



# Condition-dependent physiological and behavioural responses to anthropogenic noise



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## HIGHLIGHTS

- European eels exposed to playback of anthropogenic noise show elevated stress.
- Individuals exposed to additional noise also show a reduced anti-predator response.
- Impacts are condition-dependent, with individuals in worse condition most affected.

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## ABSTRACT

Anthropogenic (man-made) noise, a global pollutant of international concern, is known to affect the physiology and behaviour of a range of organisms. However, experimental studies have tended to focus on trait means; intra-population variation in responses are likely, but have rarely been explored. Here we use established experimental methods to demonstrate a condition-dependent effect of additional noise. We show that juvenile European eels (*Anguilla anguilla*) in good condition do not respond differently to playbacks of ambient coastal noise and coastal noise with passing ships. By contrast, the additional noise of ship passes caused an increase in ventilation rate and a decrease in startling to a looming predatory stimulus in poor condition eels. Intra-population variation in responses to noise has important implications both for population dynamics and the planning of mitigation measures.

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## 1. Introduction

Noise-generating human activities, including urbanisation, resource exploitation and transportation, have changed the soundscape of many terrestrial and aquatic ecosystems. An increasing amount of research is demonstrating that such anthropogenic noise can have a range of impacts, including on individual behaviour and physiology in some species [1–4]. However, most studies have focused on trait means: the general effect of noise on a cohort of individuals [5]. Little work has investigated how intra-population variation in intrinsic characteristics such as sex, age, and body size could affect responses to noise (but see [6–9]), despite the potential implications for population dynamics, community ecology and harvests of commercial species [10,11].

Considerable intra-population variation in body condition can arise as a consequence of a range of factors, including developmental stress and current food availability [12]. Body condition can, in turn, influence

the risk of predation, parasite infection and disease, dispersal strategies, competitive ability and reproductive performance (e.g. [13,14]). Susceptibility to pollution is also expected to be affected by body condition due to differences in the ability to maintain optimal physiological function, allocate resources or tolerate stress. Some evidence exists with respect to chemical contaminants: for example, a negative relationship was found between mussel (*Mytilus edulis*) condition and metal bioaccumulation [15], while the effect of pyrene exposure on shore crabs (*Carcinus maenas*) was stronger in starved individuals compared to their better-fed counterparts [16]. However, to our knowledge, the possibility of condition-dependent responses to anthropogenic noise remains unexplored.

Due to their socio-economic importance and the vulnerability of many species to anthropogenic pressures such as overfishing and climate change [17,18], fish are an important taxon to consider with respect to acoustic noise. All fish detect sound, often possessing specialized auditory apparatus, and thus are exposed to underwater anthropogenic noise, including from ships [19,20]. Mounting evidence shows that at least some fish species can be negatively impacted by noise (e.g. [21–25]). Juvenile European eels (*Anguilla anguilla*) pass

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through the busy shipping channels of Western Europe when moving from the ocean to rivers [26]. Eels detect sound frequencies below 300 Hz [27], which overlaps with the dominant frequencies of ship noise. Recent tank-based work used playbacks of recordings made in harbours with and without passing ships to demonstrate that juvenile eels exhibit an elevation in ventilation rate and a reduction in anti-predator behaviour when experiencing additional noise [24]. Here, we use new experiments with these established methods to test whether noise-induced physiological and behavioural responses are most pronounced in poor-condition individuals.

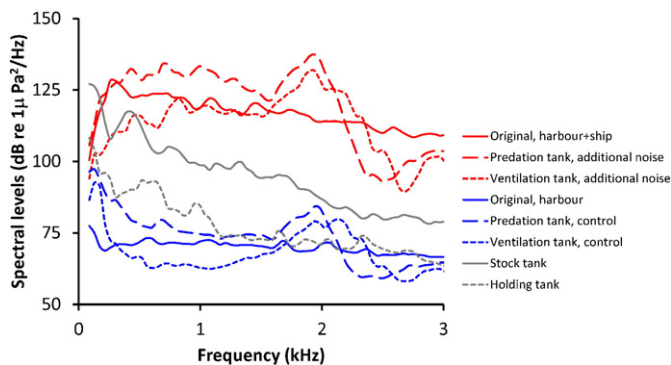
## 2. Material and methods

### 2.1. Study species and holding conditions

All procedures were approved by the University of Bristol Ethical Committee (University Investigator Number: UB/10/034). Wild glass-stage (juvenile) European eels were caught by Glass Eels Ltd., Gloucestershire, who weaned them onto a commercial diet (Perle eel food, Skretting, Norway). The eels were then transferred to 450 L stock tanks in the University of Bristol Aquarium (full transfer and husbandry details in ref. [24]). Experiments were conducted from March to June 2012; eels were moved into 50 L glass holding tanks in the experimental room for a minimum of one week prior to experiments. Ambient sounds in the stock and holding tanks were recorded using an omnidirectional hydrophone (HTI-96-MIN with inbuilt preamplifier, High Tech Inc., Gulfport MS; manufacturer-calibrated sensitivity –164.3 dB re 1 V/ $\mu$ Pa; frequency range 0.2–30 kHz) and an Edirol R09HR 24-Bit recorder (44.1 kHz sampling rate, Roland Corporation, Bellingham WA) (Fig. 1).

### 2.2. Playback tracks

Two-minute experimental playback tracks were constructed in Audacity 1.3.13 (<http://audacity.sourceforge.net/>) from original field recordings (as in refs. [9,24]). Recordings of ambient coastal noise were made at three major UK harbours (Gravesend, Plymouth, Portsmouth) when there were no ships passing close by. Recordings of ship noise were made at the same three harbours when a single ship was passing at ca. 100–400 m distance (Gravesend: Rio de la Plata, a 286 m long, 64,730 t container ship; Plymouth: Bro Distributor, a 147 m long, 14,500 t LPG tanker; Portsmouth: Commodore Goodwill, a 126 m long, 5215 t ferry). Ships were travelling at constant, relatively slow speeds (<10 knots), as enforced by port authorities for vessels entering and leaving estuarine areas. Recordings of ambient noise and ship



**Fig. 1.** Spectral analyses of field and tank-based recordings. Analyses include baseline conditions in the stock and the holding tanks, original field recordings of ambient coastal noise and ship noise, and control and additional-noise playback tracks in each type of test tank. Fast Fourier Transform (FFT) analysis of sound 0–3 kHz, using Avisoft SASLabPro v5.2.07 (Avisoft Bioacoustics): spectrum level units normalized to 1 Hz bandwidth, Hann evaluation window, 50% overlap, FFT size 1024, averaged from a 1 min sample of each recording, 43 Hz intervals presented.

passes were made using the same hydrophone, positioned at 1 m depth 20–40 m offshore, and digital recorder as described above.

Playbacks were via an underwater loudspeaker (UW-30; max output level 156 dB re 1  $\mu$ Pa at 1 m, frequency response 0.1–10 kHz; University Sound, Whitehall, Ohio, USA) in a similar setup to previous studies [9,24]. The three different tracks of each sound type were adjusted to produce approximately equal root mean square (RMS) intensity in the pressure domain to the field recordings when played back in the experimental tanks (received level, ambient coastal: ~108 dB RMS re 1  $\mu$ Pa; ship noise: ~148 dB RMS re 1  $\mu$ Pa). Examples of spectral levels from original recordings and playbacks in experimental tanks are provided in Fig. 1. Due to unresolved challenges in measuring particle velocity in small tanks at the time of the experiments, acoustic conditions were assessed in the pressure domain only. Although eels are sensitive to particle velocity as well as pressure [27], the aim of this study was not to establish absolute values for sensitivity, but rather compare physiological and behavioural responses of individuals of different condition to the same noise exposure.

### 2.3. Experimental protocols

Eels were tested once in an independent-measures design, randomly allocated to sound treatments. In both experiments, an initial period of ambient-coastal playback from one of the three harbours (A1, A2, A3) was followed by an experimental period of either another ambient-coastal track (control treatment) or a ship-noise track (N1, N2, N3; additional-noise treatment) from a different harbour. Testing blocks therefore used 12 eels, each receiving one of the 12 possible playback combinations (A1–A2, A1–A3, A1–N2, A1–N3, A2–A1, A2–A3, A2–N1, A2–N3, A3–A1, A3–A2, A3–N1, A3–N2). Playback order was randomised within testing blocks; this did not result in any chance bias in the ordering of control and additional-noise treatments within blocks (Mann Whitney U tests:  $n_1 = n_2 = 6$ , all  $U < 18$ , all  $p > 0.109$ ) or within the whole sample (ventilation-rate experiment:  $n_1 = n_2 = 78$ ,  $U = 3041$ ,  $p = 0.997$ ; predation experiment:  $n_1 = n_2 = 66$ ,  $U = 2043$ ,  $p = 0.746$ ). In both experiments, the observer was situated behind a screen and thus not visible to the eel.

To examine the condition-dependent impact of additional noise on ventilation rate, opercular beat rate (OBR) was measured. Ventilation rate is a recognised secondary indicator of stress [28], and has been shown to correlate with other physiological measures in fish, including oxygen consumption, heartrate and plasma cortisol [24,28–30]. Moreover, ventilation rate is easily measured by an observer who is blind to the acoustic experience of each fish, allows control for the baseline OBR of individual fish in a matched design, and has previously been shown to be affected by anthropogenic noise [24]. Eels were placed individually in 30-mL sealed cylindrical tubes inside the test tank containing the speaker (as per ref. [24]). Following a 2-min settling period when an ambient-noise track was playing, an observer (always J.P.) determined OBR for 1 min while the same track continued. If OBR could not be observed (e.g. when fish were turning), counting was paused; a full 1 min of beats was always counted within 90 s. The track was then switched, and 1 min of OBR determined as before. Eel activity was recorded on a 3-point ordinal scale: 0 (no swimming); 1 (some swimming in the tube); 2 (swimming in the tube and at least one vigorous outward-directed swimming motion). The water in each tube was replaced with fully-aerated water after each experimental trial; 156 individuals were tested in 13 blocks.

To examine the condition-dependent impact of additional noise on anti-predator behaviour, startle responses to a looming stimulus were assessed. This standardised method used in a variety of different research fields [24,31,32] isolates the visual component of a predatory strike. A model fish on a swinging pendulum arm, which moved through 45° to a position next to but not touching the tank wall, was placed beyond one end of the tank. An eel from a holding tank was caught in a transfer jug and left for 2 min to settle; during this time,

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