorses are mostly ambush carnivores as they wait motionless for the prey to come close to their snout. Then, the prey is drawn up due to a rapid water suction mechanism. Taking this into account, they eat various organisms as long as they fit into their snouts, especially small crustaceans, nematodes, polychaetes and small fish. Their digestive tract does not have a differentiated stomach, to what they need to eat constantly (Foster & Vincent, 2004).

Most seahorse species mature at around four months to one year of age and are monogamous at least within a single breeding cycle (Lourie et al., 2004). Over multiple cycles with the same partner, reproductive efficiency can increase, allowing for less time spent in courtship and larger broods (Foster & Vincent, 2004). They present conventional sex roles, where the female carefully chooses the male that competes more intensely (Vincent, 1994). Mating starts with a set of courtship behaviours called greetings, with both lightening their skin and actively chasing and swimming together (Figure 6A). As the courtship progresses, the pair begins to swim higher and higher in the water column (Figure 6B), until the female manages to deposit the oocytes in the male's brood pouch (Figure 6C; Vincent & Sadler, 1995). Inside the pouch, the eggs are fertilized, protected and nourished in a regulated environment until the offspring are released (Lourie et al., 2004; Vincent & Sadler, 1995). The young look like miniature adult seahorses, totally independent after birth (Figure 7A; Lourie et al., 2004).

Seahorses’ reproductive characteristics, namely their short generation period, low batch fecundity, monogamy and male parental care (Foster & Vincent, 2004), make these animals more vulnerable to environmental changes, which is worsened by overexploitation and/or other sources of threats, (Curtis et al., 2017). Furthermore, their high site-fidelity and small home ranges make them extremely vulnerable to habitat destruction (Curtis et al., 2017; Lourie et al., 2004). Living in coastal areas that are heavily impacted by human activity and all these peculiar characteristics help us to understand why many species have been included in the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN), as well as in other international and national lists of threatened species (Foster & Vincent, 2004; Vincent et al., 2011). Seahorses are now vulnerable due to the degradation of their habitats (by action of pleasure craft and dredging), by their direct collection or bycatch (in which many animals are still used for trade). Once harvested, they are traded mainly on the Asian market for Traditional Chinese Medicine (TCM), where they are believed to have a higher medicinal value or, to a lower extent, traded worldwide ornamental display (Vincent et al., 2011). Because of this, all species of the *Hippocampus* were also included in Appendix II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES; Foster et al., 2014). On top of all these vulnerabilities, seahorses are also negatively impacted by climate change (Aurélio et al., 2013; Faleiro et al., 2015), poor water quality, invasive species and noise pollution (Anderson et al., 2011; Foster & Vincent, 2004; Lourie et al., 2004).

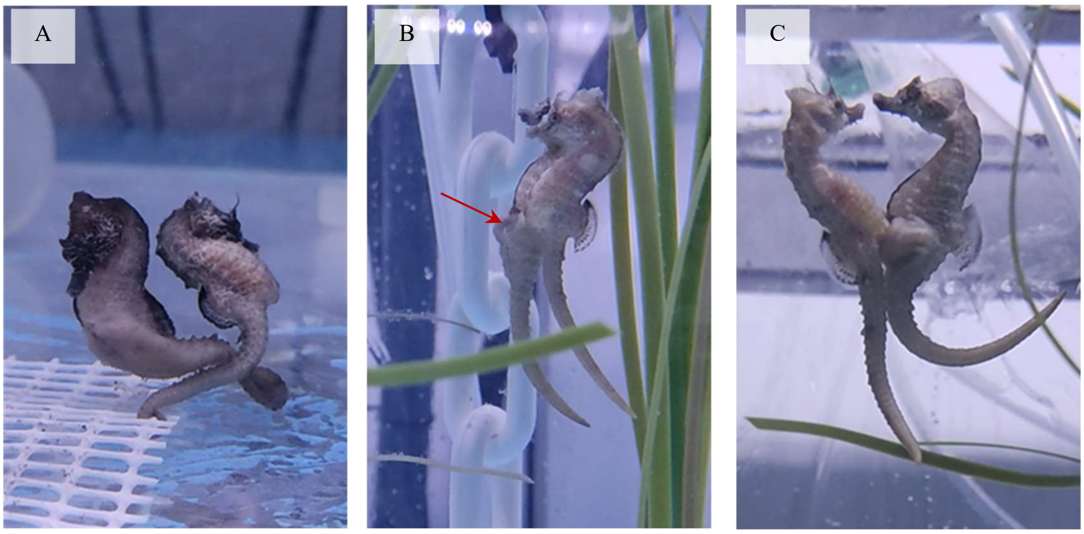

Figure 6: Short-snouted seahorse *Hippocampus hippocampus*. A) Male (left) and female (right) with lightened skin attached together. B) Male and female swimming together in the water column. The male has his pouch opened (red arrow), ready to receive the eggs. C) Female depositing the oocytes in the male's brood pouch.

1.4.1. Short-snouted seahorse *Hippocampus hippocampus*

Hippocampus hippocampus (Linnaeus, 1758) is a moderately sized seahorse with a maximum recorded height of 150 mm (Curtis et al., 2017; Lourie et al., 2004). Its short snout, usually less than one-third the length of the head, two prominent spines above each eye and a narrow coronet (Figure 7B and C) normally allows to distinguish this seahorse from other species, especially the *Hippocampus guttulatus* (Cuvier 1829), that normally co-occurs in the same habitat (Curtis, 2006; Lourie et al., 2004).

This seahorse exists in biogenic environments, usually close to seagrass areas and/or soft bottoms between rocks and algae, in European shallow coastal areas (Curtis et al., 2017; Curtis & Vincent, 2005; Lourie et al., 2004). They have very small home ranges, which suggests limited daily movements. Despite that, some adults make seasonal migrations to deeper depths in the winter season (Foster et al., 2014). The highest abundance was recorded in the Ria Formosa lagoon (Portugal; Curtis & Vincent, 2005), however a sharp decline in this population has occurred between 2000 e 2009, where 73% of *H. hippocampus* disappeared (Caldwell & Vincent, 2012). In a new survey, between 2009 and 2013, it was found that there were no significant differences in the density of this species, despite subsequent increases (Correia et al., 2015). It is believed that the causes of this initial decrease are due to human activities (e.g., fisheries, dredging and anchoring/mooring vessels) or natural changes in the Ria channels, both known for modifying the seahorse's habitat and reducing the amount of holdfasts and prey availability (Correia et al., 2015; Curtis et al., 2017; Curtis & Vincent, 2005). In the wild, the diet of this species consists mainly of little crustaceans (mainly *Mysis*), amphipods and copepods species (Kitsos et al., 2008).

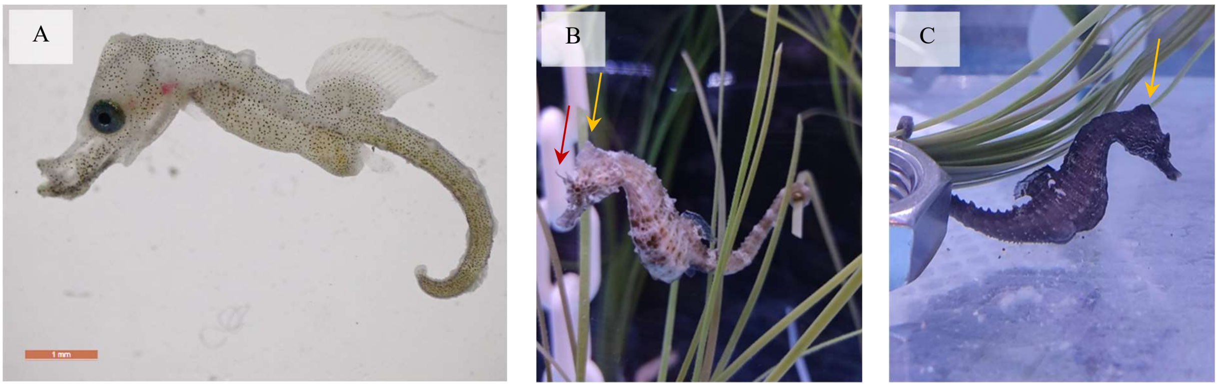

Figure 7: Short-snouted seahorse *Hippocampus hippocampus*, with two spines above each eye (red arrow) and a prominent coronet (orange arrow). A) Two days old newborn. B) Female. C) Male.

Regarding its conservation status, *H. hippocampus* has some impact on the TCM market (dried) and as an ornamental species in aquariums (live; Lourie et al., 2004). At a global level, the species *H. hippocampus* is in the category of "Data Deficient" of the IUCN Red List (Woodall, 2017), however in the Mediterranean Sea it is categorized as "Near Threatened" (Pollom, 2016). Given their vulnerability, they are also included in the List of Threatened and/or Declining Species and Habitats of the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention; OSPAR, 2008).

1.5. Objectives

Currently, little is known about the path that climate change will take and how they will influence marine ecosystems. When considering coastal areas, the need for information is even more evident due to their ecological and socio-economic importance, their high vulnerability and the fact that warming and oxygen depletion is being felt at a faster rate (Ainsworth et al., 2019; Frölicher & Laufkötter, 2018; Mackenzie & Schiedek, 2007). Alongside, seahorses' lifestyle makes them extremely vulnerable to changes in their habitat (Correia et al., 2015; Harasti, 2016). These ectothermic fishes have a thermal range normally similar to the temperature range of their natural habitat (Willmer et al., 2005). Beyond their biological limits, certain processes such as metabolism, growth, feeding, reproduction and behaviour can be affected (Aurélio et al., 2013; Faleiro et al., 2015; Mascaró et al., 2016; Qin et al., 2018). Climate-driven distributional shifts and adaption to new environmental conditions are particularly unlikely in seahorses due to their reduced swimming ability, small population distribution and high site-fidelity (Foster & Vincent, 2004).

Within this context, the objective of this dissertation was to understand the individual and combined effects of a category II marine heatwave (21.5°C) and an extreme hypoxia event (~ 27% DO) on the physiology and behaviour of the seahorse *Hippocampus hippocampus*. Specifically, I aimed to scrutinize the effects of warming and hypoxia on metabolic rates, thermal sensitivity, ventilation rates, behavioural patterns (namely resting, individual and social activities) and food intake. Concomitantly, I analyzed the impact of a slight increase in temperature associated with the onset of the marine heatwave (20.4°C) on the number and size of seahorses' oocytes.

2. Materials and methods

2.1. Species collection

Adult *Hippocampus hippocampus* were provided by Oceanário de Lisboa (Lisboa, Portugal). The previous generations of these animals are related to the Sado Estuary (Portugal). From their captive facilities, 7-month-old seahorses born at the Oceanário 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. The present experiments and analysis were reviewed and authorized by 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 acclimation

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 (Table 1). Temperature, pH, salinity and dissolved O_2 were checked daily, and ammonia, nitrite and nitrate were measured weekly and kept below detected values.

Table 1: Seawater physicochemical parameters for stock maintenance.

Parameter	Conditions	Measurement instruments
Temperature (°C)	17.0 ± 0.3	Multi3510 SET 4, WTW
pH	8.0 ± 0.1	Multi3510 SET 4, WTW
Salinity	33 ± 1	Refractometer, TMC
Dissolved O_2 (%; mg O_2 L^{-1})	~100; 7.6 ± 0.1	Multi3510 SET 4, WTW
$NH4^+$ (mg/L)	< 0.2	Ammonia / Ammonium Test Kit, TMC
NO^-_2 (mg/L)	< 0.1	Nitrite / Nitrate Combination Test Kit, TMC
NO^-_3 (mg/L)	< 10.0	Nitrite / Nitrate Combination Test Kit, TMC

Seahorses were randomly distributed into two 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 (Figure 8). Natural seawater (salinity 35) was pumped directly from the sea, filtered with 1-µm mesh and UV-sterilized (Vecton 120 Nano, TMC-

Iberia, Lisbon, Portugal), being them pumped (Reef Pump 8000, TMC-Iberia, Lisbon, Portugal) 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. Water from the sumps was continuously renewed by a water drip system also filtered with 1-µm mesh and UV-sterilized. To ensure superior water quality, each sump had a filtration system consisting in a glass wool, a sand bed filter (Reef-Filter Bio 1000, TMC-Iberia, Lisbon, Portugal), a protein skimmer (V2Skim 200, TMC) and bioballs (ouriço®, Fernando Ribeiro Lda, Queluz, Portugal) previously matured with the help of nitrifying bacteria (Nitribiotic, Tropic Marin). 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 (SPIW 312L, Whirlpool) and a chiller (HC-150A, HAILEA). Oxygen (O_2) levels were controlled by an air compressor (Mebo Blower LA-80B, NITTO KOHKI) connected to air diffusers, three in each sump and one in each aquarium (but with a lower diffusion rate).

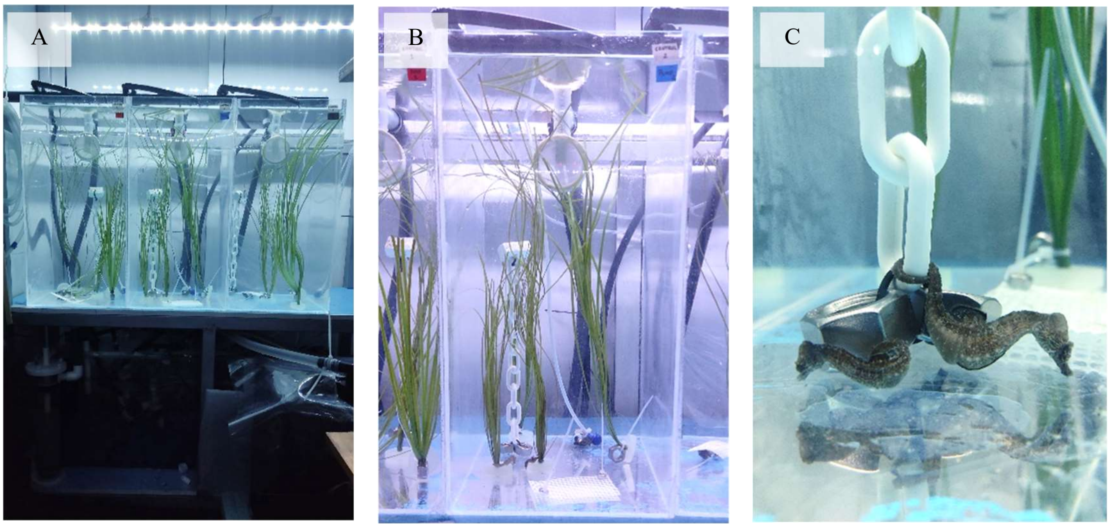

Figure 8: A) Recirculating system with three aquaria and a sump below. B) 70 L acrylic aquarium (used as a replicate). C) Female (on the left) and male (on the right) seahorses attach to a holdfast.

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 (Natureform SeaGrass, TMC-Iberia, Lisbon, Portugal), plastic chains and nets, were also added to all aquariums to provide holdfast for seahorses' attachment, habitat and to improve the well-being of the animals during the experiments.

Seahorses 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 frequent and in greater quantity *Mysis*, and less frequently and in smaller quantities enriched adult *Artemia*, copepods and cyclops (Gamma Frozen Food Slice, TMC). Live *Mysis* were introduced as much as possible (Figure 9).

After the first month, fish were wet weighted in seawater (AUW220D, Shimadzu), photographed against a white board with a metric ruler according to Lourie (2004) and measurement through ImageJ software (total height, trunk height and tail height, Figure 5). The weights ranged from 0.70 to 3.69 g, the total height varied from 55.47 to 96.29 mm, the trunk height from 16.53 to 29.31 mm and the tail height from 35.98 to 61.14 mm.

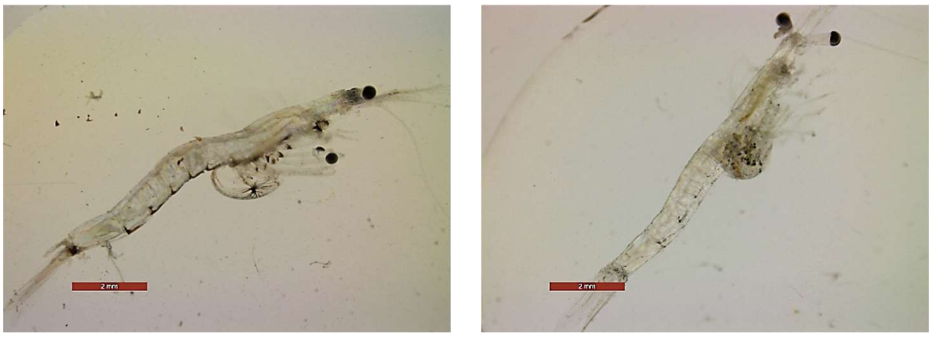

Figure 9: Live *Mysis* offered to seahorses. On the right it can be observed a pregnant female.

Seahorses were then exposed to cross-factor design of two different temperatures and two different oxygen level conditions. Thus, four different treatments arise (Figure 10):

1) control, representing the current annual mean environmental conditions of the Sado estuary (17°C, ~ 100% DO ~ 7.6 mg O_2 L^{-1});
2) marine heatwave, a projected category II marine heatwave in normoxic conditions (21.5°C, ~ 100% DO ~ 7.6 mg O_2 L^{-1});
3) hypoxia, simulating an extreme decrease in DO in the water, under normal temperature conditions (17°C, ~ 27% DO ~ 2.1 mg O_2 L^{-1}) and
4) marine heatwave and hypoxia, simulating a category II marine heatwave and an extreme hypoxia event (21.5°C, ~ 27% DO ~ 2.1 mg O_2 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 control, hypoxia and MHW treatments were the same (Figure 10).

2.2.1. Marine heatwave exposure

Based on a 30-year (1988 to 2018) SST dataset from the Sado Estuary (Portugal) region and on future climate change scenarios (Oliver et al., 2018; IPCC, 2022), a predicted category II MHW was simulated. Data was acquired from NOAA (National Oceanic and Atmospheric Administration) and analyzed through the RStudio Software (Version 1.3.1093 – © 2009-2020 RStudio, PBC) with the R package "heatwaveR" (Schlegel *&* Smit, 2018, which uses the MHWs definition of Hobday et al., 2018). The category II MHW characteristics (when the average of climatology was approximately 17.0°C) were:

1) the average total duration (13 days);
2) the maximum intensity (21.5°C);
3) the average rate of onset (0.6°C per day).

Taking this into account, seahorses were exposed to a daily increase of 0.6°C for eight days until a temperature of 21.5°C was reached. They then remained at this temperature for 11 days.

Water temperature was controlled in each aquarium by temperature controllers (STC-3000, Lamptron, accuracy ± 0.1ºC, hysteresis 0.3ºC) connected to digital heaters (Eheim GmbH & Co KG). A chiller (HC-150A, HAILEA), connected to the sump, allowed the temperature to never rise above the intended value.

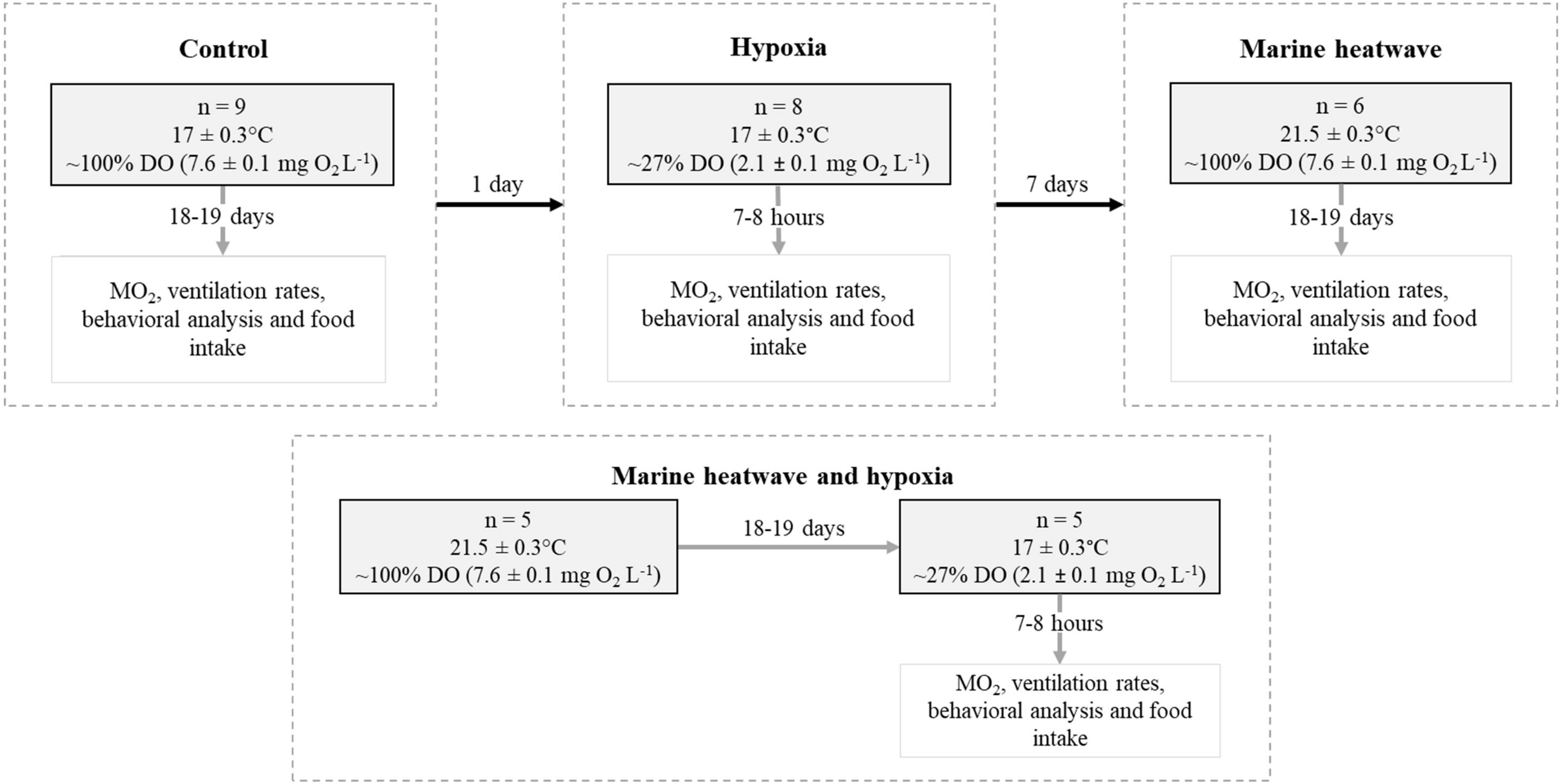

Figure 10: Number of individuals (n), seawater temperature and dissolved oxygen (percentage and concentration) established for the four experimental setups. Individuals from control, hypoxia and MHW treatments were the same. Values represent mean ± SD.

2.2.2 Hypoxia exposure

A nitrogen gas (N_2) injection was used to regulate and maintain the DO around 27% (2.1 mg L^{-1}). To make sure that the N_2 was sufficient to maintain the desired levels of O_2 during exposure, solenoid valves controlled automatically the N_2 flow injected into the water, by injecting N_2 when O_2 levels rise above 27% (2.1 mg L^{-1}) and stopping injecting when the reverse occurs. The flow was also manually adjusted with a pneumatic flow control valve (diameter 8 mm).

The hypoxic exposure had a total duration of approximately seven hours, divided into two parts: 1) 5:30h of exposure occurred in the chambers during the MO_2 measurement and 2) approximately one hour in the aquaria, after the MO_2 measurement. At the beginning of the respirometry trial, after an acclimatization period to the chamber, a decrease in O_2 concentration was implemented in the water that filled the chambers and after one hour, the desired O_2 concentration inside the chambers was obtained. After 5:30h of MO_2 measurement, the fish were again transferred to their aquarium tanks where exposure to hypoxia conditions continued for approximately an hour. To achieve desired dissolved O_2 levels in the aquariums tanks, N_2 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 (Reef Pump 2000, TMC) from the sump to the cylinder column and only then to the aquarium.

2.3. Metabolic rates, thermal sensitivity 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 (MO_2), applied to estimate the standard metabolic rates (SMR), i.e., 'the level required for a minimal resting lifestyle with no spontaneous activity, no digestion of food, and no physical, thermal, or psychological stress' (Willmer et al., 2005). Seahorses were individually placed in 606 ml (including tygon chemical tubing, Loligo® Systems) respirometry chambers which were then completely closed so that there was no external infiltration of O_2. 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 (V2Therm 100, TMC) and a chiller (HC-150A, HAILEA), regulated by a Profilux controlling system (Profilux 4, GHL) with a temperature probe. Small holdfasts were provided to animal'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 MO_2 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 5:30h, of which 2h of acclimatization and 3:30h of O_2 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. Four seahorses (or less) were measured simultaneously in each run. The duration of the measurement period ensures that, during the control and MHW treatments, the O_2 levels inside the chambers never went below 80% air saturation (Paula et al., 2022), 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 (Reef Pump 2000, TMC) submerged in a tank with treatment water, supplied and renewed the chambers with clean seawater. These pumps were controlled 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 well-aerated seawater in the case of the control and MHW treatments or with water with low concentration of O_2 in the case of hypoxia and MHW + hypoxia.

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 (OXFTC2, PyroScience) 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. A schematic illustration of the respirometry system is shown in Figure 11.

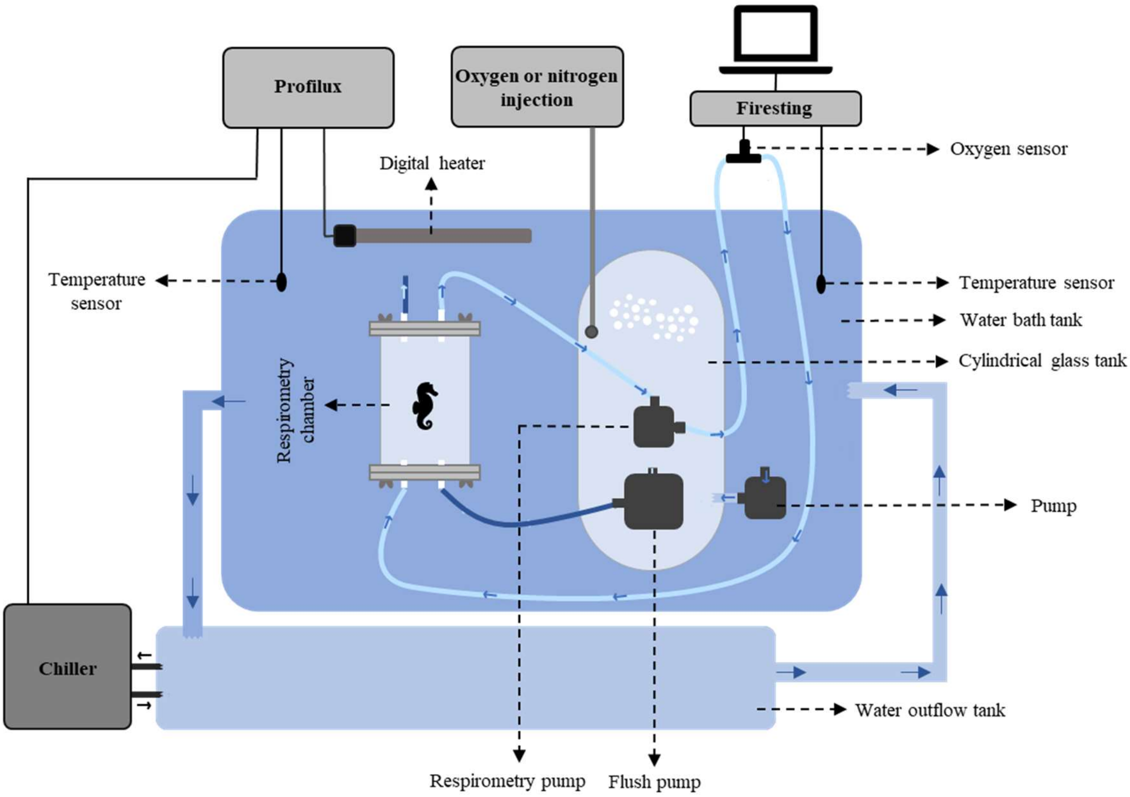

Figure 11: Experimental design of the respirometry system used for the assessment of the oxygen consumption rates of *Hippocampus hippocampus*. The blue arrows indicate the path of the water. It is solely for representative purposes, the actual system layout included four respirometry chambers.

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 [$mgO_2\ g_{fish}^{-1}\ hour^{-1}$].

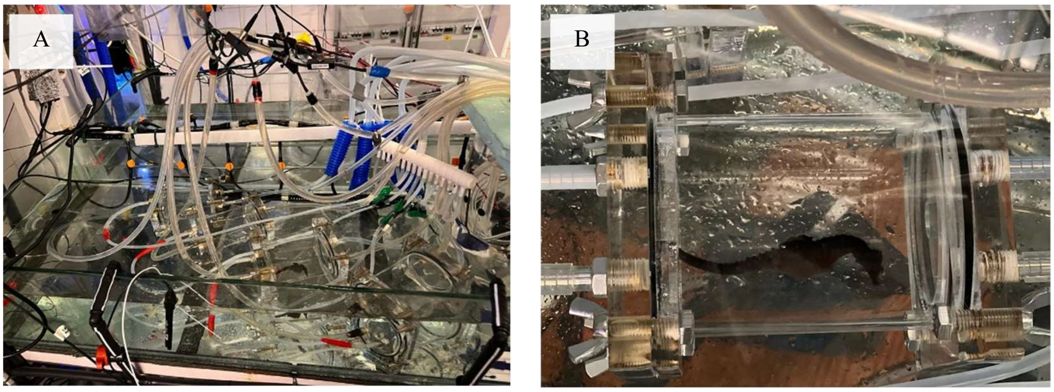

Figure 12: A) Part of the respirometry system containing four respirometry chambers in the water bath. The flush and measure circuit can be observed. B) Close-up of a respirometry chamber (horizontal acrylic cylinders) with a male seahorse inside (just for representation purposes).

The thermal sensitivity was determined for the temperature intervals of 17°C (control) to 21.5°C (MHW and MHW+hypoxia), through the temperature coefficient (Q_{10}) for standart metabolic rates, using the equation:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_1 - T_2}}$$

where R_1 and R_2 represent the standard metabolic rates at temperatures T_1 and T_2, respectively.

After the respirometry trials, seahorses were transferred to their respective aquaria where the sampling continued. After an acclimation 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 and food intake

A careful observation during the initial month of acclimatization to captive conditions allowed the formulation of a behavioural ethogram to *H. hippocampus* (Table 2). During the experimental trial, 30-minute videos were recorded to assess seahorse’s 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 of 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 2: 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.

Category	Behaviour	Description
Rest	**Stationary**	Seahorse remains completely still.
	Swinging	Light head and/or body movements while the seahorse is attached to a holdfast.
Individual activity	**Adjustment**	Seahorse often adjusts the tail on the holdfast, rotating or moving vertically along it.
	Slow body movement	Seahorse moves slowly, mainly using the tail and not the dorsal and/or pectoral fins or using them lightly to propel themselves.
	Swimming	Seahorse swims actively, constantly moving the dorsal and pectoral fins.
	Feeding	Seahorse visualizes and approaches the prey, stretching his body while attached to a holdfast or swimming towards it. It directs the snout to the prey and attacks, capturing it or not.
		Capture When the seahorse catches and ingests the prey.
		Miss When the seahorse attacks but does not catch the prey.
		Attack Sum of Capture and Miss behaviours.
Social activity	**Interaction with aggression**	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.
	Interaction without aggression	Seahorses from the same sex follow each other; 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 oocy (or attempt to).

2.5. Oocytes size

Due to egg laying during the control and marine heatwave treatments, the oocytes that fell to the bottom of the tank were collected. This egg laying can happen during the seahorse’s courtship, when the transfer of mature oocytes from the female to the male’s pouch is not successful, partially or completely. Thus, oocytes were collected after the unsuccessful egg transfer from the female to the male pouch, which occurred at 17°C (during the control) and at 20.4°C (day 6 of the MHW treatment), associated with the onset of the marine heatwave. The oocytes were then counted and photographed under a microscope. ImageJ software was used to measure oocytes height, width (Figure 12) and area.

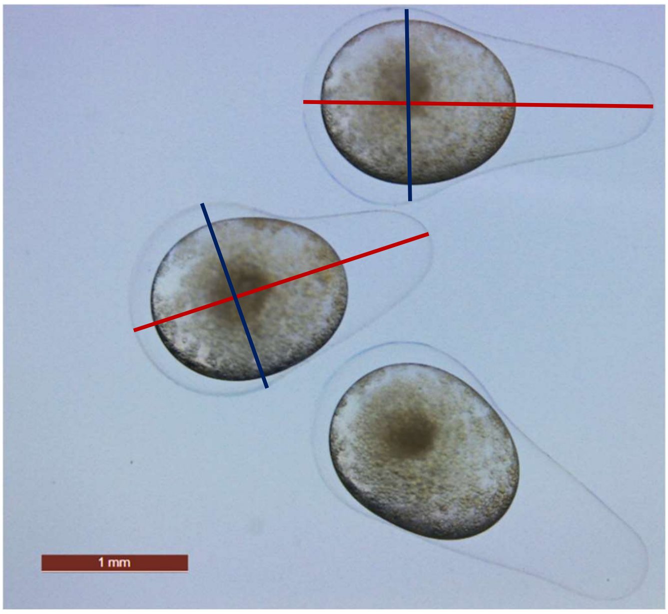

Figure 13: *Hippocampus hippocampus* oocytes and the measurements performed in them (red line is the height and blue line is the width).

2.6. Data analysis

Statistical analysis of all variables was performed in RStudio Software (Version 1.3.1093 – © 2009-2020 RStudio, PBC) via Generalized Linear Mixed Models (GLMM, Zuur et al. 2009). Treatment was used as a fixed four-level 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).

The Gaussian family (identity link function) was used to model the oxygen uptake rates. The Poisson family of distribution (log link function) was applied to the ventilation rates since they represent count data. Regarding the percentage of time spent in each behaviour and the percentage of food intake, it was used the Beta family (logit link function). Lastly, the Gamma family (log link function) was used to model response variables related to the egg size.

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 (Supplementary Table 1, 2 and 3). Post-hoc multiple comparisons between the four different treatments were also performed, using the “emmeans” package, with Tukey corrections to minimize type I errors (Lenth, 2022). The package “performance” (Lüdecke et al., 2021) was used to validate models’ performance and assumptions, i.e., independence, normality and homoscedasticity of residuals, and to check whether count data models exhibit overdispersion (higher than 1.2).

3. Results

3.1. Metabolic rates, thermal sensitivity and ventilation rates

Regarding standard metabolic rates (SMR), although there was a slight increase with temperature increase, there were no significant impacts of the MHW in the seahorses' metabolic rates (Figure 14, $p > 0.05$, GLMM analysis in Supplementary Table 4). Nonetheless, hypoxia (H) elicited a significantly decrease in the SMR of the adult seahorses (Figure 14, $p < 0.0001$, GLMM analysis in Supplementary Table 4). Furthermore, the combined effect of the two experimental factors (MHW+H) also shows to be significant ($p = 0.0077$, GLMM analysis in Supplementary Table 4), reaching a value of 2.08 ± 0.30 µmol O_2 h^{-1} g^{-1}. The thermal sensitivity (thermal coefficient, Q_{10}) values were 1.36 between control (17°C) and MHW (21.5°C) and decreased to 0.33 between control (17°C) and MHW+H (21.5°C).

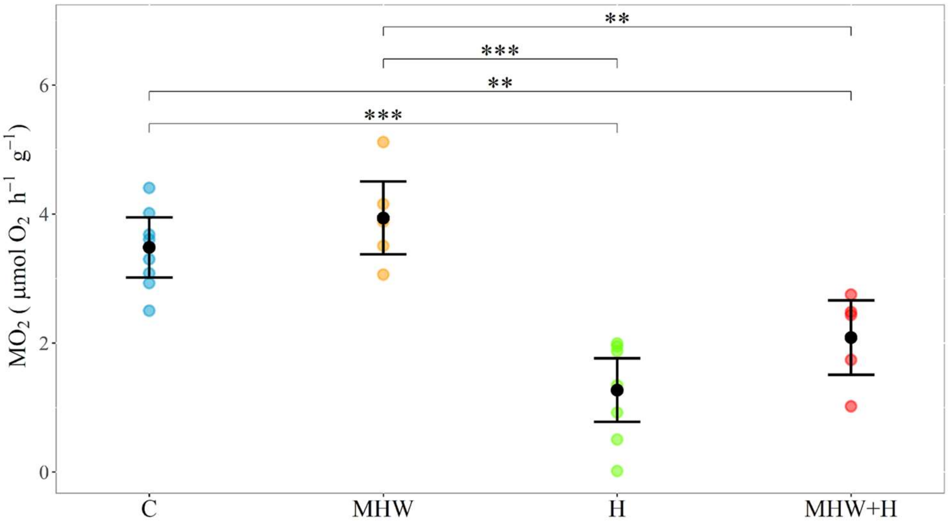

Figure 14: Oxygen consumption rates (MO2) of seahorses *Hippocampus hippocampus* exposed to the control (C, n=8), marine heatwave (MHW, n=6), hypoxia (H, n=7) and marine heatwave and hypoxia combined (MHW+H, n=5) treatments. Values correspond to predicted means ± 95% confidence intervals (C.I.). The dots with different colors represent the observations for each treatment (blue for control, orange for MHW, green for H and red for MHW+H). Significant levels (p-value < α, α = 0.05) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.

Both MHW and H promoted a significant increase in the ventilation rates, but the combined action of the two factors (MHW+H) elicited a stronger effect (Figure 15, $p < 0.0001$, GLMM analysis in Supplementary Table 4), reaching a value of 67.7 ± 4.1 beats min^{-1}, which represents an increase of 280.9% in comparison to control conditions.

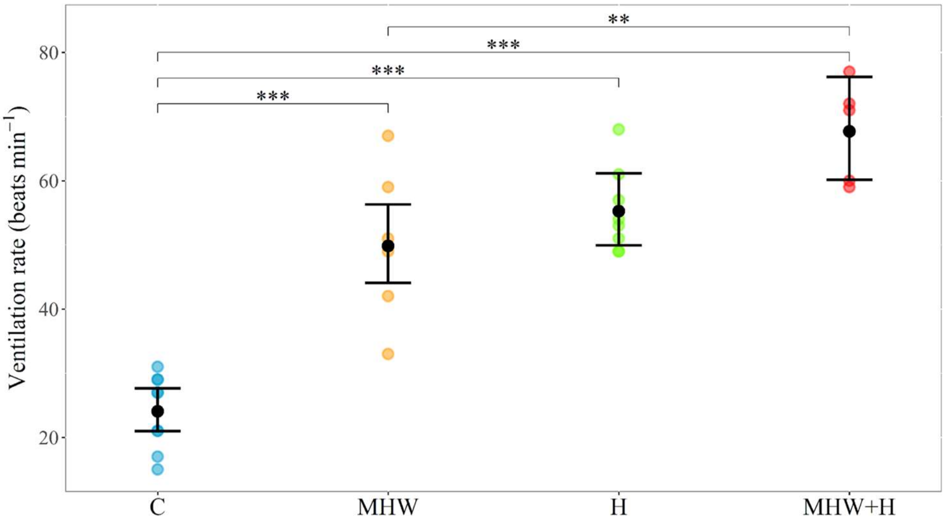

Figure 15: Ventilation rates of seahorses *Hippocampus hippocampus* exposed to the control (C, n=9), marine heatwave (MHW, n=6), hypoxia (H, n=8) and marine heatwave and hypoxia combined (MHW+H, n=5) treatments. Values correspond to predicted means ± 95% confidence intervals (C.I.). The dots with different colors represent the observations for each treatment (blue for control, orange for MHW, green for H and red for MHW+H). Significant levels (p-value < α, α = 0.05) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.

3.2. Behavioural patterns and food intake

3.2.1. Rest

Seahorses under control conditions spent an average of 80.2 ± 3.1% of their time resting, a value that decreased significantly to 58.4 ± 4.9% under MHW (Figure 16A, $p = 0.0024$, GLMM analysis in Supplementary Table 5). On the other hand, the time they spent resting increased to 98.8 ± 0.7% under H ($p = 0.0001$, GLMM analysis in Supplementary Table 5) and to 98.3 ± 0.9% under MHW+H ($p = 0.0004$, GLMM analysis in Supplementary Table 5), which represented an increase of 23.19% and 22.57%, respectively.

Regarding the stationary behaviour (Figure 16B), there were no significant changes in the time seahorses spent stationary under the MHW treatment ($p > 0.05$, GLMM analysis in Supplementary Table 5). However, exposure to H and MHW+H prompted significant changes in this behaviour (both $p < 0.0001$, GLMM analysis in Supplementary Table 5). The time that the seahorses remained stationary increased from 3.3 ± 1.4% under control conditions to 78.9 ± 5.1% in the H and to 76.9 ± 6.6% in the MHW+H. Alongside, the swinging behaviour varied significantly among all treatments (Figure 16C, $p > 0.05$, GLMM analysis in Supplementary Table 5). The percentage of time spent by seahorses swinging was 78.8 ± 4.6 under control conditions, and significant decreases in all the other treatments were observed, namely to: (i) 59.5 ± 6.8% under the MHW ($p = 0.0155$, GLMM analysis in Supplementary Table 5), (ii) 17.0 ± 4.1% under hypoxia (H) ($p < 0.0001$, GLMM analysis in Supplementary Table 5) and (iii) 20.4 ± 5.8% under the combined treatment (MHW+H, $p < 0.0001$, GLMM analysis in Supplementary Table 5).

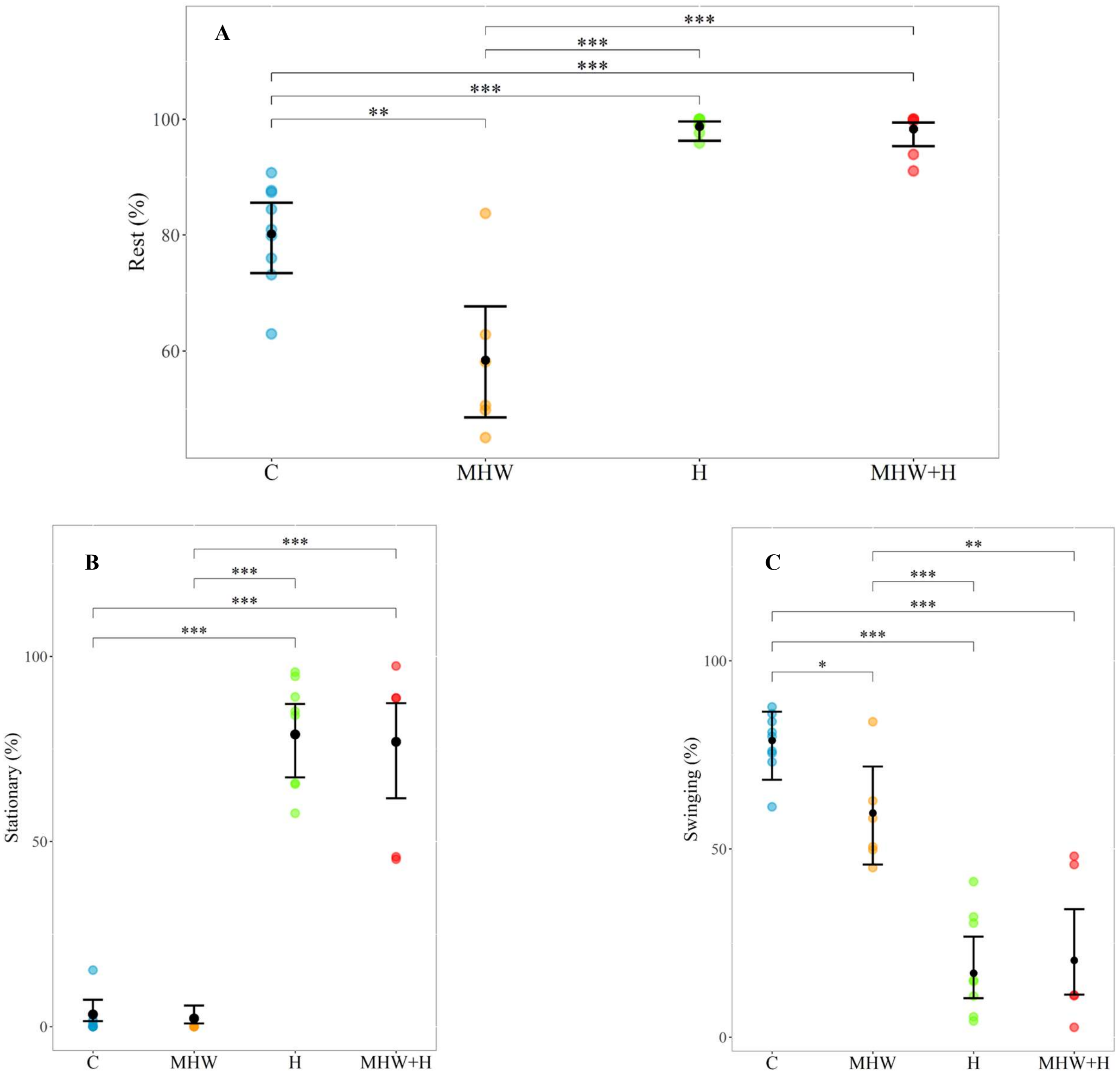

Figure 16: Behavioural patterns of seahorses *Hippocampus hippocampus* exposed to the control (C, n=9), marine heatwave (MHW, n=6), hypoxia (H, n=8) and marine heatwave and hypoxia combined (MHW+H, n=5) treatments. A) Rest category, which includes B) stationary and C) swinging behaviours. Values correspond to predicted means ± 95% confidence intervals (C.I.). The dots with different colors represent the observations for each treatment (blue for control, orange for MHW, green for H and red for MHW+H). Significant levels (p-value < α, α = 0.05) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.

3.2.2. Individual activity

The MHW treatment did not cause any significant change in the seahorses' individual activity (Figure 17A, $p > 0.05$, GLMM analysis in Supplementary Table 5), but the H and MHW+H did ($p = 0.0009$ and $p = 0.0020$, respectively, GLMM analysis in Supplementary Table 5), from an average of 16.3 ± 3.1% under control conditions to 1.6 ± 0.9% under H, and to 2.0 ± 1.1% under MHW+H. Following the same trend, only the treatments that involved the reduction of O_2 (i.e., H and MHW+H) had significant impacts on the slow body movement behaviour (Figure 17C). The control group of seahorses spent an average of 13.0 ± 2.9% of their time in this behaviour, which decreased significantly to 11.9 ± 0.5% under H ($p < 0.0001$, GLMM analysis in Supplementary Table 5) and to 14.6 ± 0.7% under MHW+H ($p = 0.0007$, GLMM analysis in Supplementary Table 5). No significant differences were found between treatments regarding adjustment (Figure 17B, $p > 0.05$, GLMM analysis in Supplementary Table 5) and swimming behaviours (Figure 17D, $p > 0.05$, GLMM analysis in Supplementary Table 5).

Seahorse feeding duration (Figure 17E), which lasted an average of 2.1 ± 0.7% under control conditions, showed no significant differences under MHW ($p > 0.05$, GLMM analysis in Supplementary Table 5). Nonetheless, the time spent feeding decreased significantly to 0.2 ± 0.1% under H ($p = 0.0204$, GLMM analysis in Supplementary Table 5) and to 0.4 ± 0.2% under MHW+H ($p = 0.0403$, GLMM analysis in Supplementary Table 5).

Regarding the number of attacks and capture success (Figure 18A and 18B, respectively), they varied significantly across all treatments in the same exact way. The number of attacks recorded under control conditions was 9.0 ± 2.8, which increased significantly to 23.8 ± 5.6 under MHW ($p = 0.0359$, GLMM analysis in Supplementary Table 5). Yet, under H there was a significant reduction of ingested *Mysis* to 1.2 ± 0.8 ($p = 0.0123$ and 0.0239, under control and the MHW temperature, respectively, GLMM analysis in Supplementary Table 5). Regarding the capture success (Figure 18B), the MHW also had a significant impact ($p = 0.0102$, GLMM analysis in Supplementary Table 5), leading to an increase of 7.1 ± 1.9 to 19.6 ± 4.3 *Mysis* ingested. Both H and MHW+H caused a significant decrease to 0.7 ± 0.4 and 0.7 ± 0.5 preys ingested, respectively ($p = 0.0252$ and 0.0281, respectively, GLMM analysis in Supplementary Table 5). The miss percentage (Figure 18C) did not follow the same trend since it did not show any significant changes among treatments ($p > 0.05$, GLMM analysis in Supplementary Table 5).

Regarding live food intake (Figure 19A), there was no significant effect on the live *Mysis* consumption between control conditions and the other treatments ($p > 0.05$, GLMM analysis in Supplementary Table 5). The consumption of frozen food (Figure 19B) only varied (increased) significantly between control (with a mean of 6.7 ± 2.5 *Mysis*) and MHW (31.5 ± 7.0 *Mysis*, $p = 0.0058$, GLMM analysis in Supplementary Table 5).

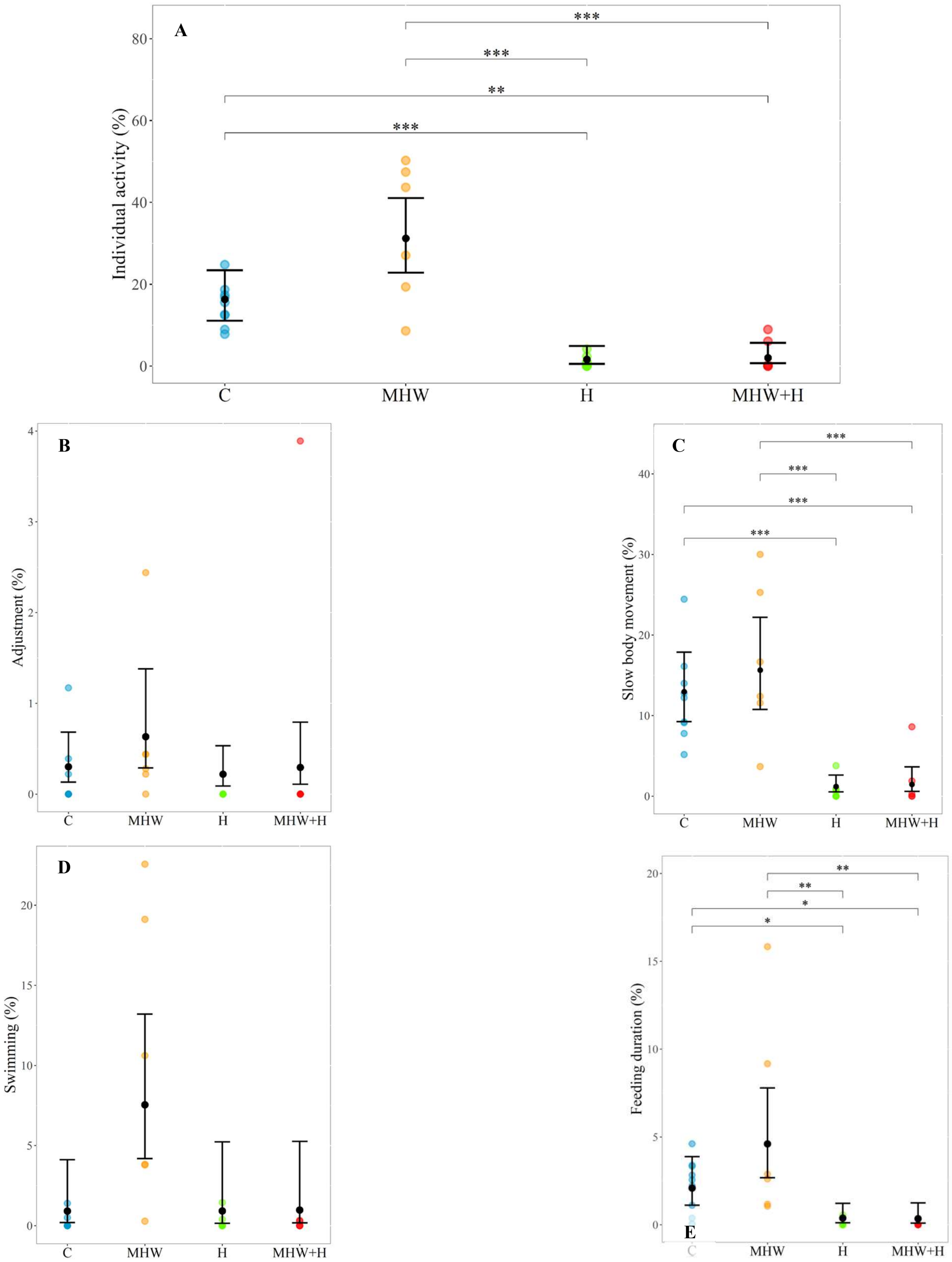

Figure 17: Behavioural patterns of seahorses *Hippocampus hippocampus* exposed to the control (C, n=9), marine heatwave (MHW, n=6), hypoxia (H, n=8) and marine heatwave and hypoxia combined (MHW+H, n=5) treatments. A) Individual activity category, which includes B) adjustment, C) slow body movement, D) swimming and E) feeding behaviours. Values correspond to predicted means ± 95% confidence intervals (C.I.). The dots with different colors represent the observations for each treatment (blue for control, orange for MHW, green for H and red for MHW+H). Significant levels (p-value < α, α= 0.05) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.

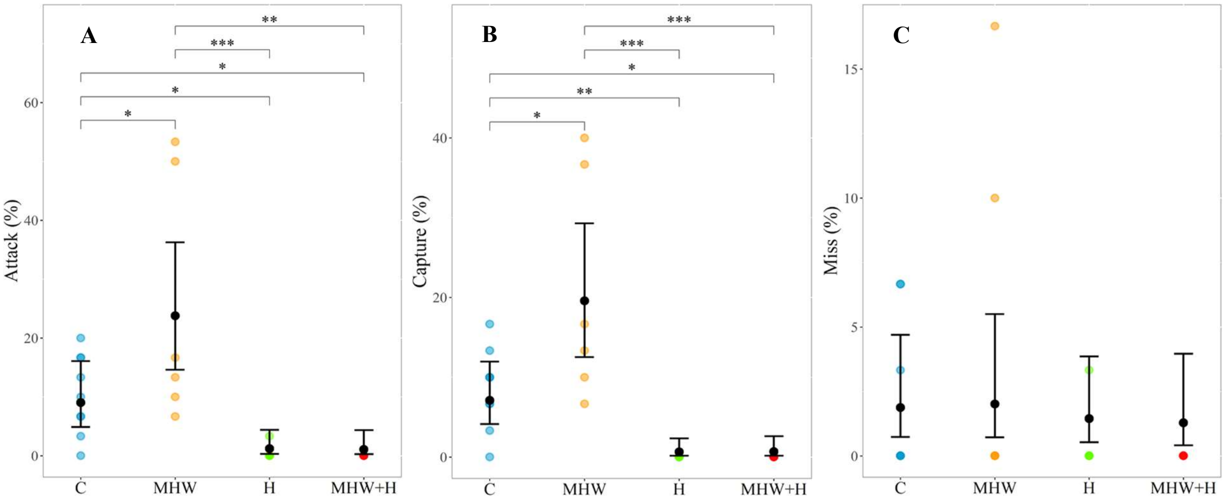

Figure 18: Feeding behaviour patterns of seahorses *Hippocampus hippocampus* exposed to the control (C, n=9), marine heatwave (MHW, n=6), hypoxia (H, n=8) and marine heatwave and hypoxia combined (MHW+H, n=5) treatments. A) Percentage of attacks, B) capture and C) miss behaviours. Values correspond to predicted means ± 95% confidence intervals (C.I.). The dots with different colors represent the observations for each treatment (blue for control, orange for MHW, green for H and red for MHW+H). Significant levels (p-value < α, α = 0.05) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.

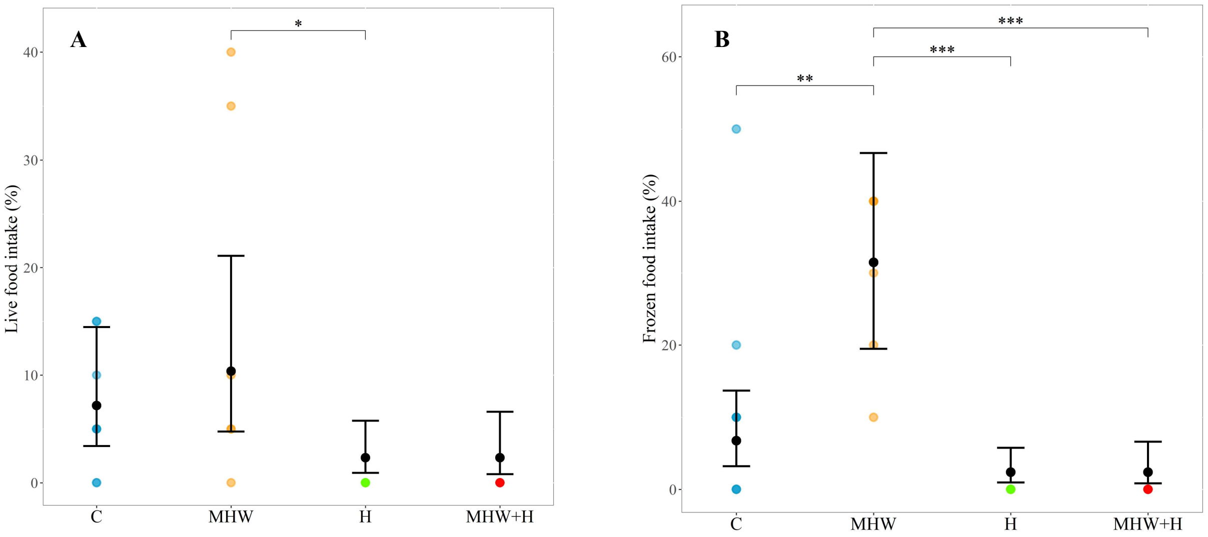

Figure 19: Food intake of seahorses *Hippocampus hippocampus* exposed to the control (C, n=9), marine heatwave (MHW, n=6), hypoxia (H, n=8) and marine heatwave and hypoxia combined (MHW+H, n=5) treatments. A) Percentage of live *Mysis* ingested. B) Percentage of frozen *Mysis* ingested. Values correspond to predicted means ± 95% confidence intervals (C.I.). The dots with different colors represent the observations for each treatment (blue for control, orange for MHW, green for H and red for MHW+H). Significant levels (p-value < α, α = 0.05) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.

3.2.3. Social activity

Regarding the social activity of seahorses, there were no significant changes between the control and the other treatments (Figure 20A, p > 0.05, GLMM analysis in Supplementary Table 5). Moreover, there were no significant changes in relation to interaction without aggression and courtship behaviours among all treatments (Figure 20B and 20C, all p > 0.05, GLMM analysis in Supplementary Table 5).

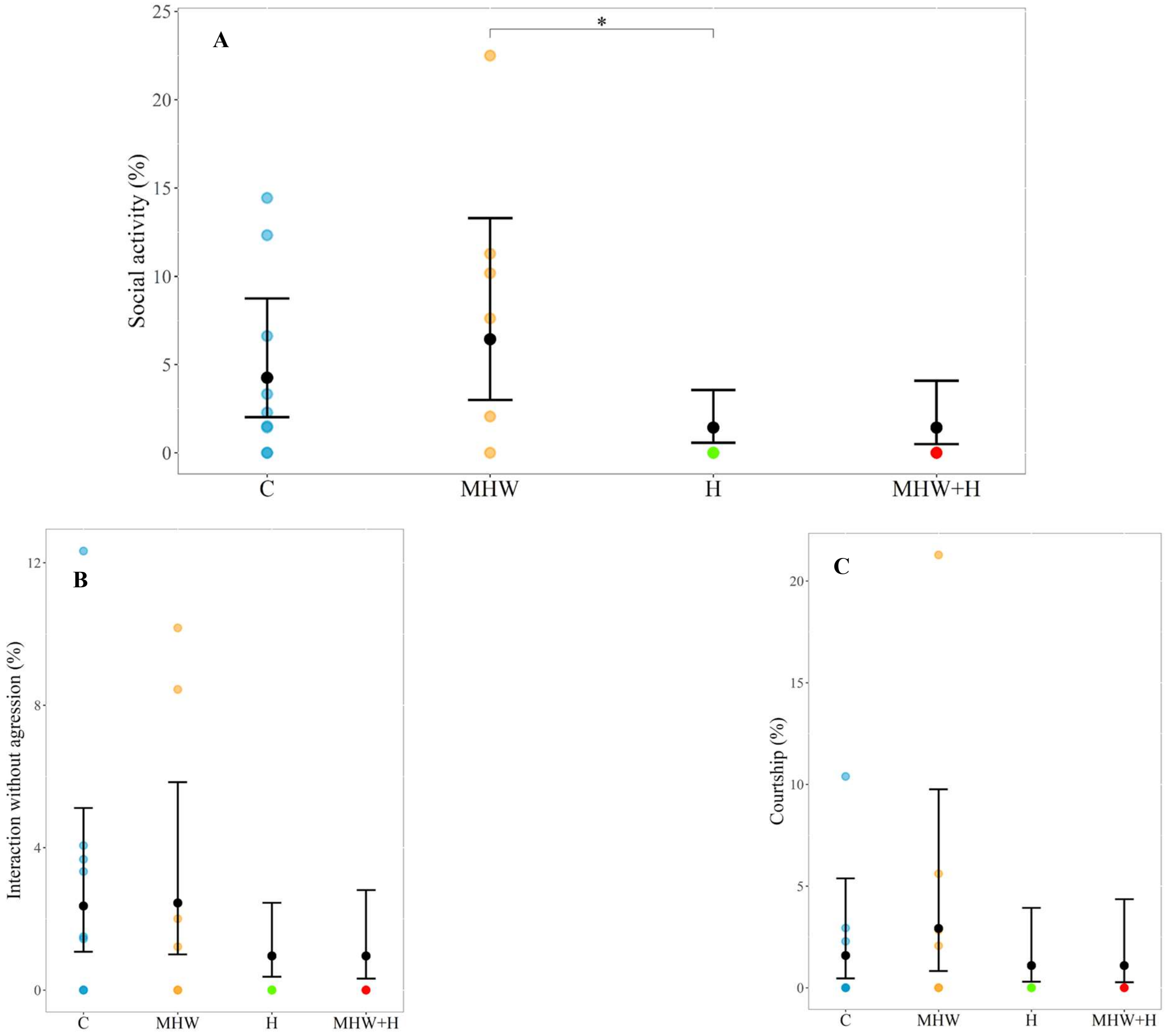

Figure 20: Behavioural patterns of seahorses *Hippocampus hippocampus* exposed to the control (C, n=9), marine heatwave (MHW, n=6), hypoxia (H, n=8) and marine heatwave and hypoxia combined (MHW+H, n=5) treatments. A) Social activity category, which includes B) interaction without aggression and C) courtship. Values correspond to predicted means ± 95% confidence intervals (C.I.). The dots with different colors represent the observations for each treatment (blue for control, orange for MHW, green for H and red for MHW+H). Significant levels ($p\text{-value} < \alpha$, $\alpha = 0.05$) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.

3.2. Oocytes

The total number of oocytes found in the individuals under control conditions was 138, which increased to 181 in the MHW. There was no egg laying under H and MHW+H conditions. Oocytes length increased significantly under MHW (2.44 ± 0.05 mm) compared to control conditions (2.17 ± 0.06 mm, Figure 21A, $p = 0.0016$, GLMM analysis in Supplementary Table 6). Similar trends were observed for oocytes' width (Figure 21B, $p < 0.0001$, GLMM analysis in Supplementary Table 6) and oocytes' area (Figure 21C, $p = 0.0002$, GLMM analysis in Supplementary Table 6).

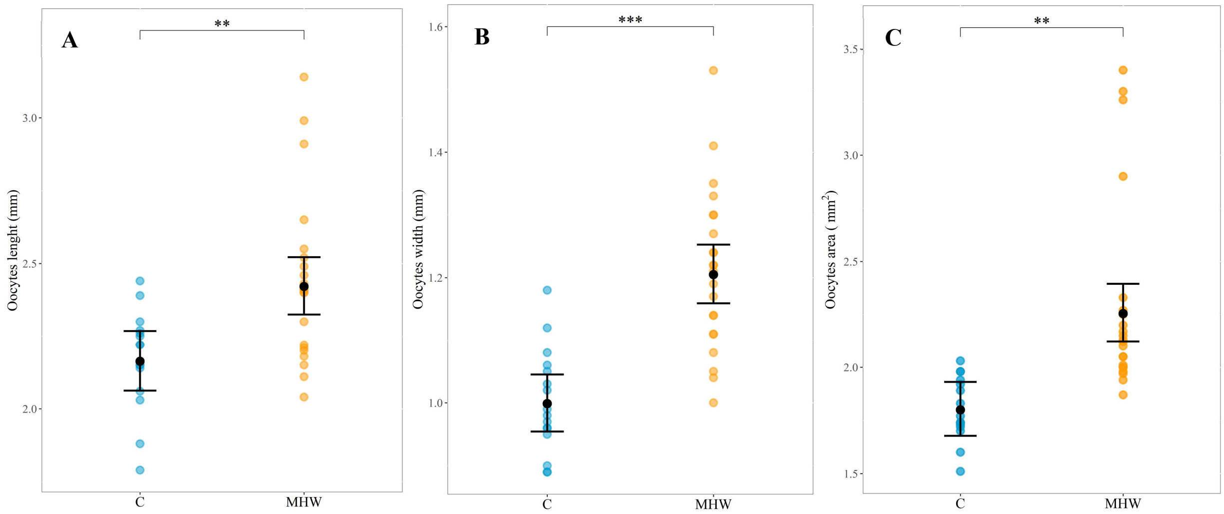

Figure 21: *Hippocampus hippocampus* oocytes A) length, B) width and C) area, exposed to the control (C, n=16) and marine heatwave (MHW, n=21) treatments. Values correspond to predicted means ± 95% confidence intervals (C.I.). The dots with different colors represent the observations for each treatment (blue for control and orange for MHW). Significant levels (p-value < α, α = 0.05) are represented as: * < 0.05, ** < 0.01 and *** < 0.001.

4. Discussion

Many marine ectothermic animals, 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 (Pigliucci, 2003, Mascaró et al., 2016). However, when these mechanisms do not exist or are not sufficient, many animals migrate to more environmentally suitable habitats. In seahorses' case, due to their specific lifestyle, i.e., their reduced swimming ability, small distribution and high site-fidelity (Foster & Vincent, 2004), this can be difficult or even impossible. Furthermore, migration can jeopardize the continuity of generations due to the possible breaking of the pair bond. The search for a new pair, coupled with the dispersion of populations and the possible lower efficiency of reproduction with the new partner, make 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, as the adaptation cannot occur as gradually as with long-term changes (Altieri & Gedan, 2014; Somero, 2010), further minimizing the possibility of migration.

Taking this into account, the physiological and behavioural effects resulting from a category II MHW and an extreme hypoxia exposure on seahorse *H. hippocampus* are reported in this study. Seahorses were, in general, prepared to deal with temperature changes associated with a category II MHW, but not so well with oxygen depletion, or even with the combined effect of the two factors.

4.1. Metabolic rates, thermal sensitivity and ventilation rates

The metabolic rates of *H. hippocampus* were not significantly affected by the MHW-related temperature increase (21.5°C). Nonetheless, the ventilation rates have increased significantly, which is presumed to be a mechanism of adapting to these new conditions. Furthermore, a thermal coefficient (Q_{10}) of 1.36 was registered, which again may suggest the presence of a compensatory mechanism to overcome the thermal stress (Johnson, 2017). The thermal coefficient is generally used to understand the thermal sensitivity of a species, to what an approximate value of 2.00 is expected for any biochemical reaction that is only affected by temperature (Clarke & Fraser, 2004; Johnson, 2017). In teleost fish, this value is usually between 1.50 and 2.00 (White et al. 2006). Taking these results into account and considering that *H. hippocampus* also successfully reproduces with temperatures of 22 to 23°C (Otero-Ferrer et al., 2012), we can assume that the temperature tested is still tolerable for these animals and may even represent optimal conditions for certain physiological processes. So far, no other study has been carried out on the metabolic impacts resulting from an environmental temperature increase on adult seahorses of this species. On the other hand, some analyzes have already been done with the co-habitant *Hippocampus guttulatus* (Aurélio et al., 2013; Faleiro et al., 2015), which we assumed to have a similar thermal sensitivity. When presented in higher warming conditions (26 to 30°C), the metabolic and ventilation rates of this seahorse species were significantly affected, but the temperatures turned out to be tolerable since behavioural activities remained the same. Despite that, they presumed that long-term exposures to these temperatures may have some negative impacts on seahorse's lifestyle and the continuation of the next generations, since the food intake during the warming experiment did not keep up with the increased energy demand, which may impact fish performance and welfare (Aurélio et al., 2013; Faleiro et al., 2015). In contrast, the tropical species *Hippocampus kuda*, which has an optimal reproduction efficiency at 26 to 28°C, begins to show negative reverse effects on gonad development, fecundity, fertilization and hatching rates and juveniles' survival at temperatures above 30°C (Lin et al. 2006).

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, are some of the first physiological responses of some species during periods of oxygen depletion. 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 the 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 oxygen 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; Seibel, 2011). 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).

Regarding the MHW and hypoxia together, the metabolic rate decreased significantly, despite having a higher mean than the observed during the hypoxia treatment (2.08 ± 0.30 vs. 1.27 ± 0.25 µmol O_2 h^{-1} g^{-1}). The ventilation rates registered the highest value of opercular beats (67.7 beats per minute), which represent an accumulation of the results of both treatments. The thermal coefficient between 17°C (normoxia) and 21.5°C (hypoxia) was 0.33, that represents a very accentuated metabolic suppression. These results, which are similar to those verified during hypoxia, suggest that O_2 reduction is the main cause of the metabolic suppression, however, they also suggest a slight temperature compensation in relation to oxygen depletion.

4.2. Behavioural patterns, food intake and egg laying

Seahorses, as already mentioned, 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 all the experimental exposures. On one hand, it decreased during the marine heatwave exposure, which indicates that the animals became more active. On the other hand, hypoxia (both at 17 and 21.5°C) had the opposite effect, once it led the animals to rest about 98% of the time, showing signs of movement lethargy. 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). Regarding the stationary behaviour, there was only a significant increase in hypoxic conditions (both at 17 and 21.5°C), which is consistent with the lower activity observed in these animals. Swinging, in contrast, showed to decrease both at higher temperatures and at lower O_2 concentrations. These results showed again signs of reduced activity during an oxygen depletion, however, the behaviour decrease during the MHW is caused by the opposite reason. During MHW, seahorses reduced the time they spent swinging as they opted for even more active behaviours, like the individual and social activities.

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, with the exception of adjustment and swimming behaviour, there was only a considerable impact during the hypoxia event, and this combined with the MHW. Once again, in situations of oxygen depletion, the animals were significantly less active. Adjustments and swimming behaviours did not show any difference along the treatments.

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, neither the social activity nor the behaviours associated with it were impacted by the different experimental conditions. These results may have occurred due to the reduced number of observations and/or the short period of time used for the analysis, since both temperature and O_2 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). Exemplifying, temperature is strongly related to the social behaviour of seahorses, due to its connection to their reproduction, as already mentioned above in *H. kuda* case (Lin et al., 2006). Oxygen concentration is also an important environmental factor, 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).

Regarding seahorses feeding behaviours, only the increase in temperature resulted in a significant increase in the food intake, due to the greater energy demand during this period. Sheng *et al.* (2006) also verified that the environmental temperature significantly affected seahorses *Hippocampus trimaculatus* feeding rate.

Last, although the results are only from one posture in each treatment, the temperature increase to 20.4°C during the onset of the marine heatwave led to a higher number and size of oocytes. These results may occur from the direct effect of temperature since, as already mentioned, temperature affects the gonad development, fecundity, fertilization and hatching rates of seahorses (Lin et al., 2006). However, other factors become quite important for the development of clusters, albeit indirectly. Food intake, especially its quantity and quality, appears to have a very important effect on the fecundity and number of spawns of female seahorses (Lin et al., 2007).

5. Final considerations

Summing up, *H. hippocampus*, as other seahorses' species, exhibit physiological and behavioural mechanisms that make them able to tolerate a short period of temperature increase such as that of a category II MHW. On the contrary, a short but extreme period of oxygen depletion, although tolerable, resulted in a metabolic suppression, coupled with a reduction in seahorse 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). The two environmental stressors together have similar impacts of those observed in the hypoxia treatment, which suggests that the oxygen depletion is the main driver of the biological changes.

These results concern short and periodic exposures where migration turns out to be even more difficult or impossible. 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; Burguer et al., 2020; IPCC, 2022; Oliver et al., 2018). However, it is also interesting and important to understand the physiological and behavioural impacts of the three major long-term climatic challenges - warming, acidification and deoxygenation, in order to understand whether the exposure period will be advantageous for the acclimatization of these animals. Furthermore, despite the reduced swimming abilities of seahorses, recent genetic studies with populations of *Hippocampus mohnikei* and *Hippocampus trimaculatus* from different zoogeographical zones show that both underwent demographic expansion more than 200 000 years ago, which suggests that these animals were able to travel between different habitats (Zhang et al., 2014). In addition, with *Hippocampus erectus*, it was also verified the existence of seasonal migrations between inshore and offshore zones, during warm and cold seasons respectively, mostly triggered by reproduction (Boehm et al., 2015, Qin et al., 2017; Foster and Vincent, 2004). Taking this into account, it is also important to understand the possibility of migration of this species in the face of climate change and what is the severity or period of exposure this can occur.

Finally, it is still important to understand if the acclimatization of adult individuals to these environmental conditions can influence the development of the next generations and how juveniles, with or without parental acclimatization, will respond to these factors (Borges et al., 2022). The early life stages of several species, including seahorses (Aurélio et al., 2013), are energy-dependent, and that energy is strongly dependent on the amount of oxygen available in the environment, which in turn also depends on the temperature of that same environment. If this infinite cascade of events has positive and negative effects on the development of adult *Hippocampus hippocampus*, it will be important to understand what will occur in even more fragile life stages.

6. References

Agusti, S., Benthuysen, J. A., Oliver, E. C. J., Chen, K., & Wernberg, T. (2020). Advances in understanding marine heatwaves and their impacts [Editorial]. *Frontiers in Marine Science,* 7(141). https://doi.org/10.3389/fmars.2020.00147

Ainsworth, T. D., Hurd, C. L., Gates, R. D., & Boyd, P. W. (2019). How do we overcome abrupt degradation of marine ecosystems and meet the challenge of heat waves and climate extremes? *Global Change Biology*, 26(2), 343–354. https://doi.org/10.1111/gcb.14901

Altieri, A. H., & Gedan, K. B. (2014). Climate change and dead zones. *Global Change Biology*, 21(4), 1395–1406. https://doi.org/10.1111/GCB.12754

Anderson, P. A., Berzins, I. K., Fogarty, F., Hamlin, H. J., & Guillette, L. J. (2011). Sound, stress, and seahorses: The consequences of a noisy environment to animal health. *Aquaculture*, 311(1-4), 129–138. https://doi.org/10.1016/j.aquaculture.2010.11.013

Aurélio, M., Faleiro, F., Lopes, V. M., Pires, V., Lopes, A. R., Pimentel, M. S., Repolho, T., Baptista, M., Narciso, L., & Rosa, R. (2013). Physiological and behavioral responses of temperate seahorses (*Hippocampus guttulatus*) to environmental warming. *Marine Biology*, 160(10), 2663–2670. https://doi.org/10.1007/s00227-013-2259-8

Barr, D. J., Levy, R., Scheepers. C., & Tily, H. J. (2013). Random effects structure for confirmatory hypothesis testing: Keep it maximal. *Journal of Memory and Language*, 68(3), 255–278. https://doi:10.1016/j.jml.2012.11.001

Benthuysen, J. A., Oliver, E. C., Feng, M., & Marshall, A. G. (2018). Extreme marine warming across tropical Australia during austral summer 2015–2016. *Journal of Geophysical Research: Oceans*, 123(2), 1301–1326. https://doi.org/10.1002/2017jc013326

Boehm, J. T., Waldman, J., Robinson, J. D., & Hickerson, M. J. (2015). Population genomics reveals seahorses (*Hippocampus erectus*) of the western mid-Atlantic coast to be residents rather than vagrants. *PloS One*,10(1). https://doi.org/10.1371/JOURNAL.PONE.0116219

Bond, N. A., Cronin, M. F., Freeland, H., & Mantua, N. (2015). Causes and impacts of the 2014 warm anomaly in the NE pacific. *Geophysical Research Letters*, 42(9), 3414–3420. https://doi.org/10.1002/2015gl063306

Borges, F. O., Sampaio, E., Santos, C. P., & Rosa, R. (2022). Impacts of low oxygen on marine life: neglected, but a crucial priority for research. *The Biological Bulletin*, 000–000. https://doi.org/10.1086/721468

Breitburg, D., Levin, L. A., Oschlies, A., Grégoire, M., Chavez, F. P., Conley, D. J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G. S., Limburg, K. E., Montes, I., Naqvi, S. W., Pitcher, G. C., Rabalais, N. N., Roman, M. R., Rose, K. A., Seibel, B. A., … Zhang, J. (2018). Declining oxygen in the global ocean and coastal waters. *Science*, 359(6371). https://doi.org/10.1126/science.aam7240

Brierley, A. S., & Kingsford, M. J. (2009). Impacts of climate change on marine organisms and ecosystems. *Current Biology*, 19(14). https://doi.org/10.1016/j.cub.2009.05.046

Brooks, M. E., Kristensen, K., Benthem, K. J. v., Magnusson, A., Berg, C. W., Nielsen A., Skaug, H. J., & Machler, M. (2017). Glmmtmb balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R journal, 9, 378–400. https://doi:10.32614/rj-2017-066

Burger, F. A., John, J. G., & Frölicher, T. L. (2020). Increase in ocean acidity variability and extremes under increasing atmospheric CO_2. *Biogeosciences,* 17(18), 4633–4662. https://doi.org/10.5194/BG-17-4633-2020

Burnett, L. E. (1997). The challenges of living in hypoxic and hypercapnic aquatic environments. *American Zoologist*, 37(6), 633–640. https://doi.org/10.1093/icb/37.6.633

Caldwell, I. R., & Vincent, A. C. J. (2012). Revisiting two sympatric European seahorse species: apparent decline in the absence of exploitation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 22(4), 427–435. https://doi.org/10.1002/aqc.2238

Cavole, L. M., Demko, A. M., Diner, R. E., Giddings, A., Koester, I., Pagniello, C. M. L. S., Paulsen, M. L., Ramirez-Valdez, A., Schwenck, S. M., Yen, N. K., Zill, M. E., & Franks, P. J. S. (2016). Biological impacts of the 2013–2015 warm-water anomaly in the northeast Pacific: Winners, Losers, and the Future. *Oceanography*, 29(2), 273–285. https://doi.org/10.5670/oceanog.2016.32

Clark, T. D., Sandblom, E., & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *Journal of Experimental Biology*, 216(15), 2771–2782. https://doi.org/10.1242/jeb.084251

Clarke, A., & Fraser, K. P. P. (2004). Why does metabolism scale with temperature? *Functional Ecology*, 18(2), 243–251. https://doi.org/10.1111/J.0269-8463.2004.00841.X

Clarke, A., & Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, 68(5), 893–905. https://doi.org/10.1046/J.1365-2656.1999.00337.X

Correia, M., Caldwell, I. R., Koldewey, H. J., Andrade, J. P., & Palma, J. (2015). Seahorse (Hippocampinae) population fluctuations in the Ria Formosa Lagoon, south Portugal. *Journal of Fish Biology*, 87(3), 679–690. https://doi.org/10.1111/jfb.12748

Curtis, J. M. R. (2006). A case of mistaken identity: skin filaments are unreliable for identifying *Hippocampus guttulatus* and *Hippocampus hippocampus*. *Journal of Fish Biology*, 69(6), 1855–1859. https://doi.org/10.1111/j.1095-8649.2006.01228.x

Curtis, J. M., Santos, S. V., Nadeau, J. L., Gunn, B., Bigney Wilner, K., Balasubramanian, H., Overington, S., Lesage, C. M., D'entremont, J., & Wieckowski, K. (2017). Life history and ecology of the elusive European short-snouted seahorse *Hippocampus hippocampus*. *Journal of Fish Biology*, 91(6), 1603–1622. https://doi.org/10.1111/jfb.13473

Curtis, J. M. R., & Vincent, A. C. J. (2005). Distribution of sympatric seahorse species along a gradient of habitat complexity in a seagrass-dominated community. *Marine Ecology Progress Series*, 291, 81–91. https://doi.org/10.3354/meps291081

Diaz, R. J., & Breitburg, D. L. (2009). The Hypoxic Environment. In A. P. Farrell & C. J. Brauner (Eds.), *Fish physiology: Hypoxia* (1st ed., pp. 1–23). Academic Press. https://doi.org/10.1016/S1546-5098(08)00001-0

Diaz, R. J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science*, 321(5891), 926–929. https://doi.org/10.1126/science.1156401

Doney, S. C. (2010). The growing human footprint on coastal and open-ocean biogeochemistry. *Science*, 328(5985), 1512–1516. https://doi.org/10.1126/science.1185198

Drost, M. (1987). Relation between aiming and catch success in larval fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 44, 304–315. https://doi.org/10.1139/f87-039

Duarte, C. M. (2014). Global change and the future ocean: a grand challenge for marine sciences. Frontiers in Marine Science, 1(63). https://doi.org/10.3389/fmars.2014.00063

Faleiro, F., Baptista, M., Santos, C., Aurélio, M. L., Pimentel, M., Pegado, M. R., Paula, J. R., Calado, R., Repolho, T., & Rosa, R. (2015). Seahorses under a changing ocean: the impact of warming and acidification on the behaviour and physiology of a poor-swimming bony-armoured fish. *Conservation Physiology*, 3(1). https://doi.org/10.1093/conphys/cov009

Faleiro, F., Narciso, L., & Vicente, L. (2008). Seahorse behaviour and aquaculture: How to improve *Hippocampus guttulatus* husbandry and reproduction? *Aquaculture*, 282(1-4), 33–40. https://doi.org/10.1016/j.aquaculture.2008.05.038

Farrell, A. P., & Richards, J. G. (2009). Defining hypoxia: an integrative synthesis of the responses of fish to hypoxia. In A. P. Farrell & C. J. Brauner (Eds.), *Fish physiology: Hypoxia* (1st ed., pp. 487–503). Academic Press. https://doi.org/10.1016/S1546-5098(08)00011-3

Felício, A. K., Rosa, I. L., Souto, A., & Freitas, R. H. (2006). Feeding behavior of the longsnout seahorse *Hippocampus Reidi* Ginsburg, 1933. *Journal of Ethology*, 24(3), 219–225. https://doi.org/10.1007/s10164-005-0189-8

Foster, S. J., & Vincent, A. C. (2004). Life history and ecology of seahorses: implications for conservation and management. *Journal of Fish Biology*, 65(1), 1–61. https://doi.org/10.1111/j.0022-1112.2004.00429.x

Foster, S., Wiswedel, S., & Vincent, A. (2014). Opportunities and challenges for analysis of wildlife trade using CITES data - seahorses as a case study. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 26(1), 154–172. https://doi.org/10.1002/aqc.2493

Fox J., & Weisberg, S. (2011). An R companion to applied regression (3rd ed.). *SAGE Publications, Inc.*

Frederich, M., & Pörtner, H. O. (2000). Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 279(5). https://doi.org/10.1152/ajpregu.2000.279.5.r1531

Frölicher, T. L., & Laufkötter, C. (2018). Emerging risks from marine heat waves. *Nature Communications*, 9(1). https://doi.org/10.1038/s41467-018-03163-6

Harasti, D. (2016). Declining seahorse populations linked to loss of essential marine habitats. *Marine Ecology Progress Series*, 546, 173–181. https://doi.org/10.3354/meps11619

Harianto, J., Carey, N., & Byrne, M. (2019). respR—An R package for the manipulation and analysis of respirometry data. *Methods in Ecology and Evolution*, 10(6), 912–920. https://doi.org/10.1111/2041-210x.13162

Harley, C. D., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J., Thornber, C. S., Rodriguez, L. F., Tomanek, L., & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, 9(2), 228–241. https://doi.org/10.1111/j.1461-0248.2005.00871.x

Hobday, A. J., Alexander, L. V., Perkins, S. E., Smale, D. A., Straub, S. C., Oliver, E. C. J., Benthuysen, J. A., Burrows, M. T., Donat, M. G., Feng, M., Holbrook, N. J., Moore, P. J., Scannell, H. A., Sen Gupta, A., & Wernberg, T. (2016). A hierarchical approach to defining marine heatwaves. *Progress in Oceanography*, 141, 227–238. https://doi.org/10.1016/j.pocean.2015.12.014

Hobday, A., Oliver, E., Sen Gupta, A., Benthuysen, J., Burrows, M., Donat, M., Holbrook, N., Moore, P., Thomsen, M., Wernberg, T., & Smale, D. (2018). Categorizing and naming marine heatwaves. *Oceanography*, 31(2), 162–173. https://doi.org/10.5670/oceanog.2018.205

IPCC. (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, In press, https://doi.org/10.1017/9781009157896.

IPCC. (2022). *Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, In press, https://doi.org/10.1017/9781009325844.

Johnson, C. (2017). Thermal response of behavior and gene expression of heat shock proteins in the lined seahorse, *Hippocampus erectus* (Master thesis, Florida Institute of Technology, Melbourne, United States of America). Retrieved from https://repository.lib.fit.edu/handle/11141/1336

Karl, T. R., & Trenberth, K. E. (2003). Modern global climate change. *Science*, 302(5651), 1719–1723. https://doi.org/10.1126/science.1090228

Kitsos, M. S., Tzomos, T., Anagnostopoulou, L., & Koukouras, A. (2008). Diet composition of the seahorses, *Hippocampus guttulatus* Cuvier, 1829 and *Hippocampus hippocampus* (L., 1758) (Teleostei, Syngnathidae) in the Aegean Sea. *Journal of Fish Biology*, 72(6), 1259–1267. https://doi.org/10.1111/j.1095-8649.2007.01789.x

Kweku, D., Bismark, O., Maxwell, A., Desmond, K., Danso, K., Oti-Mensah, E., Quachie, A., & Adormaa, B. (2018). Greenhouse effect: greenhouse gases and their impact on global warming. *Journal of Scientific Research and Reports*, 17(6), 1–9. https://doi.org/10.9734/jsrr/2017/39630

Lenth, R. (2022). Emmeans: Estimated Marginal Means, Aka Least-Squares Means. *R package version 1.8.2.*

Lin, Q., Gao, Y. L., Sheng, J. Q., Chen, Q. X., Zhang, B., & Lu, J. (2007). The effects of food and the sum of effective temperature on the embryonic development of the seahorse, *Hippocampus kuda* Bleeker. *Aquaculture*, 262: 481–492. https://doi.org/10.1016/j.aquaculture.2006.11.011

Lin, Q., Lu, J., Gao, Y., Shen, L., Cai, J., & Luo, J. (2006). The effect of temperature on gonad, embryonic development and survival rate of juvenile seahorses, *Hippocampus kuda* Bleeker. *Aquaculture*, *254*(1–4), 701–713. https://doi.org/10.1016/J.AQUACULTURE.2005.11.005

Lourie, S. A., Foster, S. J., Cooper, E. W. T., & Vincent, A. C. J. (2004). A guide to the identification of seahorses. Project Seahorse and TRAFFIC North America. University of British Columbia and World Wildlife Fund. Washington D.C.

Lüdecke, D., Ben-Shachar, M., Patil, I., Waggoner, P., & Makowski, D. (2021). Performance: an R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software*, 6(60), 3139. https://doi:10.21105/joss.03139

Mackenzie, B. R., & Schiedek, D. (2007). Daily ocean monitoring since the 1860s shows record warming of northern European seas. *Global Change Biology*, 13(7), 1335–1347. https://doi.org/10.1111/J.1365-2486.2007.01360.X

Mascaró, M., Amaral-Ruiz, M., Huipe-Zamora, I., Martínez-Moreno, G., Simões, N., & Rosas, C. (2016). Thermal tolerance and phenotypic plasticity in juvenile *Hippocampus erectus* Perry, 1810: Effect of acute and chronic exposure to contrasting temperatures. *Journal of Experimental Marine Biology and Ecology*, 483, 112–119. https://doi.org/10.1016/j.jembe.2016.07.005

McBryan, T. L., Anttila, K., Healy, T. M., & Schulte, P. M. (2013). Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. *Integrative and Comparative Biology*, 53(4), 648–659. https://doi.org/10.1093/icb/ict066

Naud, M. J., Curtis, J. M. R., Woodall, L. C., & Gaspar, M. B. (2008). Mate choice, operational sex ratio, and social promiscuity in a wild population of the long-snouted seahorse *Hippocampus guttulatus*. *Behavioral Ecology*, 20(1), 160–164. https://doi.org/10.1093/beheco/arn128

Negreiros, L. A., Silva, B. F., Paulino, M. G., Fernandes, M. N., & Chippari-Gomes, A. R. (2011). Effects of hypoxia and petroleum on the genotoxic and morphological parameters of *Hippocampus reidi*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 153(4), 408–414. https://doi:10.1016/j.cbpc.2011.02.001

Nilsson, G. E., Östlund-Nilsson, S., Penfold, R., & Grutter, A. S. (2006). From record performance to hypoxia tolerance: respiratory transition in damselfish larvae settling on a coral reef. *Proceedings of the Royal Society B: Biological Sciences*, 274(1606), 79–85. https://doi.org/10.1098/rspb.2006.3706

Niimi, A. J., & Beamish, F. W. H. (1974). Bioenergetics and growth of largemouth bass (*Micropterus salmoides*) in relation to body weight and temperature. *The Canadian Journal of Zoology*. 52, 447-456. https://doi.org/10.1139/z74-056

Oliver, E. C., Benthuysen, J. A., Bindoff, N. L., Hobday, A. J., Holbrook, N. J., Mundy, C. N., & Perkins-Kirkpatrick, S. E. (2017). The unprecedented 2015/16 Tasman Sea marine heatwave. *Nature Communications*, 8(1). https://doi.org/10.1038/ncomms16101

Oliver, E. C., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., Benthuysen, J. A., Feng, M., Sen Gupta, A., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Straub, S. C., & Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature Communications*, 9(1). https://doi.org/10.1038/s41467-018-03732-9

Otero-Ferrer, F., Molina, L., Socorro, J., Hipólito, H., Fernández, H., Fernández-Palacios, F., Izquierdo, M., & Herrera, R. (2012). Effect of different live prey on spawning quality of short-snouted seahorse, *Hippocampus hippocampus* (Linnaeus, 1758). *Journal of the world aquaculture society*, 43(2). https://doi.org/10.1111/j.1749-7345.2012.00550.x

Otto, S. P. (2018). Adaptation, speciation and extinction in the Anthropocene. *Proceedings of the Royal Society B: Biological Sciences*. 285(20182047). https://doi.org/10.1098/rspb.2018.2047

OSPAR. (2008). Case reports for the OSPAR List of Threatened and/or Declining Species and Habitats. https://www.ospar.org/site/assets/files/44259/short_snouted_seahorse.pdf. Accessed on 15 September 2022.

Paula, J. R., Repolho, T., Grutter, A. S., & Rosa, R. (2022). Access to cleaning services alters fish physiology under parasite infection and ocean acidification. *Frontiers in Physiology*, 13. https://doi.org/10.3389/fphys.2022.859556

Perkins, S. E., & Alexander, L. V. (2013). On the measurement of heat waves. *Journal of Climate*, 26(13), 4500–4517. https://doi.org/10.1175/jcli-d-12-00383.1

Pigliucci, M. (2003). Phenotypic integration: studying the ecology and evolution of complex phenotypes. *Ecology Letters*, 6, 265–272.

Pimentel, M. S., Faleiro, F., Marques, T., Bispo, R., Dionísio, G., Faria, A. M., Machado, J., Peck, M. A., Pörtner, H., Pousão-Ferreira, P., Gonçalves, E. J., & Rosa, R. (2016). Foraging behaviour, swimming performance and malformations of early stages of commercially important fishes under ocean acidification and warming. *Climatic Change*, 137(3–4), 495–509. https://doi.org/10.1007/s10584-016-1682-5

Pollom, R. (2016). *Hippocampus hippocampus* (Mediterranean assessment). The IUCN Red List of Threatened Species 2016: e.T10069A90866381. Accessed on 15 September 2022.

Pörtner, H. O., & Farrell, A. P. (2008). Physiology and climate change. *Science*, 322(5902), 690–692. https://doi.org/10.1126/science.1163156

Pörtner, H. O., & Peck, M. A. (2010). Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. *Journal of Fish Biology*, 77, 1745–1779. https://doi.org/10.1111/j.1095-8649.2010.02783.x

Pörtner, H. O., & Knust, R. (2007). Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, 315(5808), 95–97. https://doi.org/10.1126/SCIENCE.1135471/SUPPL_FILE/POERTNER.SOM.PDF

Pörtner, H. O., Mark, F. C., & Bock, C. (2004). Oxygen limited thermal tolerance in fish?: Answers obtained by nuclear magnetic resonance techniques. *Respiratory Physiology & Neurobiology*, 141(3), 243–260. https://doi.org/10.1016/J.RESP.2004.03.011

Qin, G., Zhang, Y., F C Ho, A. L., Zhang, Y., & Lin, Q. (2017). Seasonal distribution and reproductive strategy of seahorses. *ICES Journal of Marine Science*, 74, 2170–2179. https://doi.org/10.1093/icesjms/fsx042

Qin, G., Johnson, C., Zhang, Y., Zhang, H., Yin, J., Miller, G., Turingan, R. G., Guisbert, E., & Lin, Q. (2018). Temperature-induced physiological stress and reproductive characteristics of the migratory seahorse *Hippocampus erectus* during a thermal stress simulation. *Biology Open*. https://doi.org/10.1242/bio.032888

Richards, J. G. (2009). Metabolic and Molecular Responses of Fish to Hypoxia. In A. P. Farrell & C. J. Brauner (Eds.), *Fish physiology: Hypoxia* (1st ed., 443–485). https://doi.org/10.1016/S1546-5098(08)00010-1

Roessig, J. M., Woodley, C. M., Cech, J. J., & Hansen, L. J. (2004). Effects of global climate change on marine and estuarine fishes and fisheries. *Reviews in Fish Biology and Fisheries*, 14(2), 251–275. https://doi.org/10.1007/s11160-004-6749-0

Rummer, J. L., Binning, S. A., Roche, D. G., & Johansen, J. L. (2016). Methods matter: considering locomotory mode and respirometry technique when estimating metabolic rates of fishes. *Conservation Physiology*, 4(1). https://doi.org/10.1093/conphys/cow008

Russell, W. M. S. & Burch, R. L. (1959). *The principles of humane experimental technique*. Universities Federation for Animal Welfare, Hertfordshire.

Sampaio, E., & Rosa, R. (2019). Climate Change, Multiple Stressors, and Responses of Marine Biota. *Climate Action*, 1–13. https://doi.org/10.1007/978-3-319-71063-1_90-1

Sampaio, E., Rosa, I. C., Ferreira, V., Santos, C., Pörtner, H.-O., Duarte, C. M., Levin, L. & Rosa, R. (2021). Impacts of hypoxic events surpass those of future ocean warming and acidification. *Nature Ecology and Evolution*, 5(3), 311–321. https://doi.org/10.1038/s41559-020-01370-3

Schlegel, R. W., & Smit, A. S. (2018). heatwaveR: A central algorithm for the detection of heatwaves and cold-spells. *Journal of Open Source Software*, 3(27), 821. https://doi.org/10.21105/joss.00821

Seibel, B. A. (2011). Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. *Journal of Experimental Biology*, 214(2), 326–336. https://doi.org/10.1242/JEB.049171

Seitzinger, S. P., Mayorga, E., Bouwman, A. F., Kroeze, C., Beusen, A. H., Billen, G., Van Drecht, G., Dumont, E., Fekete, B. M., Garnier, J., & Harrison, J. A. (2010). Global river nutrient export: a scenario analysis of past and future trends. *Global Biogeochemical Cycles*, 24(4). https://doi.org/10.1029/2009gb003587

Sheng, J., Lin, Q., Chen, Q., Gao, Y., Shen, L., & Lu, J. (2006). Effects of food, temperature and light intensity on the feeding behavior of three-spot juvenile seahorses, *Hippocampus trimaculatus* Leach. *Aquaculture*, 256(1-4), 596–607. https://doi:10.1016/j.aquaculture.2006.02

Somero, G. N. (2010). The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine “winners” and “losers.” *Journal of Experimental Biology*, 213(6), 912–920. https://doi.org/10.1242/JEB.037473

Spillman, C. M., Smith, G. A., Hobday, A. J., & Hartog, J. R. (2021). Onset and decline rates of marine heatwaves: global trends, seasonal forecasts and marine management. *Frontiers in Climate*, 3. https://doi.org/10.3389/fclim.2021.801217

Stramma, L., Johnson, G. C., Sprintall, J., & Mohrholz, V. (2008). Expanding oxygen-minimum zones in the tropical oceans. *Science*, 320(5876), 655–658. https://doi.org/10.1126/science.1153847

Sundin, J., Rosenqvist, G., & Berglund, A. (2015). Hypoxia delays mating in the broad-nosed pipefish. *Marine Biology Research*, *11*(7), 747–754. https://doi.org/10.1080/17451000.2015.1007874

Teske, P. R., Cherry, M. I., & Matthee, C. A. (2004). The evolutionary history of seahorses (Syngnathidae: *Hippocampus*): Molecular data suggest a West Pacific origin and two invasions of the Atlantic Ocean. *Molecular Phylogenetics and Evolution*, 30(2), 273–286. https://doi.org/10.1016/S1055-7903(03)00214-8

Vaquer-Sunyer, R., & Duarte, C. M. (2008). Thresholds of hypoxia for marine biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, 105(40), 15452–15457. https://doi.org/10.1073/PNAS.0803833105

Vincent, A. C. J. (1994). Seahorses exhibit conventional sex roles in mating competition, despite male pregnancy. *Behaviour*, 128(1–2), 135–151. https://doi.org/10.1163/156853994X00082

Vincent, A. C. J., Foster, S. J., & Koldewey, H. J. (2011). Conservation and management of seahorses and other Syngnathidae. *Journal of Fish Biology*, 78(6), 1681–1724. https://doi.org/10.1111/j.1095-8649.2011.03003.x

Vincent, A. C. J., & Sadler, L. M. (1995). Faithful pair bonds in wild seahorses, *Hippocampus whitei*. *Animal Behaviour*, 50(6), 1557–1569. https://doi.org/10.1016/0003-3472(95)80011-5

Vogt, L., Burger, F. A., Griffies, S. M., & Frölicher, T. L. (2022). Local drivers of marine heatwaves: a global analysis with an earth system model. *Frontiers in Climate*, 4. https://doi.org/10.3389/fclim.2022.847995

Wang, T., & Overgaard, J. (2007). The heartbreak of adapting to global warming. Science, 315(5808), 49–50. https://doi.org/10.1126/SCIENCE.1137359

Wernberg, T., Bennett, S., Babcock, R. C., de Bettignies, T., Cure, K., Depczynski, M., Dufois, F., Fromont, J., Fulton, C. J., Hovey, R. K., Harvey, E. S., Holmes, T. H., Kendrick, G. A., Radford, B., Santana-Garcon, J., Saunders, B. J., Smale, D. A., Thomsen, M. S., Tuckett, C. A., … Wilson, S. (2016). Climate-driven regime shift of a temperate marine ecosystem. *Science*, 353(6295), 169–172. https://doi.org/10.1126/science.aad8745

White, C. R., Phillips, N. F., & Seymour, R. S. (2005). The scaling and temperature dependence of vertebrate metabolism. *Biology Letters*, *2*(1), 125–127. https://doi.org/10.1098/RSBL.2005.0378

Willmer, P., Stone, G., & Johnston, I. (2005). Environmental physiology of animals (2nd ed.). Blackwell Science.

WMO. (2022). State of the Global Climate 2021. (WMO-No. 1290). Switzerland. https://library.wmo.int/doc_num.php?explnum_id=11178

Woodall, L. (2017). Hippocampus hippocampus (Global assessment). The IUCN Red List of Threatened Species 2017: e.T10069A67618259. https://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T10069A67618259.en. Accessed on 15 September 2022.

Wu, R. S. (2002). Hypoxia: from molecular responses to ecosystem responses. *Marine Pollution Bulletin*, 45(1-12), 35–45. https://doi:10.1016/s0025-326x(02)00061-9

Xing, W., Yin, M., Lv, Q., Hu, Y., Liu, C., & Zhang, J. (2014). Oxygen solubility, diffusion coefficient, and solution viscosity. *Rotating Electrode Methods and Oxygen Reduction Electrocatalysts*, 1–31. https://doi.org/10.1016/b978-0-444-63278-4.00001-x

Žalohar, J., Hitij, T., & Križnar, M. (2009). Two new species of seahorses (Syngnathidae, *Hippocampus*) from the Middle Miocene (Sarmatian) Coprolitic Horizon in Tunjice Hills, Slovenia: The oldest fossil record of seahorses. *Annales de Paleontologie*, 95(2), 71–96. https://doi.org/10.1016/j.annpal.2009.03.002

Zhang, Y., Pham, N. K., Zhang, H., Lin, J., & Lin, Q. (2014). Genetic Variations in Two Seahorse Species (*Hippocampus mohnikei* and *Hippocampus trimaculatus*): Evidence for Middle Pleistocene Population Expansion. *PLOS ONE*, 9(8), e105494. https://doi.org/10.1371/JOURNAL.PONE.0105494

Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R.* Springer New York.

7. Appendix (supplemental material)

Supplemental Table 1: Analysis of deviance (Type II tests) for the generalized linear mixed models of the metabolic and ventilation rates of *Hippocampus hippocampus* under four different treatments - control (C), marine heatwave (MHW), hypoxia (H) and marine heatwave and hypoxia (MHW+H). Significant levels (p-value < α, α = 0.05) are represented as: * when p-value < 0.05, ** when p-value < 0.01 and *** when p-value < 0.001.

	df	χ^2	p-value	
Oxygen uptake rate (MO_2)	3	87.069	< 0.0001	***
Ventilation rate	3	141.79	< 0.0001	***

Supplemental Table 2: Analysis of deviance (Type II tests) for the generalized linear mixed models of the behavioural patterns of *Hippocampus hippocampus* under four different treatments - control (C), marine heatwave (MHW), hypoxia (H) and marine heatwave and hypoxia (MHW+H). Significant levels (p-value < α, α = 0.05) are represented as: * when p-value < 0.05, ** when p-value < 0.01 and *** when p-value < 0.001.

	df	χ^2	p-value	
Rest	3	55.326	< 0.0001	***
Stationary	3	96.761	< 0.0001	***
Swinging	3	98.028	< 0.0001	***
Individual activity	3	35.009	< 0.0001	***
Adjustment	3	4.821	0.1854	
Slow body movement	3	51.403	< 0.0001	***
Swimming	3	6.232	0.1009	
Feeding duration	3	19.814	< 0.0001	***
Attacks	3	26.306	< 0.0001	***
Capture	3	37.191	< 0.0001	***
Miss	3	0.870	0.8327	
Live food intake	3	12.408	0.0061	**
Frozen food intake	3	34.913	< 0.0001	***
Social activity	3	12.387	0.0066	**
Interaction without aggression	3	6.137	0.1051	
Courtship	3	4.152	0.2455	

Supplemental Table 3: Analysis of deviance (Type II tests) for the generalized linear mixed models of *Hippocampus hippocampus* oocytes length, width and area, under four different treatments - control (C), marine heatwave (MHW), hypoxia (H) and marine heatwave and hypoxia (MHW+H). Significant levels (p-value < α, $\alpha = 0.05$) are represented as: * when p-value < 0.05, ** when p-value < 0.01 and *** when p-value < 0.001.

	df	**χ^2**	**p-value**	
Oocytes length	1	12.45	0.0004	***
Oocytes width	1	37.88	< 0.0001	***
Oocytes area	1	22.90	< 0.0001	***

Supplemental Table 4: Summary output for the post-hoc pairwise comparisons of the metabolic and ventilation rates of *Hippocampus hippocampus* under four different treatments - control (C), marine heatwave (MHW), hypoxia (H) and marine heatwave and hypoxia (MHW+H). Significant levels (p-value < α, α = 0.05) are represented as: * when p-value < 0.05, ** when p-value < 0.01 and *** when p-value < 0.001.

	Model	**Post-hoc comparison**	**estimate**	**Std. error**	**t-ratio**	**p-value**	
Oxygen uptake rate (MO_2)	GLMM,	Treatment C - MHW	-0.456	0.34	-1.339	0.5506	
	family = Gaussian,	Treatment C - H	2.212	0.290	7.633	< 0.0001	***
	AIC = 60.9	Treatment C – MHW+H	1.397	0.378	3.696	0.0077	**
		Treatment MHW - H	2.668	0.346	7.705	< 0.0001	***
		Treatment MHW – MHW+H	1.853	0.412	4.499	0.0013	**
		Treatment H – MHW+H	-0.814	0.387	-2.105	0.1871	
Ventilation rate	GLMM,	Treatment C - MHW	0.483	0.044	-8.079	< 0.0001	***
	family = Poisson,	Treatment C - H	0.436	0.036	-9.989	< 0.0001	***
	AIC = 200.9	Treatment C – MHW+H	0.356	0.033	-11.142	< 0.0001	***
		Treatment MHW - H	0.902	0.068	-1.363	0.5338	
		Treatment MHW – MHW+H	0.736	0.064	-3.535	0.0089	**
		Treatment H – MHW+H	0.816	0.065	-2.554	0.0776	

Supplemental Table 5: Summary output for the post-hoc pairwise comparisons of the behavioural patterns of *Hippocampus hippocampus* under four different treatments - control (C), marine heatwave (MHW), hypoxia (H) and marine heatwave and hypoxia (MHW+H). Significant levels (p-value < α, α = 0.05) are represented as: * when p-value < 0.05, ** when p-value < 0.01 and *** when p-value < 0.001.

	Model	**Post-hoc comparison**	**estimate**	**Std. error**	**t-ratio**	**p-value**	
Rest	GLMM,	Treatment C - MHW	2.882	0.743	4.107	0.0024	**
	family = Beta,	Treatment C - H	0.051	0.028	-5.341	< 0.0001	***
	AIC = -117.1	Treatment C – MHW+H	0.069	0.038	-4.870	0.0004	***
		Treatment MHW - H	0.018	0.011	-6.498	< 0.0001	***
		Treatment MHW – MHW+H	0.024	0.014	-6.339	< 0.0001	***
		Treatment H – MHW+H	1.362	0.821	0.512	0.9550	
Stationary	GLMM,	Treatment C - MHW	1.501	0.796	0.765	0.8693	
	family = Beta,	Treatment C - H	0.009	0.005	-8.365	< 0.0001	***
	AIC = -151.6	Treatment C – MHW+H	0.010	0.006	-7.680	< 0.0001	***
		Treatment MHW - H	0.006	0.004	-8.205	< 0.0001	***
		Treatment MHW – MHW+H	0.007	0.004	-7.632	< 0.0001	***
		Treatment H – MHW+H	1.121	0.524	0.245	0.9947	
Swinging	GLMM,	Treatment C - MHW	2.524	0.706	3.313	0.0155	*
	family = Beta,	Treatment C - H	18.112	5.665	9.262	< 0.0001	***
	AIC = -29.0	Treatment C – MHW+H	14.480	6.615	5.851	< 0.0001	***
		Treatment MHW - H	7.175	2.105	6.716	< 0.0001	***
		Treatment MHW – MHW+H	5.736	2.610	3.839	0.0046	**
		Treatment H – MHW+H	0.799	0.362	-0.494	0.9596	

Supplemental Table 5: Continued.

	Model	Post-hoc comparisons	estimate	Std. error	t-ratio	p-value	
Individual activity	GLMM,	Treatment C - MHW	0.430	0.137	-2.642	0.0660	
	family = Beta,	Treatment C - H	11.789	6.445	4.513	0.0009	***
	AIC = -117.6	Treatment C – MHW+H	9.375	5.019	4.180	0.0020	**
		Treatment MHW - H	27.419	17.494	5.190	0.0002	***
		Treatment MHW – MHW+H	21.804	12.958	5.186	0.0002	***
		Treatment H – MHW+H	0.795	0.462	-0.394	0.9787	
Adjustment	GLMM,	Treatment C - MHW	0.475	0.227	-1.555	0.4235	
	family = Beta,	Treatment C - H	1.378	0.645	0.686	0.9012	
	AIC = -298.1	Treatment C – MHW+H	1.023	0.543	0.044	1.0000	
		Treatment MHW - H	2.904	1.458	2.124	0.1768	
		Treatment MHW – MHW+H	2.156	1.202	1.378	0.5256	
		Treatment H – MHW+H	0.742	0.406	-0.544	0.9471	
Slow body movement	GLMM,	Treatment C - MHW	0.803	0.231	-0.762	0.8704	
	family = Beta,	Treatment C - H	12.367	5.357	5.806	< 0.0001	***
	AIC = -135.7	Treatment C – MHW+H	10.025	4.979	4.641	0.0007	***
		Treatment MHW - H	15.404	6.925	6.083	< 0.0001	***
		Treatment MHW – MHW+H	12.487	6.378	4.943	0.0003	***
		Treatment H – MHW+H	0.811	0.443	-0.384	0.9802	

Supplemental Table 5: Continued.

	Model	Post-hoc comparison	estimate	Std. error	t-ratio	p-value	
Swimming	GLMM,	Treatment C - MHW	0.113	0.099	-2.485	0.0903	
	family = Beta,	Treatment C - H	0.997	0.497	-0.007	1.0000	
	AIC = -244	Treatment C – MHW+H	0.933	0.522	-0.124	0.9993	
		Treatment MHW - H	8.825	8.861	2.168	0.1634	
		Treatment MHW – MHW+H	8.262	8.036	2.171	0.1626	
		Treatment H – MHW+H	0.936	0.538	-0.115	0.9994	
Feeding	GLMM,	Treatment C - MHW	0.442	0.171	-2.114	0.1797	
	family = Beta,	Treatment C - H	5.607	3.029	3.192	0.0204	*
	AIC = -224.8	Treatment C – MHW+H	6.100	3.832	2.879	0.0403	*
		Treatment MHW - H	12.678	7.813	4.122	0.0024	**
		Treatment MHW – MHW+H	13.794	9.598	3.772	0.0054	**
		Treatment H – MHW+H	1.088	0.650	0.141	0.9990	
Attack	GLMM,	Treatment C - MHW	0.318	0.124	-2.933	0.0359	*
	family = Beta,	Treatment C - H	8.134	4.992	3.416	0.0123	*
	AIC = -171.9	Treatment C – MHW+H	9.084	6.426	3.119	0.0239	*
		Treatment MHW - H	25.576	17.334	4.783	0.0005	***
		Treatment MHW – MHW+H	28.562	21.935	4.365	0.0013	**
		Treatment H – MHW+H	1.117	0.703	0.175	0.9980	

Supplemental Table 5: Continued.

	Model	Post-hoc comparison	estimate	Std. error	t-ratio	p-value	
Capture	GLMM,	Treatment C - MHW	0. 314	0.104	-3.495	0.0102	*
	family = Beta,	Treatment C - H	11.704	7.308	3.940	0.0036	**
	AIC = -191.6	Treatment C – MHW+H	11.184	7.787	3.468	0.0109	*
		Treatment MHW - H	37.219	24.255	5.550	0.0001	***
		Treatment MHW – MHW+H	35.567	25.657	4.951	0.0003	***
		Treatment H – MHW+H	0.956	0.595	-0.073	0.9999	
Miss	GLMM,	Treatment C - MHW	0.93	0.483	-0.140	0.9990	
	family = Beta,	Treatment C - H	1.30	0.627	0.548	0.9462	
	AIC = -270.4	Treatment C – MHW+H	1.46	0.812	0.688	0.9006	
		Treatment MHW - H	1.40	0.748	0.630	0.9006	
		Treatment MHW – MHW+H	1.57	0.946	0.756	0.8732	
		Treatment H – MHW+H	1.12	0.638	0.207	0.9968	
Frozen food intake	GLMM,	Treatment C - MHW	0.158	0.0778	-3.743	0.0058	**
	family = Beta,	Treatment C - H	2.971	1.4560	2.222	0.1485	
	AIC = -190	Treatment C – MHW+H	2.971	1.6716	1.935	0.2429	
		Treatment MHW - H	18.858	10.2642	5.396	0.0001	***
		Treatment MHW – MHW+H	18.858	11.5120	4.811	0.0005	***
		Treatment H – MHW+H	1.000	0.5754	0.000	1.0000	

Supplemental Table 5: Continued.

	Model	Post-hoc comparison	estimate	Std. error	t-ratio	p-value	
Live food intake	GLMM,	Treatment C - MHW	0.668	0.338	-0.797	0.8551	
	family = Beta,	Treatment C - H	3.231	1.585	2.391	0.1084	
	AIC = -198.9	Treatment C – MHW+H	3.231	1.819	2.083	0.1896	
		Treatment MHW - H	4.834	2.614	2.914	0.0374	*
		Treatment MHW – MHW+H	4.834	2.935	2.595	0.0726	
		Treatment H – MHW+H	1.000	0.575	0.000	1.0000	
Social activity	GLMM,	Treatment C - MHW	0.646	0.313	-0.901	0.8042	
	family = Beta,	Treatment C - H	3.066	1.475	2.328	0.1220	
	AIC = -214.8	Treatment C – MHW+H	3.066	1.697	2.025	0.2095	
		Treatment MHW - H	4.745	2.495	2.961	0.0338	*
		Treatment MHW – MHW+H	4.745	2.812	2.627	0.0680	
		Treatment H – MHW+H	1.000	0.569	0.000	1.0000	
Interaction without aggression	GLMM,	Treatment C - MHW	0.965	0.479	-0.072	0.9999	
	family = Beta,	Treatment C - H	2.497	1.191	1.918	0.2495	
	AIC = -244.4	Treatment C – MHW+H	2.497	1.371	1.667	0.3640	
		Treatment MHW - H	2.588	1.361	1.808	0.2965	
		Treatment MHW – MHW+H	2.588	1.532	1.606	0.3957	
		Treatment H – MHW+H	1.000	0.565	0.000	1.0000	

Supplemental Table 5: Continued.

	Model	Post-hoc comparison	estimate	Std. error	t-ratio	p-value
Courtship	GLMM, family = Beta, AIC = -266.3	Treatment C - MHW	0.542	0.277	-1.199	0.6339
		Treatment C - H	1.448	0.697	0.769	0.8676
		Treatment C – MHW+H	1.448	0.801	0.669	0.9075
		Treatment MHW - H	2.671	1.415	1.855	0.2758
		Treatment MHW – MHW+H	2.671	1.591	1.650	0.3730
		Treatment H – MHW+H	1.000	0.567	0.000	1.0000

Supplemental Table 6: Summary output for the post-hoc pairwise comparisons of the *Hippocampus hippocampus* oocytes length, width and area under two different treatments - control (C) and marine heatwave (MHW). Significant levels (p-value < α, α = 0.05) are represented as: * when p-value < 0.05, ** when p-value < 0.01 and *** when p-value < 0.001.

	Model	Post-hoc comparison	estimate	Std. error	t-ratio	p-value	
Oocytes length	GLMM, family = Gamma	Treatment C - MHW	0.893	0.0286	-3.528	0.0012	**
Oocytes width	GLMM, family = Gamma	Treatment C - MHW	0.829	0.0253	-6.155	< 0.0001	***
Oocytes area	GLMM, family = Gamma	Treatment C - MHW	0.798	0.0376	-4.785	0.0002	***