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

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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 animal 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 remain 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.

1. 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 wind-farm operation to high-intensity impulsive sounds from seismic surveys and pile driving. Especially, impulsive sounds, which occur at both day and night (Leopold and Camphuysen, 2008; Brandt et al, 2011), have been suggested to negatively affect fishes (Popper and 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.

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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 and 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 and Janik, 2011), or diminish through habituation (Groves and Thompson, 1970; Grissom and 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.

2. Materials and methods

2.1. 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).

2.2. 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 × 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.

2.3. Sound treatment

We exposed the groups of fish eight times to a 1-h 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 and 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