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Swimming bass under pounding bass : fish response to sound exposure
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CHAPTER 5
European seabass respond more strongly to sound exposure at night and habituate over repeated sessions

Abstract

Offshore pile driving can take place day and night in series of repeated sessions of high-intensity pulse trains interspersed with relatively quiet intervals. The sound exposure may negatively affect aquatic animals, including fish. The effects may be context-dependent and vary with time of day, depending on the diel cycles of physiology and behaviour. Most studies to date have investigated behavioural changes within an exposure session, but the impacts and potential habituation to repeated sessions are unknown. Here, we exposed European seabass (*Dicentrarchus labrax*) in an outdoor pen to a series of eight impulsive sound exposure sessions over two days. Baseline behaviour before sound exposure was different between day and night; at night, the fish generally swam slower and closer to the surface in a looser shoal. Upon sound exposure, the fish increased their swimming speed, swimming depth and group cohesion, and the changes were more prominent at night. Furthermore, the fish also showed inter-trial habituation as they changed their swimming depth less with subsequent exposures. Although the observed behavioural changes do not directly imply fitness or population consequences, our findings suggest that sound impacts may be stronger at night than during the day for some fish species. Moreover, our results imply that habituation should be taken into account for appropriate sound impact assessments and potential mitigating measures.

Introduction

The increasing global energy demand has prompted the energy industries to construct more oil platforms and renewable energy farms at sea. The constructions typically involve pile driving, which produces a large amount of high-intensity impulsive sound that may negatively affect aquatic animals, including fish[1–3]. For example, fish in the vicinity of the sound source may suffer from barotrauma injuries[4–6]. However, only a small proportion of fish populations are usually close enough to receive such high-intensity sound, and they typically recover from the injuries within a few weeks[6,7]. In view of this, the potential effects of more moderate sound levels further from the sound source may be more problematic, as it is unclear how they may change the behaviour of large numbers of fish in vast areas[1,8].

In response to impulsive sound exposure, fish have been shown to change their swimming behaviour. They typically swim faster, deeper and further away from a sound source, in a tighter shoal[9–12]. It is important to understand how these changes are modulated by different acoustic parameters or environmental factors, so that potential impacts may be reduced by adjusting the current pile-driving procedures or mitigating measures. For example, a recent study revealed that European seabass recovered more slowly from impulsive sound than from continuous sound, highlighting the relevance of intermittency difference between impact-hammered pile-driving methods and more continuous drilling techniques[10]. However, given that behavioural changes are often complex and context-dependent, there is currently still need for studies testing more factors that potentially influence sound impacts[8].

Pile driving is often conducted day and night[13,14], while to date, all studies investigating underwater sound impacts were conducted during the day. Since pile driving is experienced by fish throughout their diel cycles, the sound-induced effects may also vary depending on the time of the day like with other external stressors. For example, when subjected to air exposure (lifted out of water), nocturnal green sturgeon (*Acipenser medirostris*) and Gilthead sea bream (*Sparus aurata L.*) increased plasma cortisol more at night than during the day[15,16]. In contrast, nocturnal Senegalese sole (*Solea senegalensis*) was more affected during the day[17]. It is unknown how the time of the day may influence the effects of sound exposure in a diurnal species such as the European seabass (*Dicentrarchus labrax*).

Furthermore, pile-driving operations usually consist of series of multiple exposure sessions (up to 70 sessions) over several weeks or months[13,14]. However, the impacts of sound on fish behaviour have mainly been studied within a single exposure session[18]. It is unknown if repeated exposure sessions may cause behavioural effects to accumulate, leading to stronger impact, or to diminish through habituation[19–21]. Intra-trial habituation has been shown in European seabass as their behaviour recovered to the baseline level within 30 min during sound exposure[9,10]. However, whether the fish also show inter-trial habituation over repeated trials, still needs to be demonstrated.

In this study, we exposed European seabass to a series of eight sound exposures in a large outdoor floating pen throughout the diel cycle of the fish. We expected that the fish change behaviour upon sound exposure and that the behavioural changes depend on the time of the day. We also expected that behavioural changes diminish with subsequent exposures.

Materials and methods

ANIMAL MAINTENANCE

We used mixed-sex European seabass that came from a hatchery (Ecloserie Marine de Gravelines, France) and measured about 30 cm in total body length. They all had experienced an identical series of sound exposures at least three weeks earlier in a previous experiment, using the same set-up as the current experiment[22]. The fish were kept in two cylindrical holding tanks (Ø 3.5 m, depth 1.2 m) in an 8:16 dark-light cycle at Stichting Zeeschelp, the Netherlands. The holding tanks were continuously refreshed with water from the nearby Oosterschelde marine inlet, which had a temperature ranging from 14 to 19 °C throughout the experimental period (August-October 2014). The fish were fed pellets (Le Gouessant Aquaculture, France) every other day based on a temperature-dependent prescription. The experiment was approved by the Animal Experiments Committee (DEC) of Leiden University (DEC approval no: 14047).

EXPERIMENTAL ARENA

The experiment took place in the Jacobahaven, a man-made cove in the Oosterschelde. The cove is about 200 m wide, 300 m long and 2-5 m deep depending on tides. It has a level and muddy bottom. The water is calm in summer and inhabited by wild migratory European seabass from April to November. The cove is free from boat traffic within its 2 km vicinity, making it quiet and ideal for noise impact studies.

In the middle of the Jacobahaven, we constructed a floating island (Fig. 1) using a modular system (Candock, Canada) and anchored it to the sea bottom with a stretchable system that kept the structure in place at all tides. The structure consisted of an octagonal and a square platform. The octagonal platform (Ø 11.5-12.5 m) carried an octagonal net (volume 334 m³), where test fish were held during sound exposure trials. The square working platform held an underwater speaker suspended in water, and supported a work tent (4 x 5 m) that protected the equipment from the weather and served as a working space during the experiment. The work tent was supplied with electricity via an underwater cable from Stichting Zeeschelp. The two platforms were kept at 0.5 m distance from each other to minimise unwanted sound transmission from the working platform to the octagonal platform during sound exposure. The working platform was detachable from the octagonal platform, and for every quarter of the total trials (every four fish groups), it was repositioned at another orthogonal arm of the octagonal platform. This procedure ensured that observed behavioural changes would be explained mainly by sound exposure, instead of the effects of extraneous factors, such as seabed topography and tide flows.

SOUND TREATMENT

We exposed the fish to a one-hour impulsive sound treatment consisting of 0.1 s pulses, repeated at a regular repetition interval of 2 s. The sound sample was created in Adobe Audition 3.0 using bandpassed brown noise within 200-1000 Hz, matching the hearing range of European seabass[23,24]. It was played back with an underwater transducer (LL-1424HP, Lubell Labs, Columbus, US) from a laptop through to a power amplifier (DIGIT 3K6, SynQ) and a transformer (AC1424HP, Lubell Labs).

Prior to the start of the experiment, the amplitude levels of the sound treatment were measured at 360 points within the octagonal net (120 points at 0.5, 1.5 & 2.5 m depth). The measurements were made using an M20 particle motion sensor (GeoSpectrum Technologies, Canada), which was connected to a current-to-voltage convertor that gave an output of four channels: one for sound pressure and three for the 3D particle velocity directions

(u, v & w). The data output was logged at 40 kHz on a laptop via an oscilloscope (PicoScope 3425, Pico Technologies, UK), using a Microsoft Access script. The data was subsequently analysed in MATLAB. The analysis revealed a clear gradient in amplitude levels from the furthest to the nearest points from the speaker within the experimental arena. The mean zero-to-peak sound pressure level (SPL_{z-p}) and sound velocity level (SVL_{z-p}) were 180-192 dB re 1 µPa and 124-125 dB re 1 nm/s respectively (the range reflects values from the furthest to the nearest points from the speaker). In addition, the mean single-strike sound exposure level (SEL_{ss}) and velocity exposure level (VEL_{ss}) were 156-167 dB re 1 µPa^{2}s and 99-100 dB re 1 nm^2/s respectively.

EXPERIMENTAL DESIGN

Sixteen groups of four fish (N = 16, 64 fish) were exposed to the impulsive sound treatment for eight times sequentially in two days (Fig. 2). Each group of fish was transferred to the octagonal pen in a black plastic container (56x39x28 cm) enriched with oxygen (OxyTabs, JBL, Germany) and allowed to acclimatise for at least eight hours. Half of the groups started with the first trial of the exposure series during the day and the other half at night. The exposures took place during ebb tide (starting 1.5 h after the high tide) and flood tide (ending 1.5 before the high tide), when the water depth was between 3-4 m for all the trials. Due to the tides, a subsequent trial started either 3 h or 7.5 h (alternating) after the end of the previous trial. Each trial lasted for 1.5 h and comprised 60 min of sound exposure and 15 min of silence before and after. During each trial, we recorded the light intensity, the weather condition and the water temperature, which were subsequently used as covariates in the statistical analyses. After each group of fish went through the series of eight trials, they were transferred back to the onshore holding tank.

ACOUSTIC TELEMETRY

We studied the swimming patterns of the fish with a 3D telemetry system using acoustic tags (Model 795-LG, HTI, US). The tags were programmed (Model 490-LP, HTI, US) to emit 307 kHz pings (inaudible to the fish) of 0.5 ms at four different repetition intervals (995, 1005, 1015 and 1025 ms), in order to identify the four fish in a group. The fish were tagged externally, directly under the first and second dorsal fin[25]. After tagging, the four fish were kept in a rectangular recovery tank (1.20 x 1.00 x 0.65 m), which was continuously refreshed with filtered water from the Oosterschelde. The fish stayed in the recovery tank for at least two days before being transferred to the outdoor pen. In the octagonal pen, the pings emitted from the fish were received by four hydrophones (Model 590-series, HTI, US) attached to the platform (Fig. 1). The signals were then digitised by an acoustic tag receiver (Model 291, HTI, US) connected to a laptop. The digital data were subsequently processed with computer programs MarkTags v6.1 & AcousticTag v6.0 (HTI, US) into 3D coordinates (x, y, z), with a temporal resolution of one position every second for all four fish. The 3D coordinates were then used to calculate four behavioural parameters: swimming speed, swimming depth, average inter-individual distance (group cohesion) and distance from the speaker[cf 22].

STATISTICS

We first examined the 5-minute bin of the behavioural parameters right before the sound exposure, to see if baseline behaviours varied depending on the exposure sequence (order) and the time of the day. We categorised the time of the day into 'day' or 'night', depending on whether the trial started before or after the sunrise/sunset of the day. We modelled the baseline behaviours using linear mixed model, treating group as subject variable with random effects and exposure sequence as repeated variable with an AR(1) covariance
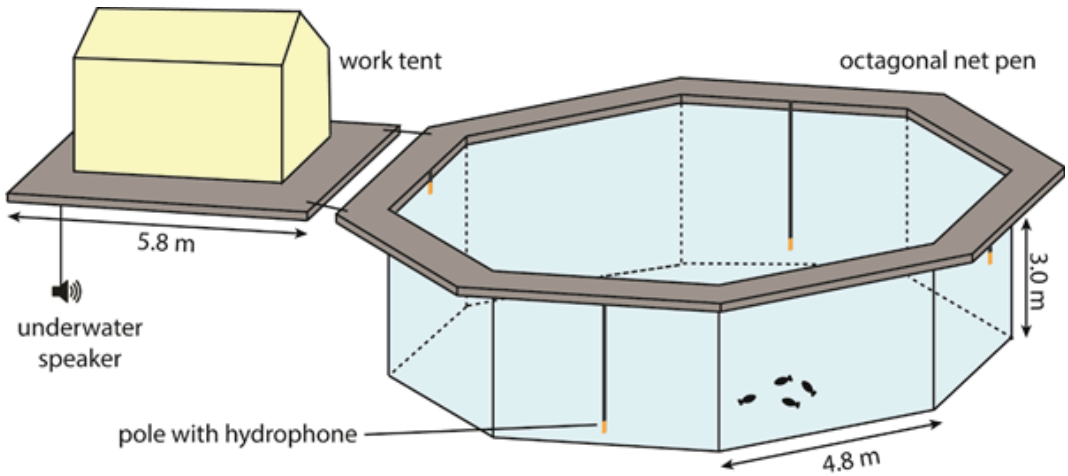
Fig. 1 Schematic of floating island where experiment was conducted. The underwater speaker hangs on the far end of the working platform at a depth of 2.2 m. The distance of the underwater speaker and the closest side of the net is 7.8 m. The four poles with hydrophones are responsible for tracking the four test fish via acoustic telemetry.
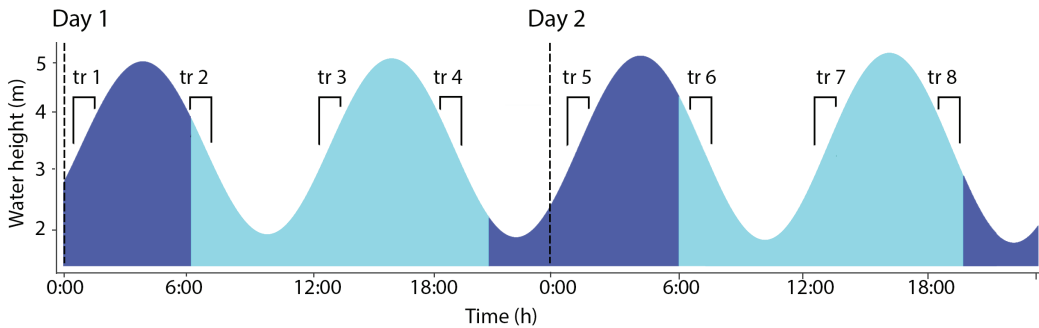

Fig. 2 Tide table showing the sound trial exposure scheme. All eight trials take place over two days when the water height is 3-4 m. Dark blue indicates night time and light blue indicates day time.

structure. In addition, we treated time of day as an explanatory factor, and tide, water temperature and exposure sequence as covariates. We selected the best model using backward stepwise method based on Akaike information criteria. Subsequently, we used the same procedure to model the behavioural changes caused by the sound exposure, which were the differences between the 5-minute bins right before and at the start of sound exposure. We also performed one-sample *t*-tests to see if the calculated differences were significantly larger than zero.

Results

We compared the pre-playback baseline behaviour of the fish between day and night (69 and 59 trials respectively) (Fig. 3a). At night, the fish swam significantly slower (linear mixed model: $F_{1,94}$ = 5.312, P = 0.023) in groups with significantly lower cohesion (linear mixed model: $F_{1,98}$ = 13.799, P < 0.001). There was a non-significant trend that they also swam higher up in the water column (linear mixed model: $F_{1,107}$ = 3.014, P = 0.085), at similar distance from the speaker. Upon sound exposure, the increase in group cohesion was significantly larger at night (linear mixed model: $F_{1,89}$ = 3.954, P = 0.050) (Fig. 3b). There was also a non-significant trend that the increase in swimming speed was also larger at night (linear mixed model: $F_{1,95}$ = 3.671, P = 0.058). Subsequent one-sample *t*-tests showed that only increases in swimming speed and swimming depth at night were significantly larger than zero (one-sample *t*-test: t_{57} = 3.782, P < 0.001; t_{57} = -2.008, P = 0.049 respectively). There was also a non-significant trend that increase in group cohesion at night was larger than zero (one-sample *t*-test: t_{53} = -1.716, P = 0.092). Within the 60 min exposure trials, all the behavioural changes reverted back to baseline levels, indicating intra-session habituation[9,10]. For inter-session

habituation, we found that changes in swimming depth diminished significantly with subsequent exposure sessions (linear mixed model: $F_{1,57}$ = 4.002, P = 0.050) (Fig. 4). For group cohesion, we found significant interaction between the time of the day and the trial order (linear mixed model: $F_{1,86}$ = 4.353, P = 0.040), which was due to a subtle decline in response over time at night and a change in response from less to more cohesion during daytime.
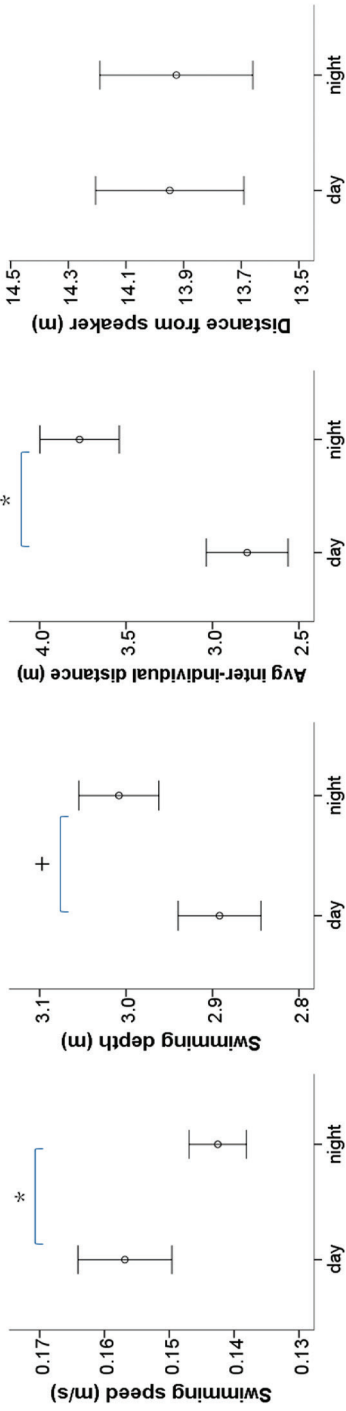
Discussion

Using a semi-natural set-up, we showed that European seabass swimming patterns varied throughout the diurnal cycle. Comparing baseline behaviour at night to during the day, the fish tended to swim slower, nearer to the surface, in a looser shoal. When exposed to sound, the fish increased their swimming speed, swimming depth and group cohesion. These changes were stronger at night. For all the behavioural changes, the fish recovered to baseline levels within an exposure session, indicating intra-session habituation. Furthermore, with subsequent sound exposure sessions, the fish gradually reduced the change in swimming depth, indicating inter-trial habituation.

STRONGER RESPONSE AT NIGHT
European seabass in our study showed clear diurnal swimming patterns. Such daily behavioural rhythm has also been shown in the dusky grouper (*Epinephelus marginatus*) and the yellowfin tuna (*Thunnus albacares*), where the fish swam closer to the surface at night[26,27]. This daily rhythmicity in movement is possibly linked to the daily rhythmicity in several hormones and metabolites[28–32]. For example, our study species, the European seabass has been shown to have daily variation in plasma glucose, insulin and cortisol[33,34]. The daily peaks of these parameters depend on whether the species is diurnal or nocturnal.
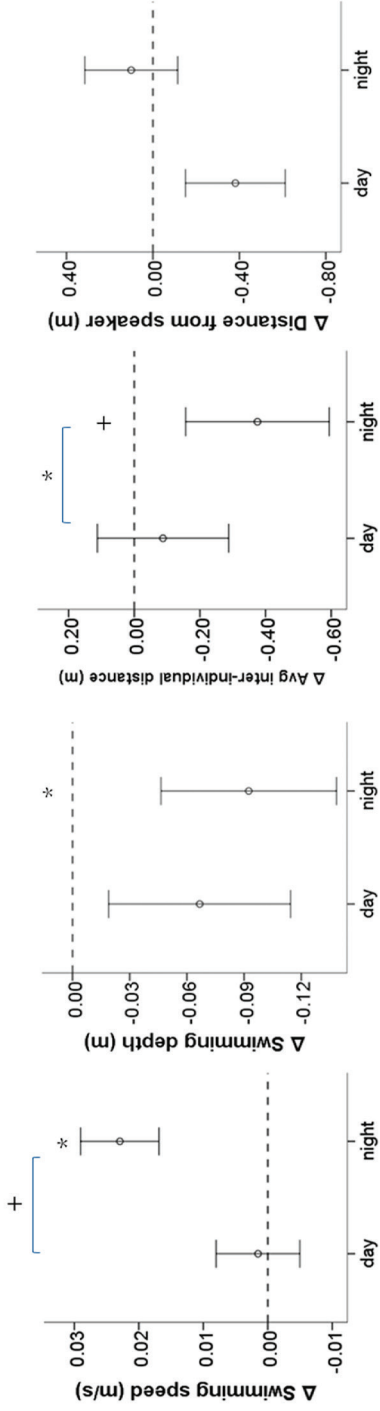
Fig. 3 (a) Baseline behaviour (mean ± SE) during the day and during the night for swimming speed, swimming depth (from bottom), average inter-individual distance and distance from the speaker. (b) Behavioural changes from before to the start of sound exposure during the day and during the night. An asterisk (*) denotes a significant difference ($P \le 0.05$) and a plus (+) denotes a non-significant trend ($0.05 < P \le 0.1$). The symbol between the bars indicates a difference between day and night, and the symbol above the bars indicates a difference from zero.
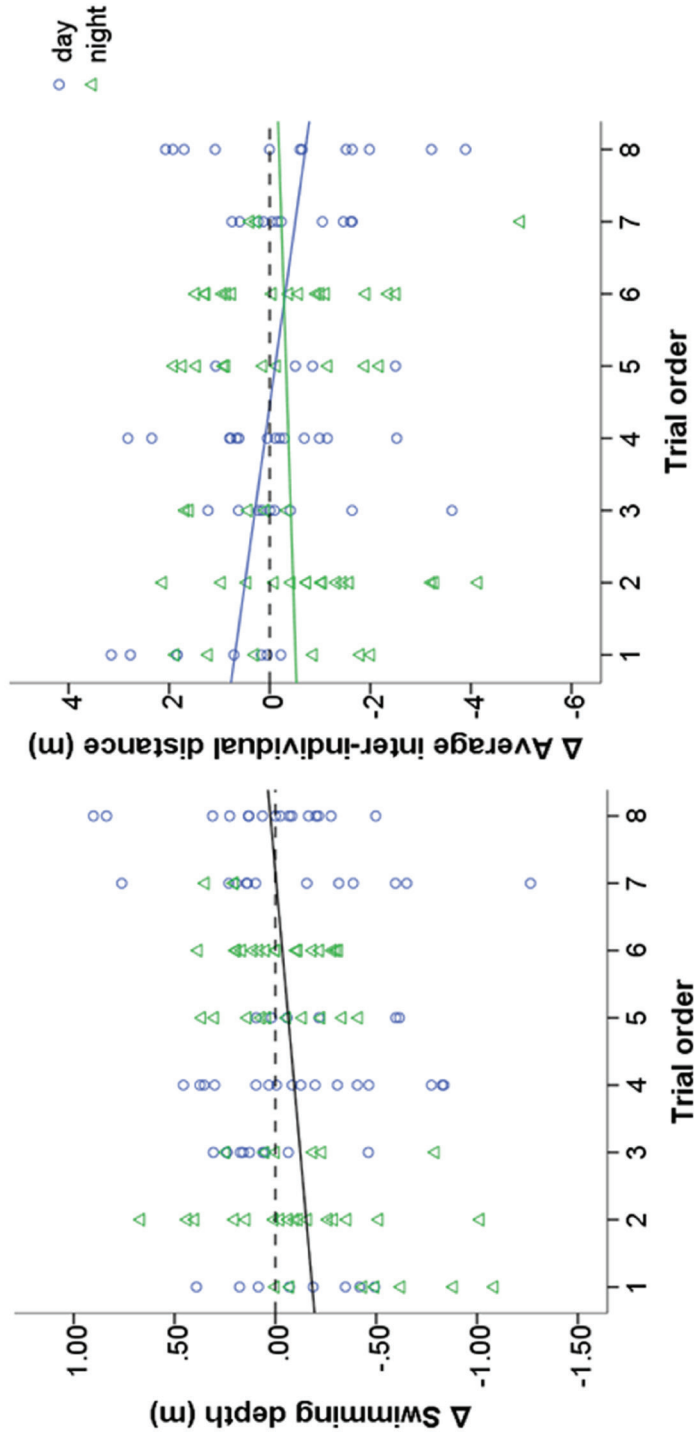
Fig. 4 Change in swimming depth and average inter-individual distance throughout the series of eight trials. The change in swimming depth diminishes with subsequent trials, indicating inter-trial habituation. The influence of trial order on the change in group cohesion is different between day and night.

Diurnal species typically produce most cortisol at the start of the day, while nocturnal species at the start of the night[15,28,35]. Such hormonal fluctuations are also affected by feeding and external stressors[15,28,31].

Upon sound exposure, European seabass in our study showed stronger behavioural changes at night compared to during the day. The influence of the time of the day on stress response has been shown in three nocturnal fish species subjected to air exposure[15–17]. Two of the species showed stronger cortisol increase at night and one during the day, suggesting that daily variation in sensitivity to stressors is species-specific. The mechanism of such differential sensitivity is still unknown, although it may be related to potential daily rhythm in the sensitivity of the glands in the hypothalamic–pituitary–adrenal (HPA) axis to corticotropin-releasing (CRH) or adrenocorticotropic hormone (ACTH)[36,37]. Rhythmicity in stress sensitivity may allow fish to effectively handle different daily activities and challenges.

The response to sound exposure during the day was particularly small compared to a previous experiment conducted two months earlier using the same setup on the same fish[22]. In the previous experiment, the fish were exposed to a series of four sound treatments varying in their temporal structures (one of the sound treatments was re-used in the current study), which took place during the day over a two-day period. This prior experience may have induced anticipation in the fish to the ensuing sound exposure in the current study, yielding lower response levels, especially during the day. Nevertheless, the fish still responded strongly to sound exposure at night, potentially because they were woken up from their resting or sleep-like state[38,39]. Such disruption can be particularly harmful to the fish as they may perform worse in daily activities. For example, when subjected to unpredictable chronic stress at night compared to during the day, zebrafish (*Danio rerio*) learned less well in an inhibitory avoidance task[40].

Despite low response levels during the day, our observation suggests that sound exposure at night may have more impact on European seabass. Although night exposure can be avoided by changing the schedules of current pile-driving practices, any modifications require careful considerations, as some species within an affected area may actually be more sensitive to stress during the day[17]. Nonetheless, our findings suggest that the responsiveness of fish to sound exposure may be affected by the natural rhythms in physiology as well as the environmental contexts. Consequently, such factors should also be considered when evaluating the efficacy and potential impacts of the schedule of a pile-driving operation.

INTER-SESSION HABITUATION
European seabass not only habituated to sound exposure within a session, they also habituated over subsequent sessions. Such inter-session habituation has been previously reported in zebrafish and the common cuttlefish exposed to repeated sound tones[41,42]. The zebrafish reduced the distance moved during startle response, while the cuttlefish reduced the probability of jetting and inking. In the current study, the European seabass reduced the change in swimming depth at the onset of sound exposure. Compared to intra-session habituation, the inter-session habituation was less prominent. For example, inter-session habituation only occurred with swimming depth, but not for the other test parameters. The lack of inter-session habituation in other parameters suggests that the fish may not have completely habituated to repeated exposures. However, it can also be explained by the more variable nature of these responses.

It is debatable whether habituation is necessarily beneficial to the fish under sound exposure[43]. On the one hand, habituation may reduce spatial and distributional changes, which is critical when a site is crucial for foraging or spawning. On the other hand, habituation may also cause fish to stay within an affected area, while still causing physiological stress[44,45], auditory masking[46] and attentional shifts[47–49]. Nevertheless, knowing that fish can habituate to repeated sound exposures, regulators and developers may be able to control the habituation rate by altering the trial intervals[9,50,51] or the interval regularity of repeated trials[18,49]. The effectiveness of such modifications still needs to be demonstrated in future studies.

CONCLUSION
Our study showed that European seabass responded more strongly to sound exposure at night and they habituated to repeated exposures. These findings demonstrate that environmental context and exposure experience may modulate sound impact on fish due to noisy human activities. Consequently, mitigation efforts aiming at minimising sound impact should take these factors into account when devising pile-driving operations. Although the implementation of implied refinements may still be problematic in the field, our study provides insights and empirical evidence that certainly help inform mitigating strategies.

Acknowledgements

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