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Anthropogenic noise compromises the anti-predator behaviour of the European seabass, *Dicentrarchus labrax* (L.)

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A B S T R A C T

Anthropogenic noise is a significant pollutant of the world's oceans, affecting behavioural and physiological traits in a range of species, including anti-predator behaviours. Using the open field test, we investigated the effects of recordings of piling and drilling noise on the anti-predator behaviour of captive juvenile European seabass in response to a visual stimulus (a predatory mimic). The impulsive nature of piling noise triggered a reflexive startle response, which contrasted the behaviour elicited by the continuous drilling noise. When presented with the predatory mimic, fish exposed to both piling and drilling noise explored the experimental arena more extensively than control fish exposed to ambient noise. Fish under drilling and piling conditions also exhibited reduced predator inspection behaviour. Piling and drilling noise induced stress as measured by ventilation rate. This study provides further evidence that the behaviour and physiology of European seabass is significantly affected by exposure to elevated noise levels.

1. Introduction

The levels and distribution of anthropogenic sound in the oceans have increased over the past sixty years in line with growth of the maritime industries (Ellison et al., 2012). Piling and drilling are among the activities that contribute to low frequency underwater noise, particularly in coastal areas. Broadband noise generated from piling is impulsive and high intensity (Bailey et al., 2010) whereas drilling creates continuous sounds (Broudic et al., 2014). Underwater noise in the low frequency range overlaps the hearing sensitivity of many fish species (Popper and Fay, 2011). Detrimental impacts are predicted for species that utilise sound for ontogenetic behaviours such as mate finding and courtship, as well as routine behaviours including species recognition, foraging, and predator-prey interactions (Codarin et al., 2009; Picciulin et al., 2010; Purser and Radford, 2011; Bracciali et al., 2012; Voellmy et al., 2014a; Shannon et al., 2016; Simpson et al., 2016). However, knowledge gaps remain concerning the ultimate endpoint of noise-induced behavioural modification at both individual and population levels.

Startle and avoidance reactions are key prey survival responses in a predator-prey situation (Webb, 1986). Noise can impact prey risk assessment as a result of reallocation of the prey's finite attention (Dukas, 2004), distracting it and preventing it from responding to predation threat (Chan et al., 2010; Simpson et al., 2015). Increased noise levels can impair the threat perception of the prey fish, potentially compromising escape (reviewed in Slabbekoorn et al., 2010). Conversely, prey may increase anti-predator vigilance and exploratory behaviour; actions which may have energy budget implications (Shannon et al., 2016). Noise can act as a stressor and may lead to altered activity and locomotion patterns (Mendl, 1999).

The European seabass, *Dicentrarchus labrax* has increasingly been used in the study of anthropogenic noise effects on fish. The hearing sensitivity of seabass is most acute at low frequencies (100–1000 Hz; Lovell, 2003); coincident with many anthropogenic noises in water (Götz et al., 2009). The scale of the behavioural responses depends on the nature of the noise (Neo et al., 2014). Increases in motility and changes in swimming performance in juveniles have been reported in response to synthetic continuous (Buscaino et al., 2010) and impulsive sounds (Neo et al., 2015). Regarding anti-predator behaviour, Everley et al. (2015) have shown that exposure to playback piling noise can reduce responsiveness to a visual stimulus. Further, startle responses are known to occur after exposure to low frequency sounds (Kastelein et al., 2008). Changes in physiological and biochemical parameters were also found in response to exposure to low frequency impulsive and continuous noise (Santulli et al., 1999; Buscaino et al., 2010; Bruintjes, 2013; Bruintjes et al., 2014; Debusschere et al., 2016).

In the current study we investigated the effects of recorded piling and drilling noise on seabass physiology and anti-predator behaviour. We hypothesised that this additional noise would result in an increased number of startle responses and increased motility, compared to

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ambient conditions, with altered responses to predator attack.

2. Materials and methods

2.1. Ethics statement

All procedures were approved by the Newcastle University animal-related research/work, operating outside the Animals (Scientific Procedures) Act 1986 (for vertebrates only) and followed the University of Exeter Ethics Committee (2013/247: Impacts of global change on aquatic organisms) approved protocols.

2.2. Study species and holding conditions

Fifty-four seabass (47.94 ± 9.09 g; 14.52 ± 1.70 cm) sourced from a commercial hatchery (Ecloserie Marine, Gravelines, France) were housed in a fiberglass holding tank (150 L × 80 W × 50 H cm) with a water depth of 30 cm (water volume = 360 L) in the Aquatic Resource Centre at the University of Exeter. In the holding tank water was refreshed continuously with a closed recirculating system and oxygen was maintained by a cylindrical ceramic airstone placed close to the water pump. Fish were fed pellets (Skretting Perla MP-L, Italy) twice daily and maintained within a 12-h photoperiod and water temperature of 16.5 °C. Individual fish were transferred in a net from the holding tank to the experimental tank, where each fish was given a thirty minute acclimation period.

2.3. Noise playback preparation and assessment

Drilling noise recordings were made in August 2014 between 17:00 and 17:30 at Yarmouth (Cowes, Isle of Wight, N50.70950, W1.51666) during the installation of one of four screw piles supporting a tidal device. Three different recordings were used to create the playback drilling noise tracks. Recordings included vessel noise (a 720 HP/530 Kw @ 2000 rpm twin engine multicat-type vessel, length overall = 20 m, beam overall = 7 m) and seabed drilling noise. Seabed sediments were characterised by course, medium and fine gravel with clay nearer the surface. Underwater sounds were recorded using a C55 Cetacean Research Technology hydrophone (Transducer Sensitivity + Preamplifier Gain – Effective Sensitivity: -165 dB, re: 1 V/μPa) connected to a Fostex FR-2LE compact audio recorder (20 Hz − 20 KHz ± 2 dB; FS 44.1/48 kHz, calibrated against a 1 KHz reference tone of known amplitude). Recordings were made five metres below the sea surface.

Three recordings of piling carried out in Swansea Bay during the installation of a lifeboat station (Swansea Bay, N51.56989, W3.97401) were used to create the playback piling noise tracks. A 1.2 m diameter monopile was hammered at 20–30 m into the sea bed. The recordings were made 2–5 m below the sea surface using a calibrated hydrophone (HTI 99HF; sensitivity without preamp: − 183 dB re: 1 V/μPa) connected to an EASDA 14 data logger (Rtsys, France). Three ambient noise recordings without vessel noise or sudden sounds were made at the same site when piling was not in operation. These were used to create the playback ambient noise tracks.

Noise samples of ten seconds duration were band-pass filtered from 0.1 to 3 KHz (FFT 1024, Hann window) using Avisoft SAS Lab Pro vs 5.2.08 (Avisoft Bioacoustics, Berlin, Germany) in order to play within the underwater speaker specifications and to minimize resonant frequencies within the experimental tank (Akamatsu et al., 2002). Each filtered noise file was looped forming a repeating 30 min playback track using Audacity (http://audacity.sourceforge.net). Drilling tracks included a ten seconds fade in and out, to simulate the gradual increase in noise level recorded in the field. There was no fade in or fade out to the piling track as piling noise has a sudden onset. Ambient noise playback tracks included five seconds fade in and out. Fade in and out times were shorter as the maximum amplitudes were lower than for drilling and piling noise tracks (Fig. 1).

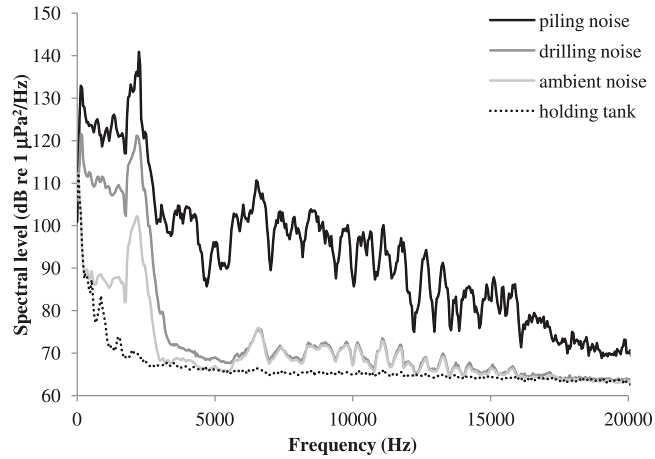

Fig. 1. Acoustic conditions in the experimental tank. Mean sound pressure spectral levels (units normalized to 1 Hz, Hann window, FFT length 1024, 50% overlap) during band-pass filtered piling (1 s averages), drilling and ambient noise (30 s averages) playbacks. An example of the holding tank recording is given for comparison.

Noise tracks were played back through a portable audio recorder (Sony PCM-M10; frequency ranges 20 Hz–20 KHz), amplifier (Kemo Electronic GmbH; 18 W; frequency response range: ∼40–20,000 Hz), and an Aqua30 underwater loudspeaker (DNH; effective frequency range 80–20,000 Hz). Sound pressure in the experimental tank was recorded during playback using a calibrated hydrophone (HTI 96-MIN; manufacturer-calibrated sensitivity − 164.3 dB re 1 V/μPa; frequency range 2–30,000 Hz) suspended mid-water in the centre of the tank (10 cm from the bottom). Before starting the experiment, playback recordings were adjusted in Audacity to achieve uniform sound pressure levels within noise treatments. The mean peak sound pressure of the piling tracks (averaged from one second recordings during pile strikes) was 152 ± 3.5 dB RMS re 1 μPa; the drilling tracks had a mean sound pressure of 132 ± 0.42 dB RMS re 1 μPa (averaged from 30 s recordings) and the mean sound pressure of the ambient tracks (averaged from 30 s recordings) was 117 ± 1.00 dB RMS re 1 μPa. A comparison between noises recorded in the field and playback noise is shown in the electronic supplementary material (Fig. S1 and S2; Table S1).

Like most fishes, hearing in *D. labrax* may be dominated by the particle motion element of sound (Popper and Fay, 2011), but because they have a swim bladder they are also likely to be sensitive to changes in pressure (Wysocki et al., 2009). For logistical reasons we only report the sound pressure levels of the playback of recordings for comparison between noise treatments. Particle motion levels in the experimental arena were also measured in the middle of the water column. Electronic supplementary material shows example particle acceleration levels (Fig. S3).

2.4. Experiment set-up and protocol

One tank (54.8 L × 45.1 W × 45.2 H cm; water depth 20 cm) made from 6 mm thick glass with a 10 mm base placed on a fiberglass bench was used for all experimental trials (Fig. S4). Thick polystyrene blocks placed between the tank and bench were used to reduce vibrations. The underwater speaker was placed at the bottom of the tank facing upward, centred and suspended beneath a 3 mm thick white Perspex© false bottom. The speaker was wrapped in medium density laminated polyethylene foam to avoid additional vibrations. A video camera (Panasonic HC-V700) was mounted above the tank and recorded the whole experiment. Perspex© panels with anti-glare sides were used to reduce reflections. A fourth wall had a window left uncovered to allow the fish to see the looming predator.

A spherical blue squash ball (40 mm diameter) fixed to a clear