European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure

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Running title: Seabass habituate to repeated sound exposures

Main finding in two lines:

Seabass behaviour in a pen varied between day and night. Responses to sound were stronger at night and seabass showed inter-trial habituation over eight repeated sound exposures in two days.

Abstract

Aquatic animals live in an acoustic world, prone to pollution by globally increasing noise levels. Noisy human activities at sea have become widespread and continue day and night. The potential effects of this anthropogenic noise may be context-dependent and vary with the time of the day, depending on diel cycles in their physiology and behaviour. Most studies to date have investigated behavioural changes within a single sound exposure session while the effects of, and habituation to, repeated exposures remains largely unknown. Here, we exposed groups of European seabass (*Dicentrarchus labrax*) in an outdoor pen to a series of eight repeated impulsive sound exposures over the course of two days at variable times of day/night. The baseline behaviour before sound exposure was different between day and night; with slower swimming and looser group cohesion observed at night. In response to sound exposures, groups increased their swimming speed, depth, and cohesion; with a greater effect during the night. Furthermore, groups also showed inter-trial habituation with respect to swimming depth. Our findings suggest that the impact of impulsive anthropogenic noise may be stronger at night than during the day for some fishes. Moreover, our results also suggest that habituation should be taken into account for sound impact assessments and potential mitigating measures.

Keywords: anthropogenic noise, *Dicentrarchus labrax*, diurnal cycle, fish behaviour, field study, impulsive sound series, inter-trial habituation

Introduction

Increasing global energy demand has prompted the energy industry to construct more oil platforms and wind farms at sea. These offshore activities produce a variety of anthropogenic noises, which range from continuous sounds produced by ship traffic and windfarm operation to high-intensity impulsive sounds from seismic surveys and pile driving. Especially, impulsive sounds, which occur at both day and night (Leopold & Camphuysen, 2008; Brandt *et al.*, 2011), have been suggested to negatively affect fishes (Popper & Hastings, 2009a, 2009b; Slabbekoorn *et al.*, 2010).

Fish in close proximity to a loud impulsive sound source may suffer from barotrauma injuries (Halvorsen *et al.*, 2012; Casper *et al.*, 2013a, 2013b). In laboratory settings fish are reported to recover from such injuries within a few weeks (Casper *et al.*, 2012, 2013b), but this may be different for free-ranging fish that need to find food and flee for predators. However, although physical damage may appear a severe impact, it only concerns a small proportion of the fish population that is close enough to receive such high-intensity sound. In view of this, the farther-ranging behavioural effects of impulsive sounds at moderate levels may be more concerning for fish populations (Slabbekoorn *et al.*, 2010; Hawkins *et al.*, 2014a).

In response to impulsive sound exposures, fish have been shown to change their swimming behaviour; typified by swimming faster, deeper, in a tighter shoal and further away from a sound source (Hawkins *et al.*, 2014b; Neo *et al.*, 2014, 2015, 2016). Such behavioural responses were actually found to be stronger for impulsive sounds compared to continuous sounds (Neo et al. 2014). Groups of European seabass (*Dicentrarchus labrax*) took longer to return to baseline swimming depth in response to impulsive sounds than to continuous sounds, while it took longer to return to baseline group cohesion levels when the exposures (either impulsive or continuous) had variable amplitude, as opposed to constant.

These results highlight the biological relevance of sound intermittency and reveal the limitations of using exclusively sound level or sound exposure level to predict response tendency or disturbance potential of aquatic animals.

Additionally, while the majority of studies investigating behavioural effects of underwater sound have been conducted during the day, impulsive sounds can be experienced by fish throughout their diel cycle which may affect their response level, like with other external stressors. For example, when subjected to air exposure (lifted out of the water), nocturnal green sturgeon (*Acipenser medirostris*) and Gilthead sea bream (*Sparusaurata L.*) increased plasma cortisol more at night than during the day (Lankford *et al.*, 2003; Vera *et al.*, 2014). In contrast, nocturnal Senegalese sole *(Solea senegalensis)* were more affected during the day (López-Olmeda *et al.*, 2013). It is currently unknown how the time of day may influence the effects of sound exposure in diurnal species such as the European seabass.

Furthermore, impulsive sounds from seismic surveys or pile-driving may be repeated, with breaks of inactivity, for several weeks or months (Leopold & Camphuysen, 2008; Brandt *et al.*, 2011). Despite this, the impacts of sound on fish behaviour have mainly been studied within a single exposure session and there are a few cases in which the effects of repeated exposures were tested. Nedelec et al. (2016) showed that the Threespot dascyllus (*Dascyllus trimaculatus*) increased hiding behaviour during playback of boat noise, but the effect was no longer significant after one and two weeks of repeated exposures. In another study, larval Atlantic cod (*Gadus morhua*) revealed no experience-related variation in responsiveness in a predator-avoidance test between different rearing noise treatments (Nedelec *et al.*, 2015). Besides these studies, there is little evidence as to whether repeated exposure sessions cause behavioural responses to accumulate, potentially leading to stronger responses through sensitization (e.g. Götz & Janik, 2011), or diminish through habituation (Groves & Thompson, 1970; Grissom & Bhatnagar, 2009; Rankin *et al.*, 2009). Earlier studies have already shown evidence for intra-trial habituation of European seabass to intermittent sounds (Neo *et al.*, 2014, 2015), but inter-trial habituation over repeated trials for this species has yet to be demonstrated.

In the current study, we exposed groups of European seabass each to a series of eight sound exposures in a large outdoor floating pen throughout the diel cycle of the fish. We aimed to answer the following questions: Do seabass vary consistently in swimming behaviour over the day? Does a sound-induced change in behaviour depend on whether it is night or day? Finally, do seabass habituate to repeated exposures of the same sound stimulus? We expected that the fish would change behaviour upon sound exposure and that the behavioural changes would depend on the time of the day. We also expected that behavioural changes would diminish over subsequent exposures.

Materials and methods

ANIMAL MAINTENANCE

We used hatchery-raised European seabass (from Ecloserie Marine de Gravelines, France), approximately 30 cm in length. Before testing, the fish were kept in a cylindrical holding tank (Ø 3.5 m, depth 1.2 m) at Stichting Zeeschelp, the Netherlands where the dark-light cycle was identical to the outdoor conditions. The holding tanks had a continuous inflow of fresh seawater from the nearby Oosterschelde estuary and water temperatures ranged from 14 to 19 °C during the experimental period (August-October 2014). We fed the seabass three times a week with food pellets (Le Gouessant Aquaculture, France), for which amounts were determined by fish number and size and adjusted based on the water temperature. Although previous experience does not affect the validity of the current test for fading responsiveness from the first to the last of a new series of sound exposures, we like to mention that the animals were also used in a previous experiment (Neo *et al.*, 2016). In that experiment, they were exposed to four sound exposures, of which one was identical to the sound exposures in the current experiment. The time between the previous and the current experiment was at least three weeks. These experiments were ethically evaluated and approved by the Animal Experiments Committee (DEC) of Leiden University (DEC approval no: 14047).

EXPERIMENTAL ARENA

The experiments were conducted in the Jacobahaven, an artificial cove located at the opening of the Oosterschelde, an estuary of the North Sea. The cove is about 200 m by 300 m in size and 2-5 m deep depending on tides with bottom sediment consisting of mud and sand. The water in the cove is relatively calm due to surrounding dams and a pier which shield the Jacobahaven from wind. Additionally, no boat traffic is allowed within 1 km of the cove, resulting in minimal levels of underwater anthropogenic noise, making it ideal for sound impact studies.

We constructed a floating platform (Fig. 1) in the center of the Jacobahaven using a modular floating dock system (Candock, Canada). We anchored it to dead weights on the bottom with an elastic cable system that kept the platform in place at all tides. The construction consisted of an octagonal walkway surrounding the pen and a square working platform for storing equipment tied to the outer perimeter of the walkway. The octagonal walkway held a net of 3 m depth and a diameter of 11.5-12.5 m (volume 334 m^3) where test fish were held during experimental exposures. The working platform carried an underwater speaker at 2.2 m depth, and supported a work tent (4 x 5 m) that shielded the equipment from weather and served as office space. The work tent was supplied with electricity via an underwater cable from Stichting Zeeschelp. We maintained a distance of 0.5 m between the platform and walkway using a physical buffer of soft buoys to minimise unwanted sound transmission from activity at the working platform to the net pen. Additionally, the working platform could be moved and reattached to one of four positions with respect to the octagonal walkway (North, East, South, and West). Every four trials, the working platform (i.e. the experimental sound source) was repositioned to the next position along the walkway, to control of the potential effects of consistent spatial preference in the experimental area across trials.

Figure 1

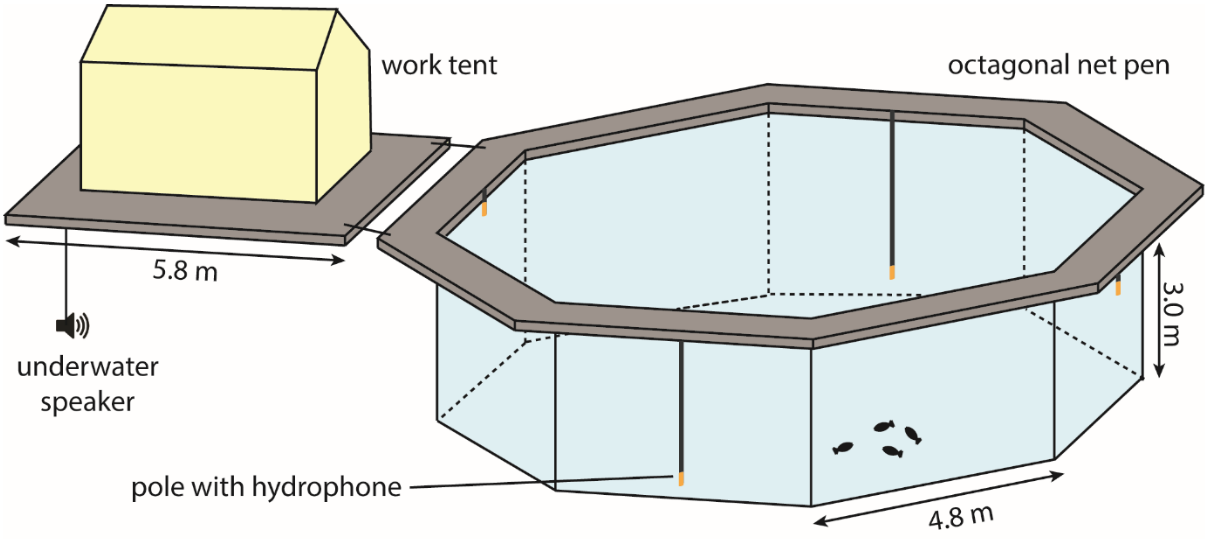


Fig. 1. Schematic of the floating platforms. The underwater speaker was suspended at the center of the far edge of the working platform. The distance from the underwater speaker to the closest side of the net was 7.8 m. The four hydrophones attached to the poles were used to track the test fish via telemetry.

SOUND TREATMENT

We exposed the groups of fish eight times 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 band-passed brown noise within 200-1000 Hz (48 dB rolloff per octave). This range matches the spectral range of highest hearing sensitivity for European seabass (Lovell, 2003; Kastelein *et al.*, 2008). However, it should be noted that these audiograms are based on sound pressure only and the methods of both papers have important limitations (cf. Ladich & Fay, 2013; Sisneros *et al.*, 2016). The sound was played back with an underwater speaker (LL-1424HP, Lubell Labs, Columbus, US) from a laptop through a power amplifier (DIGIT 3K6, SynQ) and a transformer (AC1424HP, Lubell Labs).

The amplitude levels of the sound treatment were measured at 360 points along a uniformly spaced three-dimensional grid within the octagonal net (120 points at 0.5, 1.5 & 2.5 m depth) prior to the start of the experiment. These measurements were repeated with all four working platform (i.e. speaker) positions during both flow and ebb tide (8 replicate sets). We measured the sound pressure levels (SPL) and sound velocity levels (SVL) using a M20 particle motion sensor (GeoSpectrum Technologies, Canada). The sensor was comprised of three orthogonal accelerometers and a hydrophone. The data output was logged at 40 kHz on a laptop via an oscilloscope (PicoScope 3425, Pico Technologies, UK) using an application written in Microsoft Access via Visual Basic for Applications. The data were subsequently analysed in MATLAB using a 200-1000 Hz bandwidth filter and power spectral density plots were generated using R (Fig. 2). For the particle velocity measurements, we calculated the root-mean-square, zero-to-peak and single strike energy of particle velocity for each accelerometer channel then combined the values using vector addition to result in an omnidirectional measure of particle motion which was comparable to SPL. We then averaged these values with respect to their positions relative to the working platform (8 replicates per aggregate) to calculate the presumed average sound gradient over all experimental trials. The results revealed a clear gradient in amplitude levels with an increasing distance from the speaker within the experimental arena. The mean zero-to-peak sound pressure level ($SPL_{z\text{-}p}$) and sound velocity level ($SVL_{z\text{-}p}$) were 180-192dB re 1 µPa and 124-125dB re 1 nm/s, respectively. In addition, the mean single-strike sound exposure level (SEL_{ss}) and velocity exposure level (VEL_{ss}) were 156-167 dB re 1 $\mu Pa^2 s$ and 99-100 dB re 1 nm^2/s respectively.

Figure 2

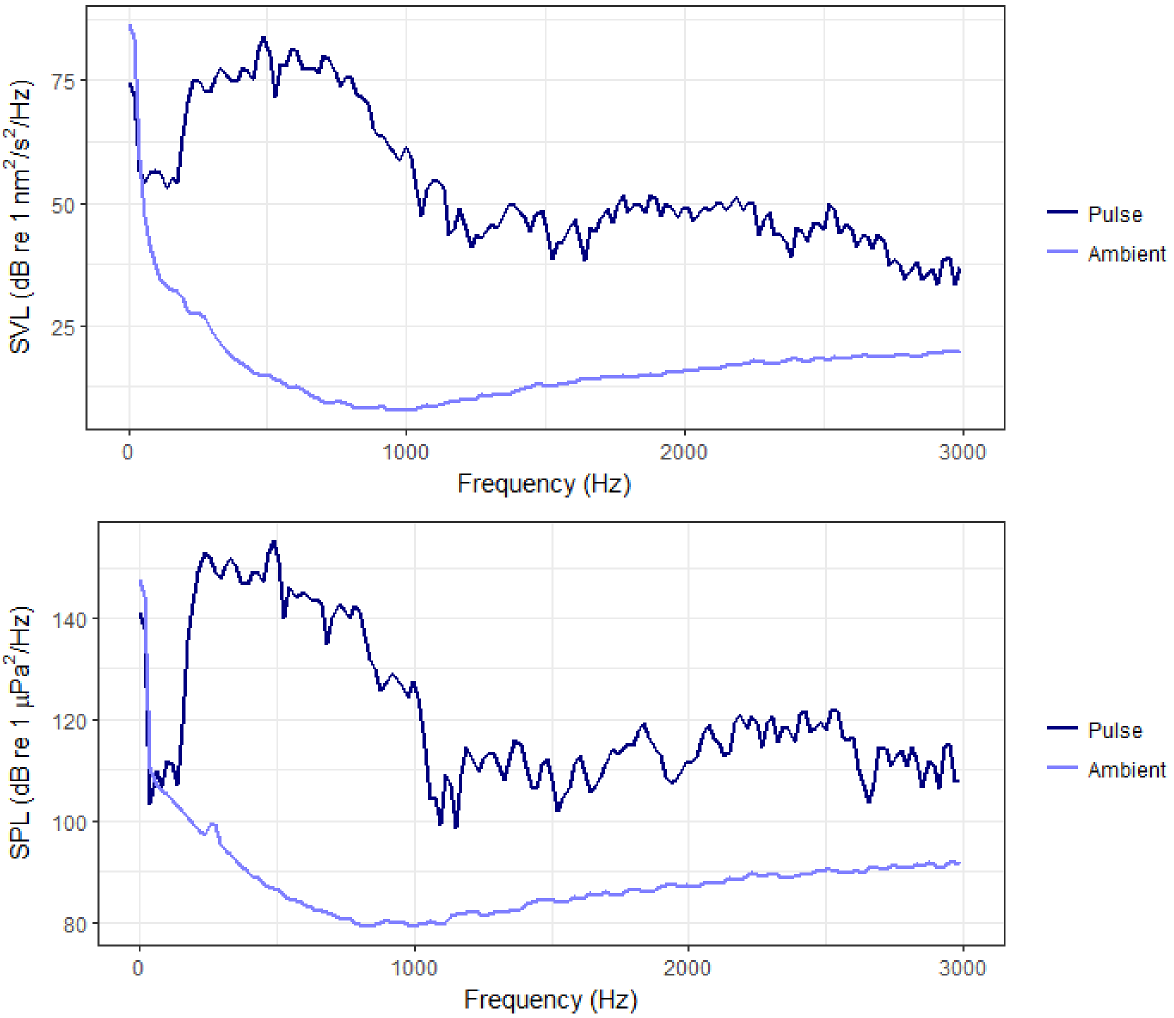


Fig. 2. Power spectral density (PSD) plots of sound velocity level (SVL, top) and sound pressure level (SPL, bottom) of a single pulse and the ambient condition in the pen. These

PSD’s were made using a sound recording in the pen at 17.5 m from the speaker and 1.5 m depth. For generating the PSD’s, we used a window length of 2048 with a Hamming window type.

EXPERIMENTAL DESIGN

We exposed each of sixteen groups of four fish (N = 16, 64 fish) to an impulsive sound treatment eight times during two consecutive days (Fig. 3). Each group of fish was transported to the net pen in a black plastic container (56x39x28 cm) with oxygen tablets (OxyTabs, JBL, Germany) to ensure sufficient oxygen levels. The fish were allowed to acclimate for at least 20 hours before the start of the first exposure. 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 ranged 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 consisted of 60 min of sound exposure and 15 min of silence before and after. We arrived at the platform 30 min before the start of the trial, where we would then record the light intensity, weather condition and the water temperature, which were used as covariates in the statistical analyses. During the trial, we waited quietly at the working platform until after the last exposure, where we then lifted the net pen, caught the fish with a scoop net and transported the group of fish back to the onshore holding tank.

Figure 3

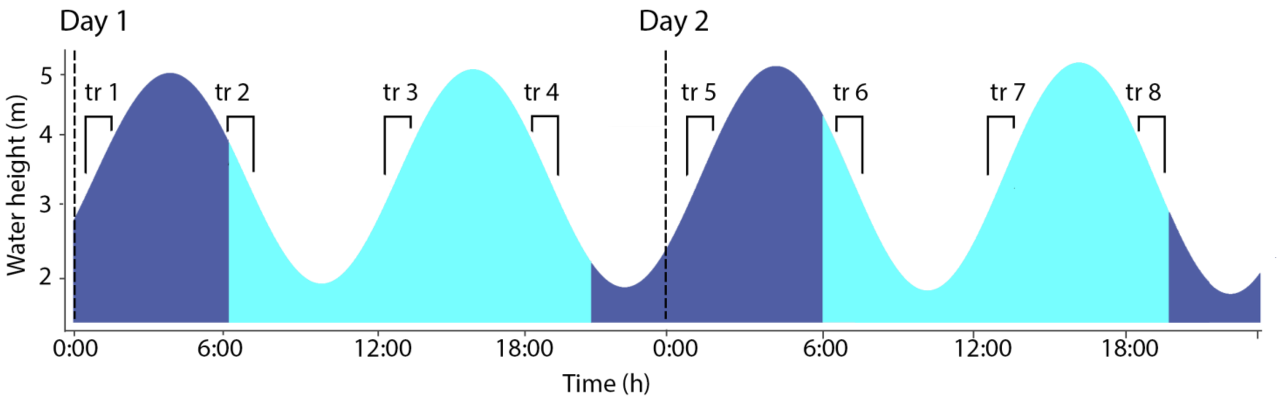


Fig. 3. Tide table showing the sound trial exposure scheme. All eight trials took place over two days when the water depth was 3-4 m. Dark blue indicates night time and light blue indicates day time.

ACOUSTIC TELEMETRY

We analysed the swimming patterns of the four seabass individuals per trial with 3D telemetry using acoustic tags (Model 795-LG, HTI, US). We set the tags to emit 0.5 ms long pings of 307 kHz (inaudible to the fish) at different repetition intervals (995, 1005, 1015 and 1025 ms) in order to identify the four unique swimming tracks. The fish were externally tagged under the first and second dorsal fin (cf. FISHBIO, 2013). Tags were reused and a maximum of 8 fish were tagged at any given time: We tagged the next group of individuals while the current group was still in the experimental trial. After the tagging procedure, the fish were kept in a recovery tank (1.20x1.00x0.65 m), which had a continuous inflow of fresh seawater from the Oosterschelde. The fish were allowed to recover for at least two days before being transported to the floating pen. In the pen, the pings from the acoustic tags were recorded by four hydrophones (Model 590-series, HTI, US) attached to the octagonal walkway (Fig. 1). The signals were then processed by an acoustic tag receiver (Model 291, HTI, US) and transferred to a connected laptop. The data were further processed with software from the manufacturer (MarkTags v6.1 & AcousticTag v6.0, HTI, US). This resulted in 3D positions per each individual per approximately 1 second intervals. The positional information was then used to calculate the group behavioural parameters: swimming speed, swimming depth, average inter-individual distance (group cohesion) and distance from the speaker (*cf* Neo et al., 2016).

STATISTICS

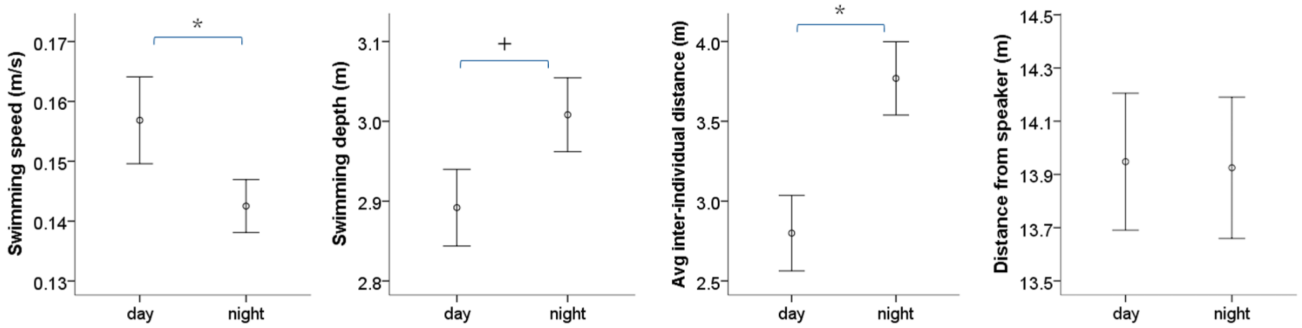
We first examined behavioural parameters in a 5 minute segment immediately before the onset of each sound exposure to see if baseline behaviours varied depending on the exposure sequence (order) and the time of the day. We categorised the time of the day into ‘day’ or ‘night’, depending on whether the trial started before or after the sunrise/sunset of the day. We modelled the baseline behaviours using a linear mixed effects model, treating the group ID as a random effect and exposure sequence (1 to 8) and time of day (day/night) as continuous and categorical fixed effects, respectively. In addition, we also used time of day, tide, and water temperature as additional fixed effects covariates. We selected the best model using backward stepwise selection based on Akaike information criteria (AIC). Subsequently, the same modelling procedure was applied to the behavioural changes caused by the sound exposure, where the responding variable was instead the change in swimming behaviour values between the 5 minute segments immediately before and after the onset of each 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. 4a). 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. 4b). 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 (Neo *et al.*, 2014, 2015, 2016). 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. 5). 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.

Figure 4 [next page]

(a) Baseline behaviour



(b) Behavioural changes

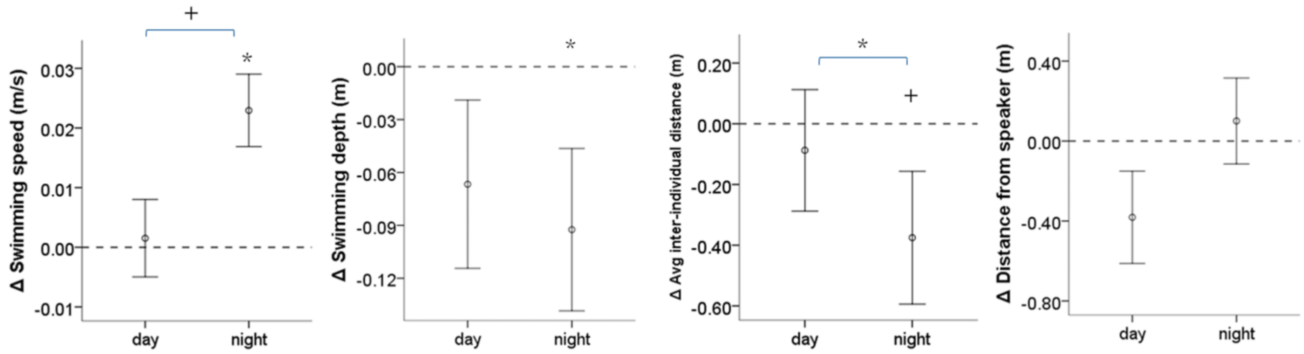


Fig. 4. (a) Baseline behaviour (mean ± SE) during the day and during the night for swimming speed, swimming depth (from bottom), average inter-individual distance and distance from the speaker. (b) Behavioural changes from before to the start of sound exposure during the day and during the night. An asterisk (*) denotes a significant difference ($P \leq 0.05$) and a plus (+) denotes a non-significant trend ($0.05 < P \leq 0.1$). The symbol between the bars indicates a difference between day and night, and the symbol above the bars indicates a difference from zero.

Figure 5 [next page]

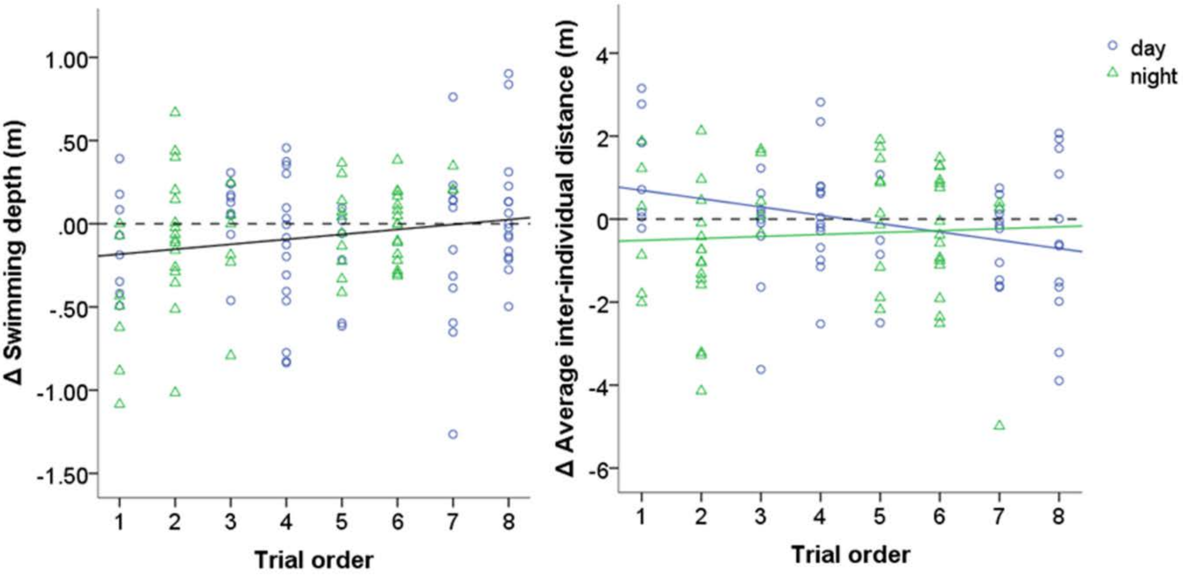


Fig. 5. Change in swimming depth (left) and average inter-individual distance (right) throughout the series of eight trials. The change in swimming depth diminishes with subsequent trials, indicating inter-trial habituation. The influence of trial order on the change in group cohesion is different between day and night.

Discussion

We showed significant variation in swimming patterns throughout the diurnal cycle of European seabass in semi-captive conditions in an outdoor floating pen. Comparing baseline behaviour at night to during the day, the fish swim significantly slower and in a looser shoal, and also tended to stay nearer to the surface (non-significant trend). When exposed to sound, the fish increased their swimming speed, swimming depth and group cohesion. These changes were stronger at night (significant for speed and depth and a non-significant trend for group cohesion). Additionally, the observed changes in swimming depth gradually reduced for subsequent sound exposures, indicating inter-trial habituation.

STRONGER RESPONSE AT NIGHT

The European seabass in our study were spatially restricted by the floating pen and relatively shallow water but showed clear diurnal swimming patterns. Such daily behavioural rhythms have also been shown in free-ranging dusky grouper (*Epinephelus marginatus*) and yellow fin tuna (*Thunnusal bacares*), where the fish swam closer to the surface at night (Mitsunaga *et al.*, 2013; Koeck *et al.*, 2014) or in sprat (*Sprattus sprattus*), who form dense schools during the day and disperse during the night (Hawkins *et al.*, 2012). This daily rhythmicity in movement is possibly driven by diel cycles in hormones and metabolites (Kühn *et al.*, 1986; Pavlidis *et al.*, 1999; De Pedro *et al.*, 2005; Polakof *et al.*, 2007). For example, our study species, the European seabass, has been shown to have significant daily variation in plasma glucose, insulin and cortisol (Planas *et al.*, 1990; Cerdá-Reverter *et al.*, 1998). The daily peaks of these parameters depend on whether the species is diurnal or nocturnal. Diurnal species typically produce most cortisol at the start of the day, while nocturnal species at the start of the night (Montoya *et al.*, 2010; Oliveira *et al.*, 2013; Vera *et al.*, 2014).

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 during exposure to some external stimulus has been shown in three nocturnal fishes (Lankford *et al.*, 2003; López-Olmeda *et al.*, 2013; Vera *et al.*, 2014). Two of the species showed stronger cortisol increase at night and one during the day in response to experimental exposure to air (taking fish out of the water), 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 rhythms in the sensitivity of the associated endocrine glands (Engeland & Arnhold, 2005; Dickmeis, 2009). The response to sound exposure during the day was particularly small compared to a previous experiment conducted before the current experiment using the same setup on the same animals. In the previous experiment, the fish were exposed to a series of four sound treatments varying in their temporal structure (one of the sound treatments was re-used in the current study), which took place during the day over a two-day period (Neo *et al.*, 2016). 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 (Zhdanova, 2006, 2011). Such disruption can be particularly harmful to the fish as it may affect their daily activities. For example, when subjected to unpredictable and chronic exposure to stressors at night compared to during the day, zebrafish (*Danio rerio)* learned less well in an inhibitory avoidance task (Manuel *et al.*, 2014).

Despite low response levels during the day, our observations suggest that sound exposure at night may have more impact on European seabass than during daytime. However, application of these findings with regard to managing anthropogenic marine activities requires careful consideration, as some species within an affected area may actually be more sensitive to stress during the day (López-Olmeda *et al.*, 2013). Also, care should be taken when extrapolating results from hatchery-reared fish in a constrained set-up to wild free-ranging fish. 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 potential impacts of noisy offshore activities.

INTER-SESSION HABITUATION

European seabass not only habituate to sound exposure within a session, as shown in previous experiments (Neo et al 2014, 2015, 2016), they also habituated over subsequent exposures, as shown in the current study. Such inter-trial reduction in behavioural response has also been reported for the coral reef fish, *Dascyllus trimaculatus*. Its hiding behaviour during boat noise diminished during a two-week period with repeated playback of boat noise. This reduced behavioural response was in line with diminished elevated ventilation rates after one and two weeks (Nedelec *et al.*, 2016). Other relatively long-term studies that looked into physiological measures showed similar results. Post-larval European seabass, that had been exposed to impulsive sound for 12 weeks, no longer showed elevated ventilation rates upon exposure of the same noise type (Radford *et al.*, 2016). In a split-brood experiment using larval Atlantic cod, two days of noise treatment reduced growth whereas the growth had converged again at the end of the experiment which lasted for 16 days (Nedelec *et al.*, 2015).

In the current study, the European seabass reduced the change in swimming depth at the onset of sound exposure. Compared to the intra-trial habituation of earlier studies (Neo et al. 2014, 2015, 2016), the inter-trial habituation was less prominent. For example, inter-trial habituation only occurred with swimming depth, but not for the other test parameters. The lack of inter-trial 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. Furthermore, the behaviour of the fish was constrained by the floating pen set-up and absolute levels or the nature of behavioural changes in our study should not be taken to extrapolate to the outside world. Nevertheless, relative differences with context (day and night) or variation among subsequent exposures provide conceptual insights and can be considered a proof of principle.

It is debatable whether habituation is necessarily beneficial to the fish under sound exposure (Bejder *et al.*, 2009). 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 (Anderson *et al.*, 2011; Filiciotto *et al.*, 2013), auditory masking (Vasconcelos *et al.*, 2007) and attentional shifts (Purser & Radford, 2011; Simpson *et al.*, 2014; Shafiei Sabet *et al.*, 2015). Hence, more insights into the consequences of fish habituation to repeated sound exposures (Davis, 1970; Chanin *et al.*, 2012; Neo *et al.*, 2015) and specific features such as interval regularity of repeated trials (Nedelec *et al.*, 2015; Shafiei Sabet *et al.*, 2015; current study), are critical for valid impact assessments..

CONCLUSION

Our study showed that European seabass responded more strongly to sound exposure at night and that 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 or seismic survey operations. Our study did not aim at assessing absolute thresholds to extrapolate to real-world conditions, but the natural water body conditions and the relatively large swimming area in the floating pen provide fundamental insights and may help in predicting variation in potential for sound impact between day and night and between brief and long-term or repeated exposure conditions. However, studies on free-ranging fish and exposure conditions in deeper water are needed to gain critical knowledge for impact assessments and potential for mitigation.

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