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Sound exposure changes European seabass behaviour in a large outdoor floating pen: Effects of temporal structure and a ramp-up procedure☆

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ABSTRACT

Underwater sound from human activities may affect fish behaviour negatively and threaten the stability of fish stocks. However, some fundamental understanding is still lacking for adequate impact assessments and potential mitigation strategies. For example, little is known about the potential contribution of the temporal features of sound, the efficacy of ramp-up procedures, and the generalisability of results from indoor studies to the outdoors. Using a semi-natural set-up, we exposed European seabass in an outdoor pen to four treatments: 1) continuous sound, 2) intermittent sound with a regular repetition interval, 3) irregular repetition intervals and 4) a regular repetition interval with amplitude 'ramp-up'. Upon sound exposure, the fish increased swimming speed and depth, and swam away from the sound source. The behavioural readouts were generally consistent with earlier indoor experiments, but the changes and recovery were more variable and were not significantly influenced by sound intermittency and interval regularity. In addition, the 'ramp-up' procedure elicited immediate diving response, similar to the onset of treatment without a 'ramp-up', but the fish did not swim away from the sound source as expected. Our findings suggest that while sound impact studies outdoors increase ecological and behavioural validity, the inherently higher variability also reduces resolution that may be counteracted by increasing sample size or looking into different individual coping styles. Our results also question the efficacy of 'ramp-up' in deterring marine animals, which warrants more investigation.

1. Introduction

The rise of underwater noise pollution resulting from human activities at seas may threaten the health and stability of fish populations (Hawkins et al., 2014a; Normandeau Associates, 2012; Popper and Hastings, 2009a, 2009b; Radford et al., 2014; Slabbekoorn et al., 2010). This concern needs to be corroborated by understanding how critical fish behaviours change in response to the exposure of man-made noise (Hawkins et al., 2014a; Slabbekoorn et al., 2010). For example, man-made noise has been shown to affect fish by changing their swimming patterns (Hawkins et al., 2014b; Neo et al., 2014; Neo et al., 2015a; De Robertis and Handegard, 2013; Sarà et al., 2007), territorial dynamics (Sebastianutto et al., 2011), antipredator vigilance (Simpson et al., 2014; Voellmy et al., 2014a), foraging efficacy (McLaughlin and Kunc, 2015; Payne et al., 2015; Purser and Radford, 2011; Shafiei Sabet et al., 2015; Voellmy et al., 2014b) and other fitness-related activities (Boussard, 1981; Picciulin et al., 2010). These studies were conducted using different sound sources, which reflected the diversity of man-made noise sources in reality, and varied in their spectral, amplitudinal and temporal characteristics (Slabbekoorn et al., 2010). Different acoustic features likely differ in their relative importance in exerting behavioural effects, but such findings cannot be properly interpreted without deeper fundamental understanding (Hawkins et al., 2014a; Normandeau Associates, 2012).

It was only recently that the temporal characteristics of sound were shown to affect the on-set and recovery of behavioural changes for fish (Neo et al., 2014, 2015a). For example, the behavioural recovery of captive European seabass (*Dicentrarchus labrax*) in a large basin was faster when exposed to continuous sound than

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to impulsive sound (Neo et al., 2014). In addition, impulsive sound exposure induced initial and delayed behavioural changes that were influenced by the pulse repetition interval (PRI) (Neo et al., 2015a). Moreover, amplitude fluctuations were shown to affect shoaling behaviour of the seabass (Neo et al., 2014). The latter effect is interesting as amplitude fade-in, usually called 'ramp-up' or 'soft-start', is widely recognised and has been applied as a mitigation strategy (Hawkins et al., 2014a; JNCC, 2010; Normandeau Associates, 2012; Weilgart, 2007). A gradual rise in sound level, before a pile-driving or seismic shooting operation at full power, is assumed to drive away marine mammals and fish, in order to prevent injuries caused by intense sound exposure close to the sound source. However, the efficacy of the procedure still needs to be demonstrated (Cato et al., 2013).

Behavioural studies often carry implications that are difficult to ascertain because of interpretation discrepancies and generalisation uncertainties inherent to different experimental approaches. For example, tank-based and laboratory studies examining the behavioural impact of sound on captive fish have methodological advantages but also apparent extrapolation limitations (Calisi and Bentley, 2009; Hawkins et al., 2014a; Popper et al., 2014; Slabbekoorn, 2014). Such confined set-ups have high internal validity but lack ecological validity, wherein the acoustic fields likely differ from natural waters in a complex and unpredictable manner (Parvulescu, 1967), and the fish behaviour different and more constrained than in the wild (Hawkins et al., 2014a; Radford et al., 2014). However, this concern has not been substantiated with empirical evidence showing in what ways these limitations result in different behavioural observations between tank-based and open-water studies. Comparisons of behavioural responses to the same stimuli in the same social setting in both tank-based and open-water conditions could improve the external validity of test results and may provide additional insights into the underlying mechanisms (Brewer, 2000; Campbell, 1957).

Field studies on free-ranging animals have the highest ecological validity, but conducting well-replicated and well-controlled sound exposure studies at sea is exceedingly costly and logistically challenging. Moreover, discrepancies between contradictory results from different field studies can often not be sufficiently explained (see Hawkins et al., 2014b), due to unknown and potentially confounding or modulating factors. Consequently, a semi-natural approach with semi-controlled setting and a size-appropriate enclosure in the fish natural environment may sometimes be an optimal compromise (Calisi and Bentley, 2009; Slabbekoorn, 2014).

In this study, we used European seabass in a large floating pen in a man-made cove within a tidal marine inlet, to test the impacts of sound exposure with different temporal structures. We tested four sound treatments varying in intermittency (continuous vs impulsive), repetition interval regularity and the presence of 'ramp-up' to test the following hypotheses: 1) Upon sound exposure, fish change their swimming speed, swimming depth, group cohesion and swim further away from the sound source; 2) the behavioural changes are affected by the different temporal structures, including intermittency, repetition interval regularity and the presence of 'ramp-up'; 3) the behavioural changes are in agreement with previous indoor studies which had the same experimental design (Neo et al., 2014, 2015a).

2. Materials and methods

2.1. Animal maintenance

Mixed-sex European seabass from a hatchery (Ecloserie Marine de Gravelines, France) with a total body length of about 30 cm were used in this study (Neo et al., 2014, 2015a). Before and after the experiment, 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 research institute in Zeeland, the Netherlands. The water in the holding tanks was continuously refreshed with water from the nearby Oosterschelde marine inlet and the water temperature varied from 17 to 22 °C throughout the experimental period (June–August 2014). The fish were fed pellets (Le Gouessant Aquaculture, France) every other day based on a temperature-dependent prescription. All experiments were in accordance with the Dutch Experiments on Animals Act and approved by the Animal Experiments Committee at Leiden University (DEC approval no: 14047).

2.2. Experimental arena

The experiment was conducted 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 relatively calm in the summer and is home to wild European seabass. No external boat traffic is allowed within about 2 km of the cove, making it quiet and ideal for noise impact studies.

In the middle of the Jacobahaven, a floating island consisting of two platforms (Fig. 1) was constructed from a modular floating system (Candock, Canada) and anchored to the sea bottom with dead weights, chains and stretchable bungee ropes that kept the island in place at all tides. The octagonal platform (Ø 11.5–12.5 m) supported a custom-made octagonal net (volume 334 m^3), in which test fish were held during sound exposures; the square working platform supported a work tent (4 × 5 m), which protected all equipment from the weather and served as a working space during the experiment. The two platforms were kept at 0.5 m distance from each other to minimise unwanted noise 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, it was repositioned at another orthogonal arm of the octagonal platform. The use of four different positions facing the four cardinal directions was intended to minimise the influence of extraneous factors (e.g. seabed topography, tide flows) on fish swimming patterns.

2.3. Treatment series

We exposed the fish to a series of four sound treatments: continuous, impulsive regular, impulsive irregular and impulsive regular with 'ramp-up' (Fig. 2a). In order to vary only the temporal parameters of interest in the treatments while keeping all other sound parameters constant, the sound treatments were created in Audition 3.0 (Adobe, San Jose, US) using filtered brown noise (band-passed: 200–1000 Hz). The continuous treatment consisted of uninterrupted sound elevation with constant amplitude. The other three impulsive treatments consisted of a pulse train with 0.1 s pulses, repeated at either a regular PRI (pulse repetition interval) of 2 s, or an irregular PRI of 0.2–3.8 s (random; average 2 s). The 'ramp-up' treatment consisted of 20 min of fade-in from ambient level to the same amplitude as the other treatments. All sound samples were created in Adobe Audition 3.0 using filtered brown noise (band-passed: 200–1000 Hz; matching the hearing range of European seabass (Kastelein et al., 2008; Lovell, 2003)) and 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).

To examine the soundscape of the whole experimental arena, we measured both sound pressure level (SPL) and sound velocity