

Anthropogenic noise compromises anti-predator behaviour in European eels

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Abstract

Increases in noise-generating human activities since the Industrial Revolution have changed the acoustic landscape of many terrestrial and aquatic ecosystems. Anthropogenic noise is now recognised as a major pollutant of international concern, and recent studies have demonstrated impacts on, for instance, hearing thresholds, communication, movement and foraging in a range of species. However, consequences for survival and reproductive success are difficult to ascertain. Using a series of laboratory-based experiments and an open-water test with the same methodology, we show that acoustic disturbance can compromise anti-predator behaviour – which directly affects survival likelihood – and explore potential underlying mechanisms. Juvenile European eels (*Anguilla anguilla*) exposed to additional noise (playback of recordings of ships passing through harbours), rather than control conditions (playback of recordings from the same harbours without ships), performed less well in two simulated predation paradigms. Eels were 50% less likely and 25% slower to startle to an “ambush predator” and were caught more than twice as quickly by a “pursuit predator”. Furthermore, eels experiencing additional noise had

diminished spatial performance and elevated ventilation and metabolic rates (indicators of stress) compared with control individuals. Our results suggest that acoustic disturbance could have important physiological and behavioural impacts on animals, compromising life-or-death responses.

Introduction

Noise-generating human activities in aquatic environments, such as commercial shipping, recreational boating, pile-driving, seismic exploration and energy production, are widespread and occur with increasing frequency (McDonald *et al.*, 2006; Normandeau Associates, 2012). In terrestrial environments, the prevalence of transportation networks, resource extraction and urban development, for example, is similarly greater now than ever before (Watts *et al.*, 2007; Barber *et al.*, 2009). In addition to increasing the amount of noise, human activities often generate sounds that are very different from those arising from natural sources (Hildebrand, 2009; Popper & Hastings, 2009; Normandeau Associates, 2012). Consequently, anthropogenic (man-made) noise has changed the acoustic landscape of many areas around the globe and represents a very real, and often novel, challenge to animals. It is therefore recognised as a major component of environmental change and a pollutant of international concern; for example, anthropogenic noise is included in the US National Environment Policy Act and the European Commission Marine Strategy Framework Directive, and as a permanent item on the International Maritime Organisation Marine Environmental Protection Committee agenda.

Recent studies on a wide variety of taxa have demonstrated impacts of anthropogenic noise across a range of scales, from individual organisms to community ecology (see reviews in Tyack, 2008; Barber *et al.*, 2009; Slabbekoorn *et al.*, 2010; Normandeau Associates, 2012; Morley *et al.*, 2014). At an individual level, the most dramatic effects are injury or even death experienced by some, but not all, species when very close to certain, particularly impulsive, sound sources (Keevin & Hempen, 1997; Halvorsen *et al.*, 2012). Further from the source, there may be physiological effects, including temporary threshold shifts (transient reductions in hearing sensitivity) and stress, although again this has been found in only some of the species tested (e.g. Popper *et al.*, 2005, 2007; Wysocki *et al.*, 2006, 2007). Furthest from the source, behavioural effects are the most likely to occur, and thus it is behavioural changes that represent the most widespread impact of anthropogenic noise (Normandeau Associates, 2012).

Elevated or changed sound levels could affect animal behaviour in three main ways, which are not mutually exclusive. Noise could act as a stressor (Wright *et al.*, 2007; Kight & Swaddle, 2011), could act as a distracting stimulus, diverting an individual's limited amount of attention from their primary tasks (Mendl, 1999; Chan & Blumstein, 2011), or could mask crucial acoustic cues or signals (Brumm & Slabbekoorn, 2005; Radford *et al.*, 2014a). Numerous studies have considered how anthropogenic noise consequently affects communication, movement patterns and foraging (Barber *et al.*, 2009; Slabbekoorn *et al.*, 2010; Radford *et al.*, 2014a). However, it is often difficult to translate these effects into meaningful predictions about individual fitness and population-level consequences (NRC, 2005; Morley *et al.*, 2014), because animals may be able to move away from the source, disturbances may be sporadic and compensation may prevent long-term impacts (NRC, 2005; Bejder *et al.*, 2006; Normandeau Associates, 2012). In contrast, studying anti-predator behaviour offers a direct link to individual fitness: a reduced likelihood of escape affects survival and there is no way to compensate for capture by a predator.

Our knowledge of the natural world is the product of a complementary suite of scientific approaches conducted both in captive and field conditions. To explore the impacts of a range of other environmental stressors (e.g. global warming and ocean acidification), a valuable starting point has been to test for specific effects in carefully controlled laboratory conditions (e.g. Dixon *et al.*, 2010; Scott & Johnston, 2012). This allows the tight control of potentially confounding factors, but accepts the compromise that the “stressor experience” does not fully recreate real-world conditions (Slabbekoorn, 2014). Here we use a series of controlled laboratory-based experiments and an open-water test with the same methodology to investigate the potential for acoustic disturbance to compromise anti-predator behaviour, a topic which has received little empirical attention, and to explore possible underlying mechanisms. We use a well established laboratory model species, the European eel (*Anguilla anguilla*), which is known to detect frequencies below 300 Hz (Jerkø *et al.*, 1989), overlapping with the dominant frequencies of ship noise (Normandeau Associates, 2012). In the wild, juvenile eels pass through the busy shipping channels of Western Europe when moving from the deep ocean to rivers (Schweid, 2009). During this transition, they encounter two main types of predation threat: ambush predation when seeking refuge in benthic habitat and pursuit predation in open water. Thus eels make a valuable model system for studying impacts of noise on anti-predator behaviour.

Specifically, we studied whether the behaviour and physiology of juvenile eels is affected by additional noise (playback of recordings of a ship passing through a harbour) compared with control conditions (playback of recordings from the same harbours without ship noise). We tested the prediction that additional noise would result in eels exhibiting diminished or maladapted responses to predatory threats in ambush and pursuit paradigms. A number of mechanisms may underpin the demonstrated changes in anti-predator responses when eels are exposed to playback of additional noise (Kight & Swaddle, 2011). So, we then measured spatial and physiological performance, specifically testing for compromised lateralised behaviour and altered metabolic and ventilations rates as indicators of stress.

Material and methods

Study species and holding conditions

Glass-stage European eels, collected in the River Severn in May 2011, were reared and weaned onto a commercial diet (Perle eel food, Skretting, Norway) by Glass Eels Ltd., Gloucestershire before transfer to the University of Bristol aquarium facilities. All experiments were conducted using this stock population during July–September 2011 when eels measured 8–12 cm.

Eels were held in 450 l glass stock tanks and moved into 50 l glass holding tanks in the experimental room for a minimum of one week prior to experiments. During experiments, eels were fed once daily; water temperature was $16^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$; lighting was provided 13:11 day:night; tanks contained artificial plants and tube shelters; filtration was by twice-weekly water changes and water movement was maintained by slow-bubbling airstones to avoid unnecessary noise. 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 $1\text{V}/\mu\text{Pa}$; frequency range 0.2–30 kHz) and an Edirol R09HR 24-Bit recorder (44.1 kHz sampling rate, Roland Corporation, Bellingham WA; recording level calibrated using pure sine wave signals from a function generator with a measured voltage recorded in line on an oscilloscope). Stock and holding tank recordings were made 2 cm above the tank floor, at the end of the large long-term stock tank where the eels typically rested, and in the centre of the smaller holding tanks ([Fig. 1](#)). Due to unresolved challenges in measuring particle velocity in small tanks, we assessed acoustic conditions in the pressure domain

only. Although eels are sensitive to particle velocity as well as pressure (Jerkø *et al.*, 1989), in this study we do not attempt to establish absolute values for sensitivity, but rather explore impacts of additional noise on behaviour and physiology (see Rogers 2014 for an excellent examination of the complex pressure and particle velocity conditions in tanks).

Playback tracks

The 2-min playback tracks used in all the experiments were created using Audacity 1.3.13 (<http://audacity.sourceforge.net/>) from recordings made at three major UK harbours (as per Wale *et al.*, 2013a,b). Field recordings were made of both ambient harbour noise and ship noise generated by a single passing ship 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, 5,215 t ferry). Ships were travelling at constant, relatively slow speeds (<10 knots), as enforced by port authorities for vessels entering and leaving estuarine areas. Weather conditions during recordings ranged from 0 to 1 on the Douglas Scale of sea state, with a still to moderate wind speed. Recordings were made using the same hydrophone, positioned at 1 m depth 20–40 m offshore, and solid-state recorder as described above (see [Fig. 1](#) for spectral analyses).

Sound levels of the three different ambient-harbour (control treatment) and ship-noise (additional-noise treatment) playback tracks were adjusted to produce equal RMS intensity in the pressure domain to the field recordings when played back using an UW-30 underwater speaker (max output level 153 dB re 1 μ Pa at 1 m, frequency response 0.1 to 10 kHz, Lubell Labs Inc., Columbus OH). Recordings in test tanks were made 2 cm above the tank floor, in the centre of the tank for the predation/lateralisation tanks, and 10 cm from the speaker wall (where fish were placed) for the ventilation/metabolic rate tank (Fig. 1). The three control playback tracks were modified (uniform amplification or attenuation) to play at ~108 dB RMS re 1 mPa (106.8, 108.6, 107.5) at the position of the fish (or in the centre of the tank for the pursuit and lateralisation experiment), which was substantially greater than the noise floor in the tanks (96 dB RMS re 1 mPa), while additional-noise playback tracks were modified to play at ~148 dB RMS re 1 mPa (149.0, 148.6, 147.8).

General experimental design

Our interest was in the effect of additional noise on behaviour and physiology relative to that exhibited by individuals from the same cohort from the same holding conditions that experienced control playbacks; any treatment-based effect is not the consequence of captive conditions *per se*. In each experiment, individual eels were tested once in an independent-samples design. All experiments (except the assessment of metabolic rate) involved an initial period of a control playback from one of the three harbours (C1, C2, C3), followed by a switch to playback of either a control recording or an additional-noise recording (N1, N2, N3) from a different harbour. As such, there were 12 possible combinations of control to control or control to additional-noise playback: C1–C2, C1–C3, C1–N2, C1–N3, C2–C1, C2–C3, C2–N1, C2–N3, C3–C1, C3–C2, C3–N1 and C3–N2. Testing blocks therefore utilised 12 eels, each one receiving one of the 12 possible playback combinations. The order in which these playback combinations were presented was randomised within each testing block; subsequent analysis confirmed that this did not result in any chance bias in the ordering of control and additional-noise treatments (Mann Whitney *U* tests on ranked orders: all $P > 0.670$). For the assessment of active metabolic rate, eels experienced only one playback track (C1, C2, C3, N1, N2 or N3), and thus testing blocks utilised six eels which each received one track in randomised order. Subsequent analysis again confirmed that there was no chance bias in the ordering of control and additional-noise treatments ($P = 0.934$). The lack of an order bias within blocks rules out the possibility that an unmeasured potential confounding effect, such as an accumulation of stress hormones in the water, could explain any treatment-based differences found.

All datasets were analysed using two-way ANOVAs to assess the impact of acoustic treatment (control or additional-noise), while controlling for any potential effect of testing block (which was never significant, all $P > 0.159$); the interaction term was never significant (all $P > 0.120$). The non-significant effect of block indicates that there were no changes across time in, for example, the ability of the catcher in the pursuit predation paradigm.

Predation experiments

Predation experiments were conducted under UK Home Office licensing (PPL 30/2860) in a 120 x 40 cm (water depth: 40 cm; wall thickness: 4 mm) glass tank, with an upward-facing loudspeaker suspended

underneath a false 4 mm thick Perspex bottom. The experiment considering an ambush (sit–wait–strike) predatory threat used the well-established looming stimulus approach, which isolates the visual component of a predatory strike; this method has been used to assess fish anti-predator responses in a range of research fields (Batty, 1989; Fuiman & Cowan, 2003). A model fish on a swinging pendulum arm, which moved through 45° to a position next to the glass, was placed beyond one end of the tank. The tank was illuminated from above and the observer was behind a screen. An eel from a holding tank was caught in a transfer jug and left for 2 min to settle; during this time, all eels returned to a normal sedentary mode. A control playback track was switched on in the experimental tank (by A.N.R., who operated the sound system throughout) and the eel released into the end furthest from the model fish. After 1 min acclimatisation, when eels freely explored the tank, the track was switched to either another control track or an additional-noise track. When the eel first passed the ‘predator window’ (a 20 cm wide stretch of glass in front of the predator), the predator was remotely released by S.D.S., who was listening to loud music through headphones and so was unaware of the acoustic treatment in the tank. Eels in the two playback treatments did not differ significantly in the time until they first passed the predator window (ANOVA: $F_{1,43} = 0.07$, $p = 0.800$). The entire trial, including any responses to the looming predatory stimulus, was recorded on video (Casio EX-FH20, Tokyo, Japan) for later analysis. S.D.S. scored the videos without sound (and thus ‘blind’ to the acoustic treatment), to determine whether the eel startled (exhibit a directional change in swimming trajectory between consecutive frames) and, if it did, the time taken to startle (from initiation of model release). The occurrence of a startle reaction indicates detection and response to a stimulus as a potential threat, and is the first stage in a typical defence cascade (Cacioppo *et al.*, 2000). The water in the experimental tank was stirred between trials to homogenise any olfactory cues, and we tested 48 eels in four blocks, changing the water between each block.

To consider how additional noise affects the response to a pursuit (chase and catch) predatory threat, eels were chased through a maze with a handnet until captured. The maze was created in the experimental tank by the addition of two static 70 cm “[” shaped Perspex baffles in a staggered formation. Transfer and settling of eels, and initiation of playbacks, was the same as for the ambush predator experiment (above). When the track was switched, catching was initiated from the far end of the tank from the current position of the eel. The catcher (always S.D.S.) could only chase the eel (no ambush

tactics were allowed); he was ‘blind’ to the acoustic treatment throughout (see above); and operated from a fixed position at the side of the tank wearing the same external clothing during all blocks. We compared the time taken to catch each eel depending on whether a control or additional-noise track was playing in the tank. The water in the experimental tank was stirred between trials to homogenise any olfactory cues, and we tested 60 eels in five blocks, changing the water between each block.

Potential underlying mechanisms

To assess the impact of additional noise on characteristic spatial behaviour, we assessed the degree of lateralisation displayed in both noise treatments, using the standard measure of the proportion of turns in a preferred direction at symmetrical junctions in a maze (Domenici *et al.*, 2012). Lateralised behaviour is important in fishes for predator inspection, spatial cognition and schooling (Brown *et al.*, 2004; Braithwaite, 2006). We used the same tank as in the predation experiments, with the same Perspex baffles positioned in parallel to create a simple “]” maze with a central corridor and T-junction at each end. The tank was illuminated centrally from above, a ceiling-mounted mirror was used to give an aerial view for monitoring eel behaviour, and the observer was behind a screen to remain hidden from the eel even in the mirror. For each trial, an eel from a holding tank was caught in a transfer jug and left to settle for 2 min. A control track was then switched on in the experimental tank and the eel transferred to one end of the maze. The eel was given 5 min to explore the arena and then the direction of the next 10 turns made by the eel (five at each end) was recorded; at no stage did eels need to be guided into the maze during the experiments. Playback was then switched to another control track or an additional-noise track and the direction of the subsequent 10 turns recorded to assess any change in directional preference exhibited. The water in the experimental tank was stirred between trials to homogenise any olfactory cues and changed after each block of 12 eels; 48 eels were tested in total.

To explore whether the changes in anti-predator behaviour are potentially underpinned by physiological state, we considered two standard measures that are commonly used as indicators of stress: ventilation (opercular beat rate) and active metabolic rate (oxygen usage) (Barton, 2002). For opercular beat rate, individual eels were placed into 30 ml gas-tight sealed tubes (polystyrene 1 mm wall thickness; estimated to be 90–95% acoustically transparent based on typical acoustic impedance of polystyrene vs.

water) inside the test tank at a fixed location 10 cm from the speaker. The plastic test tank was 34 x 20 cm (water depth: 16 cm; wall thickness: 2 mm), with a sideward-facing loudspeaker suspended at one end. Eels were allowed to settle for 2 min while a control track was playing. An observer (always S.D.S.), ‘blind’ to the acoustic treatment (see above), then counted opercular beats for 1 min. If opercular beat could not be observed, counting was paused; for every individual tested, a full 1 min of beats was counted (always within 90 s). The track was then switched to another control track or an additional-noise track, and 1 min of opercular beats was counted as before. Time was monitored and the track was switched by a second observer (A.N.R.). The water in the tubes was replaced with fully-aerated water after each eel and we tested 24 individuals in two blocks.

To determine oxygen usage in both the laboratory and an open-water study, individual eels were placed in the same tubes as used for the measurement of opercular beat rate, with the tops sealed underwater to avoid air bubbles. After an eel was placed in a tube, the playback track was randomly selected to avoid any biases arising from preferential capture. Dissolved oxygen content of the water at the start of each trial was tested (Morris *et al.*, 2005) (laboratory study: Dissolved Oxygen Meter 781, Strathkelvin Instruments, SI Ltd., North Lanarkshire, UK; open-water study: Dissolved Oxygen and Temperature Meter HI 9164, Hanna Instruments Inc., USA). Sealed tubes were placed 10 cm from a speaker, either in a test tank (laboratory study) or in a mesh tray suspended 20 cm below the surface (total water depth: 4 m) and 1 m from the wall of the Cumberland Basin (Bristol Harbour, 51°26′53″N, 2°37′10″W; open-water study). After 2 min exposure to the playback track, a water sample was taken from the tube and the dissolved oxygen content tested. In each experiment, we tested 48 eels in four blocks and analysis was conducted on the percentage change in dissolved oxygen content (to account for minor variation in the starting value).

Results

Predation experiments

In the experiment considering an ambush predatory threat, individuals exposed to additional noise at the time of “attack” were 50% less likely to startle than those attacked during the control treatment (chi-squared test: $\chi^2_1 = 8.57$, $n = 48$, $P = 0.003$; Fig. 2a). Moreover, individuals that did startle during

additional-noise playback were 25% slower to do so than those that startled in the control treatment (ANOVA: $F_{1,23} = 10.26$, $P = 0.004$; Fig. 2b). The startle response is crucial for prey to survive attacks by ambush predators and, if successful, is likely to terminate the predator–prey interaction as the predator returns to its ambush position.

In the experiment considering a pursuit predation threat, eels experiencing additional noise were caught more than twice as quickly as those in the control treatment (ANOVA: $F_{1,54} = 10.78$, $P = 0.002$; Fig. 2c). In the wild, the probability of successful escape is likely to increase the longer that capture can be avoided, either because the prey may find a suitable shelter to hide or because the predator gives up the chase as energetically too costly.

Potential underlying mechanisms

The initial high level of lateralisation (proportion of turns in a preferred direction) apparent during playback of a control track was unchanged with a switch to a different control track (Fig. 3a), but declined with a switch to additional-noise playback (Fig. 3b); there was a significant loss in directional bias with additional noise (ANOVA: $F_{1,43} = 40.24$, $P < 0.001$; Fig. 3c). In the context of our pursuit predation paradigm, lateralisation may be important for rapid decision-making and optimal utilisation of a spatially complex environment when attempting to reduce the likelihood of capture.

There was no significant change in opercular beat rate when one control track was exchanged for another, but eels exhibited a significant increase in opercular beat rate when a control track was replaced with an additional-noise track (ANOVA, change in beat rate between periods: $F_{1,21} = 21.80$, $P < 0.001$; Fig. 3d). In the laboratory-based assessment of active metabolic rate, eels exposed to playback of additional noise showed a significant increase in oxygen usage compared to those in the control treatment ($F_{1,43} = 13.77$, $P = 0.001$; Fig. 3e); there was no noticeable difference in movement (almost all individuals in both treatments remained stationary during playback). The same treatment-related difference in active metabolic rate was found in the open-water test: eels used significantly more oxygen during additional-noise playback than during control playback ($F_{1,43} = 49.82$, $P < 0.001$).

Discussion

We found that acoustic disturbance detrimentally affects anti-predator performance of juvenile eels in experimental ambush and pursuit predation paradigms. The influence of noise on anti-predator behaviour has received little research attention (two previous studies indicated that crabs were less likely to detect a looming stimulus; Chan *et al.*, 2010; Wale *et al.*, 2013a), but we suggest that information on such direct determinants of survival is particularly valuable. Further investigation is needed to test the extent to which acoustic disturbance may also affect predators, and to determine the interactive effect on the performance of both parties during predation events.

In addition to effects on anti-predator behaviour, we also provide evidence that acoustic disturbance can cause changes in spatial behaviour and physiological state. Using a standard test of lateralisation, we found that additional-noise playback caused a reduction in characteristic lateralised behaviour in eels. Since lateralised behaviour in fish is important for spatial cognitive tasks and anti-predator behaviour, including predator recognition (Brown *et al.*, 2004; Braithwaite, 2006), the observed loss of lateralisation may have compromised optimal space use by eels in the pursuit predation experiment, hence reducing time to capture. A study demonstrating impacts of ocean acidification on lateralisation suggests that brain function may be more fundamentally affected by elevated CO₂ conditions (Domenici *et al.*, 2012). Whether noise also influences brain function at a more fundamental level remains to be tested.

The noise-induced alteration in physiological state could indicate a more general allostatic stress response, with the intensity, duration and frequency of multiple aspects of behaviours likely to be affected (Broom & Johnson, 1993; McEwan & Wingfield, 2003; Wingfield, 2005); a previous study measuring cortisol levels in fish has also suggested that noise is a stressor (Wysocki *et al.*, 2006, but see Wysocki *et al.*, 2007). If this response includes a reduction or cessation of normal locomotor activity (Metcalf *et al.*, 1987), for example, then the likelihood of escape from a predator may be reduced. An allostatic stress response could also potentially affect attention (Chan & Blumstein, 2011), as would noise acting as a distractor (Chan *et al.*, 2010), compromising anti-predator behaviour that relies on cognitive processes including detection, classification and decision-making (Mendl, 1999; Shettleworth, 2010). Furthermore, the addition of noise may have masked (Brumm & Slabbekoorn, 2005) the noise of the

handnet in the pursuit predation experiment, but not the visual or tactile cues; masking is unlikely as an explanation in the looming stimulus experiment where the “predator” was not coupled to the tank.

Playback of field recordings are known to be qualitatively different from the original noise source and acoustic conditions in small tanks are complex (Parvulescu, 1967; Okumura *et al.*, 2002). However, our primary aim was to investigate whether acoustic disturbance, rather than a particular noise source, has the potential to have an impact. Our finding of a similar alteration in active metabolic rate using the same methods in a tank and in open water also suggests that the demonstrated impacts are not simply an artefact of the reflections and reverberations that occur in tank environments. From a biological perspective, captive animals are usually more constrained than in the wild, and individuals receive husbandry regimes that do not fully replicate natural conditions of resource availability. However, experiments in tanks allow tight control of conditions and contexts, as well as detailed data collection, and have proved a valuable stepping stone in the study of other environmental stressors (Dixson *et al.*, 2010; Scott & Johnston, 2012). Our experiments show that near to the sound source there are impacts of acoustic disturbance with implications for survival, while sonar-based tracking of free-swimming fish indicates changes in swimming behaviour near to vessels (Handegard *et al.*, 2003; Hawkins *et al.*, 2014). Ultimately, studies in open-water conditions are needed to investigate the spatial scale of impacts that carry fitness consequences.

There is clearly further potential for scientific exploration in carefully controlled conditions of how acoustic disturbance affects animals, for example considering intra-population variation, context-dependency, prior experience and recovery (Radford *et al.*, 2014b), and testing whether frequent or continuous exposure to noise may lead to changes in response due to tolerance, habituation or sensitisation (Bejder *et al.*, 2009; Wale *et al.*, 2013b). In an applied context, the main challenge moving forwards is to deliver to regulators evidence that can be directly useful to management and policy decisions, considering dose-dependent responses to real-world anthropogenic noise sources. For now, our laboratory-based findings that additional noise compromises anti-predator and lateralised behaviour and physiological state, with potential survival consequences, suggest that further work on this contemporary global environmental change issue is warranted.

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Figure legends

Figure 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 harbour noise and ship noise, and control and additional-noise playback tracks in each type of test tank. Fast Fourier Transform (FFT) analysis of sound 50–5000 Hz, using Avisoft SASLabPro v5.2.07 (Avisoft Bioacoustics): spectrum level units normalised to 1 Hz bandwidth, Hann evaluation window, 50% overlap, FFT size 1024, averaged from a 15 s sample of each recording, 43 Hz intervals presented.

Figure 2 Anti-predator responses of eels during playback of control and additional-noise tracks. (a) Number of eels exhibiting a startle response or not to a looming stimulus ($n = 24$ for each treatment). (b) Mean \pm SE time taken to startle to looming stimulus by those individuals in (a) that exhibited a startle response (control treatment: $n = 19$; additional-noise treatment: $n = 9$). (c) Mean \pm SE time to capture free-swimming eels in a maze with a handnet ($n = 30$ for each treatment).

Figure 3 Potential underlying mechanisms for the impact of acoustic disturbance on eel anti-predator behaviour. (a and b) Lateralisation of eels during initial playback of control tracks ('before') and during subsequent playback ('during') of either another control track (a) or an additional-noise track (b) ($n = 24$

in each case). (c) Mean \pm SE change in directional bias exhibited by eels following the switch in playback track (n = 24 in each case). (d) Mean \pm SE ventilation rate of eels during playback of control or additional-noise tracks. Ventilation was recorded for 1 min while eels were exposed to a control track ('before') and then for a further 1 min after switching the playback ('during') to either an additional-noise track or another control track (n = 12 in each case). (e) Mean \pm SE oxygen depletion in tubes with eels exposed to playback of control or additional-noise tracks (n = 24 for each treatment).