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Effects of anthropogenic noise as a source of acoustic stress in wild populations of *Hippocampus guttulatus* in the Ria Formosa, south Portugal

Jorge Palma  | Marisa Magalhães | Miguel Correia  | José Pedro Andrade 

CCMAR, Universidade do Algarve, FCT,
Edifício 7, Campus de Gambelas, Faro, Portugal

Correspondence

Jorge Palma, CCMAR, Universidade do
Algarve, FCT, Edifício 7, Campus de Gambelas,
Faro 8005-139, Portugal
Email: jpalma@ualg.pt

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Abstract

1. This study evaluated the effects of underwater noise as a source of acoustic stress in the wild populations of *Hippocampus guttulatus* in the Ria Formosa, south Portugal. Two different scenarios of underwater noise were tested: transient motor boat sound (63.4–127.6 dB) and constant sound produced by the motor of the boat directly above the animals (up to 137.1 dB).
2. Observations were obtained in the wild between 4 and 10 m depth throughout a 3 min period, using a video camera and a hydrophone set, and compared with a control sample.
3. A significant increase ($P < 0.05$) in the respiratory rate was observed in 87% of the observed fish. Opercular movements per minute (OMPM) increased from 35.7 ± 10 (control sample) to 41.2 ± 15.5 after the first minute, to 45.5 ± 13.3 after the second (both under transient sound) and to 49.7 ± 12.5 after the third (under constant sound exposure). Differences in means between the control fish and fish observed during the second ($P < 0.01$) and third minute of observation ($P < 0.0001$) were significant. Concordantly, a significant increase ($P < 0.05$) in the OMPM of fish observed in the first minute and the third minute was noted. In addition to the OMPM increase, 37.5% of the animals abandoned the observation location presumably in an attempt to avoid the negative sound stimuli.
4. The noise caused by boat traffic generated an immediate physiological response, expressed as an increase in OMPM, and a behavioural response resulting in site abandonment, which together can be considered as a negative impact on the seahorse populations. This work contributes to an increasing number of studies that have shown that boat traffic can induce ecological and environmental consequences to aquatic species. Future research should evaluate how navigation exclusion areas could have a positive impact on the seahorse populations inhabiting shallow coastal areas.

KEYWORDS

acoustic stress responses, behaviour, *Hippocampus guttulatus*, seahorses, underwater noise

1 | INTRODUCTION

Seahorses, as with other small marine fish species, are an integral part of the marine biodiversity and ecosystem function (Foster & Vincent, 2004). Seahorses have a unique life history, characterized by a sparse distribution, low mobility, site fidelity, small home ranges, low fecundity, mate fidelity and lengthy parental care to small broods, which might render them vulnerable to overfishing and environmental disruptions, including habitat damage and degradation (Foster & Vincent, 2004). In addition, seahorses inhabit shallow coastal areas worldwide, where anthropogenic impacts tend to be most frequent and severe (Bell, Lockyear, McPherson, Marsden, & Vincent, 2003). These constraints help to explain why 14 of the 42 seahorse species are listed as Vulnerable or Endangered on the 2018 IUCN Red List of Threatened Species, one as Near Threatened, 17 as Data Deficient and 10 as Least Concern (IUCN, 2018).

In shallow coastal habitats, anthropogenic impacts are the sum of several human activities, including persistent or short-term polluting events, fishing activity (with direct and indirect impact, either as bycatch or seafloor degradation, yacht mooring, bottom dredging and boat traffic. The Ria Formosa lagoon (south Portugal) is no exception, as these anthropogenic disturbances are present and play a significant role in the occurrence and conservation of the natural populations of the long snout seahorse, *Hippocampus guttulatus*. During the spring–summer period, human activities peak, leading to a substantial increase in the underwater noise.

Fish have a well-developed auditory system with the ability to discriminate sounds produced in specific frequency bands (Fay, Popper, & Webb, 2008; Kasumyan, 2008), and many of them rely on their hearing for sensing their conspecifics and other species' activities in their surroundings (Popper, Fewtrell, Smith, & McCauley, 2004). Thus, human-induced underwater noise has the potential to cause disturbance at a physiological level (e.g. reduced hearing sensitivity) (Graham & Cooke, 2008), as excessive noise has been found to either temporarily alter hearing thresholds in fish or to destroy hair cells of auditory maculae (Popper et al., 2004). Seahorses, like most fishes, are considered to have a generalist hearing (typically able to detect sounds up to 1 or 1.5 kHz) due to their low-frequency sensitivity range and the absence of bony or gaseous vesicular connection to the swim bladder. It is probable, therefore, that they detect and process both particle motion and sound pressure components with relative contributions varying according to the sound pressure level, distance from the sound and its frequency (Anderson, 2013).

Over the past decades, human activities have produced increasing background underwater noise pollution (Hildebrand, 2009; Ross, 2005), changing the acoustic characteristics of many coastal, pelagic and deep marine ecosystems (Celi et al., 2016). In particular, boat traffic of all sizes has greatly increased, and noise emissions account for >90% of the acoustic energy that humans emit into the sea (National Research Council, 1994). Different boats produce different underwater noises depending on the type of engine, and even small boats can generate considerable amounts of noise. For example, small boats with more powerful outboard motors can produce sounds of about

175 dB re 1 μ Pa (Conservation and Development Problem Solving Team, 2000). Boat traffic generates different-intensity noises, which can cause low to severe physiological impacts (e.g. hearing sensitivity loss or decay), but as acoustic pollution is constantly produced over time, it may affect large areas, inducing serious hazards not only to individual animals, but also to entire populations (Panigada et al., 2008; Slabbekoorn et al., 2010).

According to Merchant, Witt, Blondel, Godley, and Smith (2012), sources of anthropogenic underwater noise can be categorized as impulsive or continuous, each associated with particular effects on marine fauna, and each requiring an adapted management approach to mitigate potential impacts to the species (Merchant et al., 2012). Impulsive noise consists of brief, discrete sounds of sudden occurrence (e.g. acoustic pulses from explosions, pile driving or seismic air guns). Continuous anthropogenic noise is primarily generated by shipping (Merchant et al., 2012), which includes all aquatic vehicles (e.g. jet skis, boats, jetfoils and large vessels). The former can induce acute effects on animals, including permanent or temporary auditory damage, physiological stress and antipredator responses (e.g. displacement) (Merchant et al., 2012), whereas the latter is associated with acoustic masking of biologically important cues (Clark et al., 2009; Francis & Barber, 2013), foraging disruption (Blair, Merchant, Friedlaender, Wiley, & Parks, 2016), increased physiological stress (Slabbekoorn et al., 2010) and developmental deficiencies (de Soto et al., 2013). Consequently, noise pollution is not only a threat to marine organisms but it also may affect the composition, health and services of ecosystems (Peng, Zhao, & Liu, 2015). Hastings, Popper, Finneran, and Lanford (1996) noted that underwater sounds equal to or greater than 180 dB at 50–2000 Hz would be harmful to fishes and Gisiner et al. (1998) observed physiological effects of intense sound on marine fishes including swim bladder injuries, eye haemorrhages, decreased egg viability and decreased growth rates.

As a result of this, anthropogenic noise is now recognized as a major pollutant, appearing in international legislation such as the Marine Strategy Framework Directive 56/2008 CE. This study aimed to determine the effects of underwater anthropogenic noise caused by boat traffic as a source of acoustic stress in wild populations of *H. guttulatus* in the Ria Formosa lagoon.

2 | MATERIAL AND METHODS

2.1 | Area description

This study was conducted in the Ria Formosa lagoon, south Portugal (36°59'N, 7°51'W), a shallow estuarine lagoon connected to the Atlantic Ocean by six inlets (Newton & Mudge, 2003). The Ria Formosa is a highly productive system composed of a network of channels and tidal creeks and characterized by a high water-turnover rate (Curtis & Vincent, 2005). Ria Formosa has been classified by the Portuguese authorities as a natural park since 1987, and is an integrant area of the European network of protected areas (Natura, 2000) and a protected area under the Ramsar convention on wetlands of international importance.

2.2 | Sound and video recording in the wild: In-situ controlled observations

In the Ria Formosa lagoon, the anthropogenic underwater noise is mainly produced by outboard motor boats; so, in this study, to mimic that, a 4.10 m long outboard motor boat equipped with an 40 hp Yamaha motor was used. This choice represents the most common combination of boat and outboard motor used in the Ria Formosa and can therefore be considered as the most frequent and common source of underwater noise.

All sound recordings were collected using an underwater hydrophone under two different circumstances: transient/navigation sound and constant sound. The transient/navigation sound was recorded with the boat in transit passing at the observation point in a sequence of figures of eight for a predetermined period of time (2 min; Figure 1c), whereas the constant sound was obtained over a period of 1 min, with the boat anchored directly above the observation point (Figure 1d). Constant sound was recorded immediately after the previous recording (navigation sound) to simulate an excessive sound exposure.

Sounds (including underwater sounds) are normally expressed in decibels (dB). The relevant technical features of the hydrophone (digitalHyd SR-1 hydrophone; MarSensing Lda, Faro, Portugal) used in this study are as follows:

- sampling frequency, 50,781 samples per second;
- cutting frequency, 25 kHz;
- programmable gain of 1, 2, 4, 8, 16, 32 or 64;
- converting analogue/digital 16-bit;
- data memory, MMC card 2 GB;
- autonomy of memory, about 5 h 40 min (in continuous acquisition).

The hydrophone was equipped with a programmable amplifier set for a 2× gain and a nominal sensitivity of −162 dB re 1 V/1 μPa and was

calibrated by recording test tones from a reference calibrator. The frequency distribution and decibels of chronic (constant) noise or the peak levels and deviations of acute (transient/navigation) noise were measured.

A digital camera (Canon PowerShot G12, Canon Corp., Tokyo, Japan) with an underwater housing was used to obtain individual videos for each focal fish. A specially designed Plexiglas® (Evonik, Essen, Germany) structure was built and used to house the hydrophone and the camera so these devices could be placed close to each observed seahorse, operating simultaneously. When a seahorse was located, one diver placed the structure close to the seahorse (at ~30 cm distance) and started the video recording (Figure 1a). Then, a buoy connected to the Plexiglas structure through a cable was released to provide a signal to the skipper to start boat operations (Figure 1b). Boat operations started 1–2 min later, allowing the diver to move away and eliminate any kind of interference with the observed fish. Transient and constant sounds were then collected as already described. Each seahorse was observed only once for a 3 min period (2 min under transient sound and 1 min under constant sound) after which the buoy was recovered by the diver. This procedure was repeated every time a new seahorse was found.

Recordings were collected during a 1–2 h period around the peak of slack high tide. During this period, tidal currents are reduced to a minimum, allowing both higher water visibility and the diver to operate freely. During the dives, the seahorse species, sex, depth and temperature were recorded. Sound and video files were saved in memory cards incorporated in each device and later downloaded; sound files were analysed with Audacity® software and videos files using the Gom Player® software package (Gretech Corporation, Seoul, South Korea).

Sound files were analysed and ranked according to their intensity and frequency. Ambient detectable sounds were registered and the sources characterized for later removal during data analysis. Video files (one per animal) were observed to detect potential stress responses and compared with the *ex situ* control samples. Reactions to sound stimuli were categorized into two main behaviours and/or

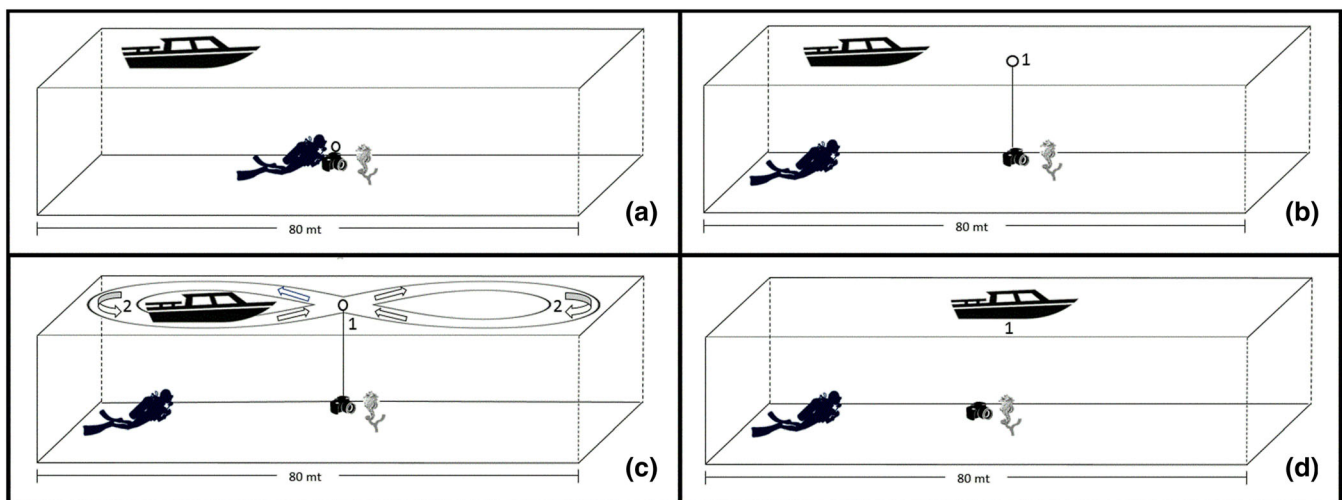


FIGURE 1 Underwater protocol for image and sound acquisition. (a) Diver approach to target fish and equipment deployment. (b) Signal to the skipper to start boat operations—buoy release (1). (c) Boat in transit passing at the observation point in a sequence of figures of eight for a predetermined period of time (2 min). (d) Boat stationary producing a constant sound directly above the observation point (1 min)

physiological reactions: site abandonment as a direct reaction to underwater sound, and number of opercular movements per minute (OMPM), thus indicating a possible stress reaction to the sound stimuli. The OMPM were counted to quantify the breathing activity and average breathing per minute both from a gender (male/female OMPM ratio) and pooled perspective.

Samples were collected in three different locations, where seahorse presence and depth were the selection criteria. Site 1 was a shallow area (4–6 m depth), whereas site 2 was a deeper area (7–14 m depth) (Figure 2). Site 3 is also a shallow area (5–6 m depth) with seahorse presence and is one of the locations in the Ria with higher boat traffic, so it was chosen to obtain *H. guttulatus* observations under normal boat traffic conditions (Figure 2). Recordings were collected during the spring and summer, the seasons with greatest boat traffic.

2.3 | Sound and video recording in the wild: Normal boat traffic

In order to record seahorse reaction to sound produced by normal boat traffic, the seahorses were observed in site 3 (Olhão channel), one of the channels in the Ria Formosa with higher boat traffic. The experimental protocol was the same as already described, with the exception that sound occurrence was not controlled; thus, matching the fish observation and sound occurrence was random. Fifteen videos were recorded, and the same behaviour and physiological reactions were analysed when seahorses were exposed to normal boat traffic conditions (referred to as wild).

2.4 | Sound profile with depth

In order to determine the variation of sound intensity with depth, sound intensity was measured metre by metre from 1 to 12 m depth under a constant boat noise (same as the third minute exposure) for 30 s.

2.5 | Ex-situ observations for control

A control sample to set the basal *H. guttulatus* OMPM values was recorded at the Aquaculture Research Station of Ramalhete (CCMAR/UALG) in the absence of any kind of sound stimuli. Observed fish (captive born) were kept in two 250 L plastic tanks assembled in a flow-through system at a density of 24 fish per tank. The same equipment (Plexiglas structure with the camera and hydrophone) was gently set inside the observation tanks to minimize any inherent stress due to its presence. Prior to the start of the observations, observed fish were kept in a sound-free environment for 24 hr without any potential sources of stress (e.g. air stone bubbling, water splashes). Average temperature and dissolved oxygen in the observation tanks were similar to those recorded under natural conditions. Sixteen videos (each 3–4 min long) were obtained and later observed to determine the stress-free *H. guttulatus* breathing behaviour.

2.6 | Sound analysis with Audacity software

The sound files obtained were analysed using Audacity software and transformed to the decibel system. The decibel system is a sound

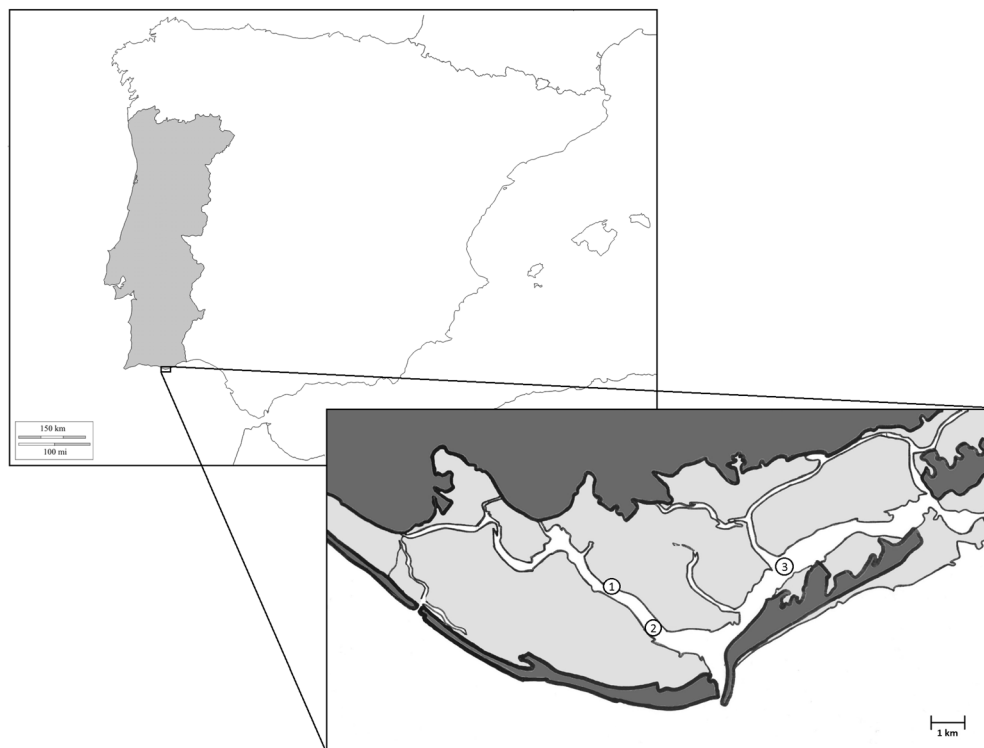


FIGURE 2 Site locations. Site 1: approximately 36°59'30.55"N, 7°53'55.96"W; site 2: approximately 36°59'04.56"N, 7°53'40.52"W; site 3: approximately 36°59'11.17"N, 7°51'42.70"W

intensity measurement expressed as power per area. The decibel values were calculated using the equation

$$\text{dB level} = 10 \log_{10} \frac{I}{I_0}$$

where I is the intensity and I_0 a base intensity (threshold of hearing is $10^{-12} \text{ W m}^{-2}$).

So, when the intensity $I = 1$ we have

$$10 \log_{10} \left(\frac{1}{10^{-12}} \right) = 10 \log_{10} (10^{12}) = 10 \times 12 = 120 \text{ dB level}$$

Decibels and decibels relative to full scale (dBFS) are logarithmic forms of sound measurement, and the value of a single decibel relative to full scale will increase the closer it gets to 0 dBFS and decrease as it tends to infinity (log measurement as just described). The difference in the perceived loudness between 0 dBFS and -6 dBFS is going to be greater than the perceived loudness between -6 and -12 dBFS even though the gap between the decibel levels is the same between the two. Essentially, 6 dB is correlated with a doubling of the sound level.

The software package Audacity (<http://www.audacityteam.org/>) was used to analyse the sound data obtained with the hydrophone, in a default output scale from 10^{-12} (approximately zero) to 1 W m^{-2} . Sound volume in this experiment was measured in decibels relative to full scale, with 0 dBFS being the reference point (Figure 3) and transformed to decibels taking into account the programmable amplified gain of the hydrophone and nominal sensibility, with 162 dB re 1 V/1 μPa being the sensibility, which was added to the negative values of decibels relative to full scale obtained to obtain the real decibel values.

If values go above zero to the positive ranges in the program, sound starts to distort and clip. Clipping appears in Audacity in the form of a red line, where a positive value occurs. Removing those lines and, consequently, the corresponding sounds (produced by equipment operation by the diver; e.g. sounds created by the camera and hydrophone positioning generated higher sounds due to their proximity of the sound receptor), which include crackles and other peak sounds

that do not correspond to the sound of the motor boat, is crucial for a proper interpretation of the sound files. Operational noises increase and distort the target sound, sometimes even suppressing it, changing the peak levels and waveform from the desired form that corresponds to the waveform of interest, which in this study is the boat transitions during the observations.

In a sound recording, there are peak volume levels (dark grey on Figure 3) that correspond to the highest sound levels and the average loudness over time (mid grey on Figure 3) of the clip or root-mean signal. Both these levels were measured in this experiment (during boat transitions and constant sound) and the waveforms were analysed.

2.7 | Statistical analysis

Differences in OMPM mean values among control, first, second, and third minute and wild were tested using one-way ANOVA with post hoc Tukey's multiple comparisons test ($P = 0.05$). Statistical analyses were conducted using the package GraphPad Prism (version 6.01 for Windows; GraphPad Software, San Diego, CA, USA).

3 | RESULTS

3.1 | Sound analysis

In order to perceive the difference between transient sound and constant sound, a spectrogram (expressed as frequency per amplitude, i.e. hertz per decibel) was produced. Higher sounds (presented in dark grey in the spectrogram) corresponded to the boat's closest proximity to the buoy (and therefore to the observed seahorses), whereas light grey corresponded to the fading sound of the boat moving away from the buoy (Figure 4). Data showed a similar sound exposure for *H. guttulatus* during the first and second minutes of boat transitions, $112.2 \pm 2.4 \text{ dB}$ and $112 \pm 3.4 \text{ dB}$ respectively, and a slightly higher sound exposure, $116.5 \pm 3.6 \text{ dB}$, during the third minute under

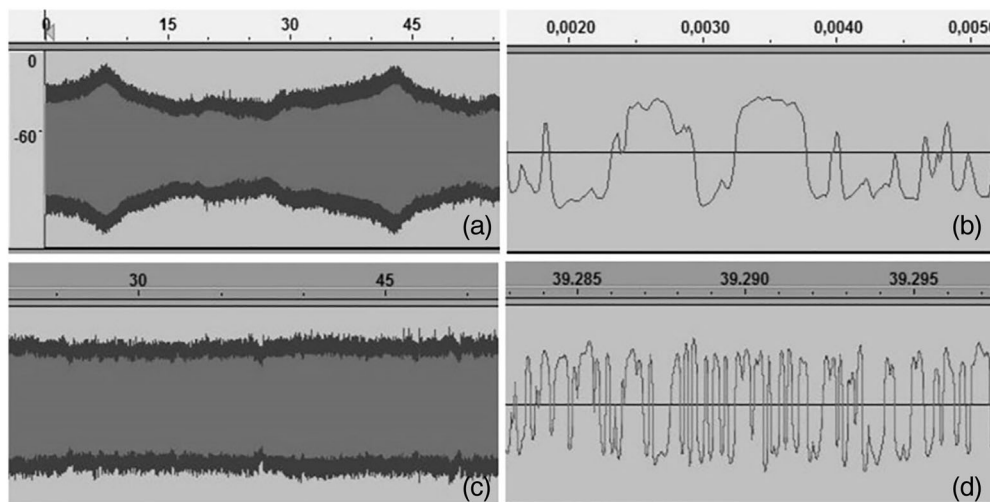


FIGURE 3 Observed soundwaves; (a) transient sound, (b) transient sound with zoom, (c) constant sound and constant sound with zoom, (d) using Audacity®. Left images: peak levels in dark grey and root mean signal average loudness in mid grey

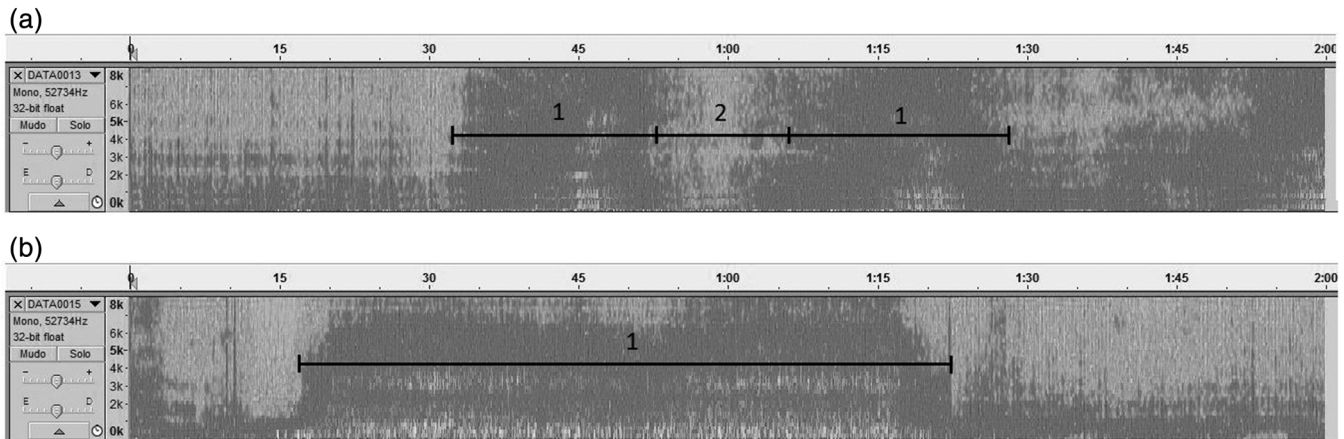


FIGURE 4 Frame spectrogram of transient (a) and constant sound (b): (1) approaching boat (loud sound exposure - dark grey); (2) departing boat (low sound exposure - mid grey)

constant sound exposure. The minimum sound exposure was 63.4 dB at the far-off positioning of the boat to the buoy (60–80 m) and 137.1 dB the maximum (during constant sound observations). Sound exposure measured during the observations in the wild was 114.1 ± 2.7 dB. The sound intensity at different depths was very similar, with an overall variation of just 2.7 dB.

3.2 | Video analysis

The control sample resulted from 48 *H. guttulatus* valid observations (28 males and 20 females). In the in-situ experiments, 57 *H. guttulatus* were observed, but only 46 were found viable for analysis (29 males and 17 females); 75.9% ($n = 22$) male seahorses were marked as 'pregnant'. For the wild/normal boat traffic sample, 15 *H. guttulatus* (11 males, 4 females) were observed and 11 were viable for further analysis. Observation exclusions were due to technical reasons, including camera displacement, blurred image, animal mispositioning or animal displacement before the start of the observation period related to an eventual discomfort caused by the diver's approach during camera and hydrophone positioning.

The *H. guttulatus* basal OMPM obtained from the control sample was 35.7 ± 10 , whereas in the in-situ trial it averaged 41.2 ± 15.5 OMPM, 45.5 ± 13.3 OMPM and 49.7 ± 12.5 OMPM at the end of the first and second minutes of transitions and at the end of the third minute under continuous sound exposure respectively. These values represent an OMPM increase of 15.4%, 27.5% and 39.2% after the first, second and third minutes respectively compared with the basal control value. The average OMPM in seahorses observed in the wild/normal boat traffic was 45.6 ± 10.1 , a value 27.7% higher ($P < 0.05$) than the observed basal value.

Hippocampus guttulatus individuals increased their breathing frequency by 14 OMPM (control sample vs. end of third minute of observations) and by 8.5 OMPM (end of the first minute vs. end of the third minute). No significant differences ($P > 0.05$) were observed between the number of OMPM in the control fish and fish exposed to the transient boat sound during the first minute, but from that point onwards

the OMPM increased significantly ($P < 0.05$). Significant differences between the control values and those observed during the second ($P < 0.01$) and third minute ($P < 0.0001$) were observed. A significant increase ($P < 0.05$) in the OMPM in fish observed in the first minute and the third minute was also observed. In a pairwise comparison between the control sample and the seahorses observed in the wild, a significant increase in the OMPM of the wild animals ($P < 0.05$) was observed, corresponding to a 9.9 OMPM increase (27.7%).

In the in-situ observations, 87% ($n = 40$) of the seahorses increased their respiratory rate (OMPM) throughout the observation period, and 37.5% of those animals ($n = 15$) ended up moving away from the observing location. Thirteen per cent ($n = 6$) did not show any response to the induced stimuli, a value significantly lower ($P < 0.05$) than the number of fish that reacted to the sound stimuli. Results also indicated that males had a higher breathing frequency than females no matter what the sound exposure scenario. In the control sound-free environment, male OMPM values (39.9 ± 9.7) were significantly higher ($P < 0.05$) than those of females (29.8 ± 9.8) (Figure 5). When exposed

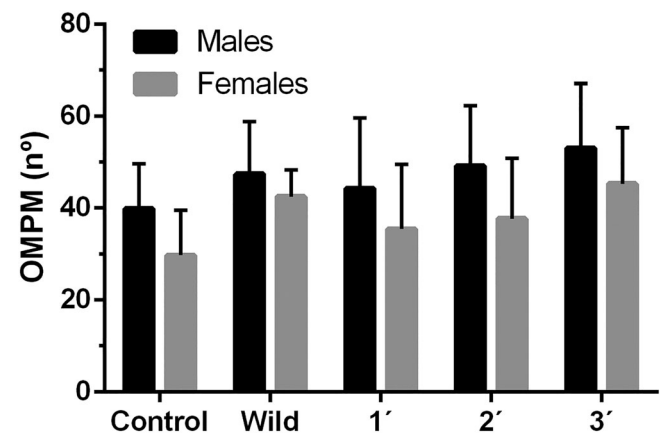


FIGURE 5 Number of opercular movements per minute (OMPM) of male and female *Hippocampus guttulatus* observed in the control, sound trials and in the wild

to the same decibel increase in the in situ and wild conditions, it was observed that males maintain a higher breathing frequency than females in all observed situations, a difference that became significantly different ($P < 0.05$) during the second minute of transient sound exposure in the in-situ observations (Figure 5).

4 | DISCUSSION

Worldwide, seahorse species suffer environmental pressures and are particularly vulnerable to population decline due to their distinctive life history, behaviour and ecology and habitat preference for shallow coastal areas, where anthropogenic disturbances tend to be most frequent and severe (Bell et al., 2003). In the Ria Formosa lagoon, *H. guttulatus* have decreased in abundance by 94% (Caldwell & Vincent, 2012) compared with the data reported by Curtis and Vincent (2005) less than a decade before. Correia (2015) pointed to natural silting events, fishing activity (including the use of illegal fishing gears with direct [bycatch] and indirect [habitat degradation]), yacht anchoring and boat traffic as the main causes for such a decline. Ria Formosa lagoon sustains intensive human action and boat traffic, including fishing boats, ferryboats, recreational boats and yachts. All boats, irrespective of their size, create underwater noise through propeller singing, propeller cavitation, propulsion or the use of other equipment and machinery (Richardson, Greene, Malme, & Thomson, 1995). As many fish species rely on their hearing for sensing activity in their surrounding environment (Popper et al., 2004), the noise from boats has the potential to cause disturbance.

Compared with light, sound propagates much more effectively in water than in air. In water, sound propagation velocity is different, as it is not as absorbed as it is in the air due to less sound buffering. In this study, the sound intensity measurements at different depths were quite similar, with an overall minimal variation. Thus, no matter the depth range seahorses inhabit in the Ria Formosa, they are likely to be equally exposed to a similar sound intensity; therefore, sound avoidance is impossible, a constraint with obvious implications on seahorse welfare in the Ria Formosa lagoon, as they are impacted regardless of the depth they inhabit.

Exposure to intense sounds can damage the auditory capacity of both terrestrial and aquatic vertebrates, including fish, and hence exposure to loud underwater sounds may result in a reduced hearing sensitivity (Smith, 2012). Besides the induced hearing loss, noise exposure relates equally to acoustic communication, stress and intraspecific communication (loud ambient noise can mask biologically relevant sounds) and can trigger stress responses with unfavourable consequences for the animal health, growth and reproduction (Anderson, 2013). The external seahorse morphology is different from other bony fish, but the internal organs, including the gas bladder, are morphologically similar, so the effect of sound is likely to be similar to other fish.

Previous studies have identified sublethal physiological disturbances in fish as a response to underwater noise. As recreational boating continues to grow in popularity, its impact has been demonstrated in a number of fish species; for example, largemouth bass

(*Micropterus salmoides*) (Graham & Cooke, 2008), damselfish (*Chromis chromis*), brown meagre (*Sciaena umbra*) and red-mouthed goby (*Gobius cruentatus*) (Codarin, Wysocki, Ladich, & Picciulin, 2009), and gilthead seabream (*Sparus aurata*) (Celi et al., 2016). However, to the best of our knowledge, published studies that have focused on seahorse stress responses to chronic noise exposure are very scarce and focused on captive-bred specimens (Anderson, Berzins, Fogarty, Hamlin, & Guillette, 2011; Fish, 1953). In captivity, seahorses are exposed to increased ambient noise caused by water flow, air pumps, air bubbles and refrigeration unit motors (Anderson et al., 2011), which will induce stress responses to chronic noise exposure. Anderson et al. (2011) observed both behavioural and physiological impacts on captive *Hippocampus erectus*. Tail adjustments and time spent stationary were observed and interpreted as irritation behaviours, and piping and clicking were respectively considered pathological and distress behaviours. As for the physiological implications, these authors observed that animals add a faster decay in their morphological indices, variable heterophilia and higher plasma cortisol concentrations when maintained in loud tanks.

In the wild, Masonjones and Babson (personal communication), mentioned in Anderson et al. (2011), found an increased incidence of gas bladder disease, behavioural differences, longer gestation rates and fewer, smaller and slower growing offspring in dwarf seahorse, *Hippocampus zosterae*, when exposed to boat motor noises. In the present study, 87% of the observed animals showed visible reaction signs (expressed by increased OMPM) to sound stimuli during the induced sequence of transient and constant sound exposure. In addition, 37.5% of those same animals abandoned their holdfast (regardless of the holdfast type) and moved away, a behaviour interpreted as an attempt to avoid or reduce the distress caused by the noise. As cryptic species, seahorses remain stationary grasped to holdfasts/shelter, only abandoning it in very specific occasions (e.g. food search in the absence of currents or during courtship behaviour). If forced to do it during unsafe situations (e.g. during strong current periods), given their poor swimming ability, it can constitute a problem and become a fragmentation factor for the populations. Seahorses are a prime example of sedentary marine fish, which remain within a small area with easy access to their needs: food, shelter/protection and mating opportunities. If forced to disperse, fish may become displaced far enough from their previous habitat, away from their home range, and find themselves unable to return, and thus forced to settle in a less favourable location, with potentially lower habitat quality and absence of their conspecifics. Seahorse dislocation from preferred habitats is normally caused by both natural (e.g. storms, strong currents, silting events) and anthropogenic (e.g. fishing, habitat degradation) impacts. However, anthropogenic underwater noise can be regarded as an additional impacting factor that may influence these populations differently in direct dependence of sound production, frequency and intensity.

In the present study, only 13% of the seahorses did not show any reaction to the noise stimuli. As this value is significantly lower ($P < 0.05$) than the 87% of fish that reacted to sound exposure, the explanation for this may rely on specific physiological constraints. Either there is a small portion of extremely resilient fish that became

adapted to environmental noises, or, as mentioned earlier, these fish may already suffer from auditory decay and are unable to fully perceive the environmental noise. Nonetheless, without further information it is not possible to identify the reasons for this behaviour.

Additionally, it was observed that males had a higher breathing frequency than females in all situations tested (control, induced sound stimuli and normal boat traffic in the wild). As both genders react similarly to the noise stimuli with a proportional increase in their breathing frequency, males end up exhibiting a higher breathing frequency under any given circumstance. Having a higher basal OMPM than females, an increase in the opercular movements due to stress conditions will cause males greater physiological stress than females. According to Masonjones (2001), oxygen consumption by male *H. zosteræ* is significantly increased from early to late gestation from 10 to 52% above 'pre-pregnant' levels. This observation is explained by the biological need that male seahorses (responsible for pregnancy and parental care) have to provide oxygen to their offspring. As observations were mainly made during spring and summer time (which corresponds to the *H. guttulatus* breeding season) and most males were marked as 'pregnant', observations and data seem to corroborate this assumption. Moreover, the OMPM increase cannot be regarded independently, as it represents an increased physiological activity that implies an increased metabolic rate determining possible metabolic alterations.

Habitat and species conservation is a present-day necessity to minimize habitat and biodiversity loss. The management of ecosystems as a whole is fundamental, and it is acknowledged that multiple human actions can have cumulative and multiplicative impacts on these systems and can lead to irreversible changes in the functioning of them (Curtin & Prellezo, 2010). Several studies have showed that ecological systems and the services that they generate can be transformed by human action into less-productive or otherwise less-desired states (Folke et al., 2004). Within these negative human actions, underwater noise is a spatially extensive pollutant, and there is growing evidence that suggests it may have highly detrimental impacts on natural communities (Francis & Barber, 2013). To our knowledge, this was the first study addressing this research topic under natural conditions, and the results showed that underwater noise pollution is indeed a detrimental factor to the life quality of the local seahorse populations. Human use of the lagoon is extensive, and any kind of prohibition to navigation is virtually impossible; however, navigation exclusion areas or reduced boat speed could be implemented. The results can be used as guidelines to accommodate this information in management and conservation plans and contribute to minimizing or even preventing the impacts of this stressing factor on these and hopefully in other aquatic species.

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ORCID

Jorge Palma  <https://orcid.org/0000-0002-5790-3433>

Miguel Correia  <https://orcid.org/0000-0003-1846-1174>

José Pedro Andrade  <https://orcid.org/0000-0002-5482-9776>

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