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Anthropogenic noise is a recognized global pollutant that could potentially impact many organisms, including fishes. One of the acoustic sources producing high impulsive noise and vibration is pile driving. However, the potential impacts of real pile driving on fish species has received little attention, mainly due to the logistical challenges involved. Here, we investigated the impact of pile driving on the oxygen uptake (a secondary stress response) of black seabream *Spondyliosoma cantharus* and European plaice *Pleuronectes platessa* using an experimental pile driver setup in a flooded ship-building dock. Each individual fish was tested in ambient and pile driving conditions using a counterbalanced paired design to control for potential order effects. During pile driving, black seabream increased oxygen uptake compared to the ambient control condition suggesting higher stress levels. Plaice did not show differences in oxygen consumption between the pile driving and ambient treatment. These results show the impact of pile driving on secondary stress responses in fish, highlight species-specific differences concerning acoustical impacts, and showcase the possibility of carrying out large-scale semi-field acoustic experiments.

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1. INTRODUCTION

Research concerning the impact of anthropogenic sound on aquatic species has greatly increased in the last two decades (reviewed in, e.g., Slabbekoorn *et al.*, 2010; Williams *et al.*, 2015). In fish species, experiments have been conducted using several methods of acoustic exposure including: (1) playbacks of recorded sounds (Smith *et al.*, 2004; Wysocki *et al.*, 2006; Bruintjes and Radford, 2013, 2014), (2) real boat or ship passes (Sarà *et al.*, 2007; Graham and Cooke, 2008; Jacobsen *et al.*, 2014; Simpson *et al.*, 2016), (3) seismic airguns (Engas *et al.*, 1996; Popper *et al.*, 2005; Miller and Cripps, 2013; Thompson *et al.*, 2013), and (4) impact pile driving (Debusschere *et al.*, 2014, 2016).

The dimensions of the piles that are used to reinforce offshore constructions shows large variation (see e.g. Dähne *et al*., 2013, Debusschere *et al*., 2014, Bruintjes *et al*. 2016). Moreover, many elements will influence the sound intensity, amplitude and frequency structure of the sound during pile driving including the pile's physical quantities, hammer type, pile driving method, strike rate, water depth, and the substrate of the area the pile is being installed.

The impacts of real pile driving on fishes have received little attention, mainly due to logistical difficulties. It is important to study the potential impact of pile driving in natural or semi-natural settings using *in situ* pile driving to replicate realistic sound pressure and particle motion propagation in both the water column and seabed.

Studies using juvenile European sea bass (*Dicentrarchus labrax*) and *in situ* pile driving, have shown a decrease in oxygen uptake (a secondary stress response), and no impact on mortality (Debusschere *et al.*, 2014, 2016). Other studies, using sound playbacks have reported an increase in primary and secondary stress characteristics, including increased oxygen consumption, higher ventilation rates and increased blood cortisol levels (Smith *et al.*, 2004; Wysocki *et al.*, 2006; Simpson *et al.*, 2015; Bruintjes *et al.*, 2016; Purser *et al.*, 2016). However, there is a lack of studies investigating the impacts of pile driving on the physiology of adult fish.

The current study investigated the impact of simulated impact pile driving on the oxygen uptake of black seabream (*Spondyliosoma cantharus*) and European plaice (*Pleuronectes platessa*). It was predicted that both fish species would increase their oxygen uptake during exposure to pile driver as a stress response.

2. MATERIAL AND METHODS

Experiments were conducted in a former shipbuilding dock at the Offshore Renewable Energy Catapult in Blyth, UK. The dock measured 93 x 18 m with a 3 m average water depth and a 3.5 m simulated seabed consisting of North Sea sand and small stones (Bruintjes *et al.,* in review). Simulated impact pile driving was produced by a Wrag penna post-driver with a 200 kg hammer powered by a tractor. The post-driver struck a pile made from a steel pipe (7.5 m long, 16.5 cm diameter, 0.65 cm thick) with a steel plate (1.51 x 1.64 m, 1.4 cm thick) welded at 50 cm from the bottom to ensure stable acoustic conditions during pile driving. During pile driving, the hammer struck the post 10 times per minute.

During 30 minute pile driving exposure, individuals were subjected to a cumulative Sound Exposure Level (SEL_{cum}) of 184.41 dB re 1 μPa^2. During 30 minute ambient conditions the fish were exposed to a SEL_{cum} of 159.33 dB re 1 μPa^2.

Black seabream (n=14) had an average mass of 538.4 ± 27.6 g (mean ± Standard Error [SE]) and measured 27.6 ± 0.7 cm Standard Length [SL] (mean ± SE). Plaice (n=21) had an average mass of 171.7 ± 10.5 g (mean ± SE) and an average SL of 21.3 ± 0.4 cm. All fish were wild caught in UK coastal waters.

Oxygen uptake of both fish species was measured for 30 min during exposure to pile driving and during ambient conditions in a counterbalanced paired design (i.e. each fish was tested in pile driving and in ambient conditions in a counterbalanced order to control for a potential order effect). Between both experiments (pile driving followed by ambient conditions, or vice versa) individuals had one hour to recover.

At the start of the experiment, individual fish were placed in airtight containers (for bream: radius 29.5 cm, height 20.7 cm [volume 10 L], for plaice 27.4 x 20.0 x 7.5 cm [volume 1.125 L]), a water sample was taken and the container lid was sealed underwater to prevent air bubbles. The water samples were analyzed for dissolved oxygen content with a handheld oxygen meter (HI 9164, Hanna Instruments Inc., USA). The containers with the fish inside were placed in a mesh tray, lowered 3 m below the water surface and rested on top of the seabed at 10 m from the pile driving setup. The containers rested on the seabed to expose the fish to pressure waves and particle motion via the water column as well as any potential ground roll waves via the sediment. After 30 min of exposure to pile driving or ambient conditions, the containers with the fish were raised to the dock side, a water sample was taken and the dissolved oxygen content of the water was again analysed. The difference between the initial dissolved oxygen content of the water and the content following 30 minutes of exposure, were used for analyses. The analyses were conducted on the percentage change in oxygen consumption (e.g. Simpson *et al*., 2015, 2016).

3. RESULTS

Black seabream significantly increased their oxygen consumption during exposure to pile driving (mean O_2 consumption ± Standard Error [SE]; 20.0 ± 1.8 %;) compared to ambient conditions (mean O_2 consumption ± SE; 13.2 ± 2.6 %) (Paired sample t-test: $t_{13} = -2.26$, $p = 0.042$; Figure 1a). The oxygen consumption of plaice did not differ between exposure to pile driving (mean O_2 consumption ± SE; 14.4 % ± 1.6 %) and ambient conditions (mean O_2 consumption ± SE; 15.8 ± 1.0) (Paired sample t-test: $t_{23} = -0.91$, $p = 0.374$; Figure 1b).

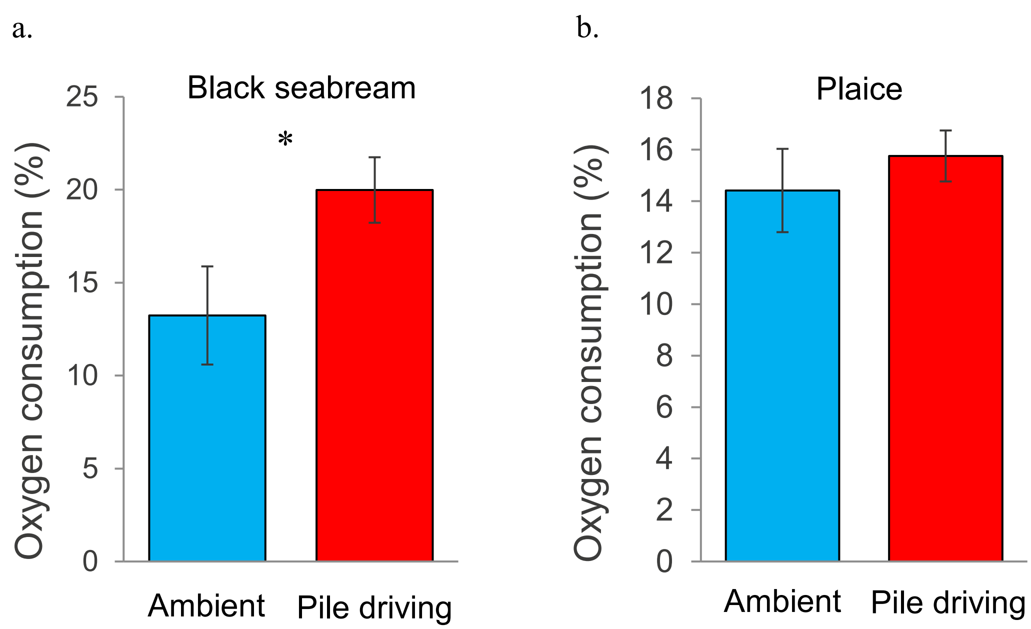

Figure 1. Oxygen consumption of black seabream (a) and plaice (b) during ambient and pile driving conditions. Shown are means ± one standard error (SE); * = $p < 0.05$.

4. DISCUSSION AND CONCLUSION

Impact pile driving increased oxygen uptake in black seabream, while plaice did not show differences in their oxygen consumption. The increased oxygen uptake suggests heightened stress during exposure to pile driving (Barton, 2002). Other species, such as European eels (*Anguilla anguilla*) and European sea bass also increased oxygen uptake when exposed to playback of anthropogenic sounds (Simpson *et al.*, 2015; Radford *et al.*, 2016), whereas an *in situ* pile driving study using European sea bass found a decrease in oxygen uptake (Debusschere *et al.*, 2016).

Although black seabream and plaice were tested under identical acoustic exposures, the results clearly differed, showcasing the importance of collecting species-specific data. Other studies that have tested multiple fish species using identical acoustic regimes have found both different outcomes between species (Popper *et al.*, 2005; Picciulin *et al.*, 2010; Voellmy *et al.*, 2014b), as well as similar results (Amoser and Ladich, 2003; Voellmy *et al.*, 2014a; Bruintjes *et al.*, 2016).

Although these experiments used simulated impact pile driving rather than real-word offshore operations, studies using realistic anthropogenic noise sources in large-scale semi-field conditions – such as this study – could advance current knowledge of the impacts of anthropogenic noise on marine organisms.

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