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Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms

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Effective foraging behaviour is essential for animals to survive and reproduce, and depends on many intrinsic and environmental factors. There is increasing evidence that man-made (anthropogenic) factors can affect the behaviour of a wide range of taxa. However, few experimental studies have investigated how foraging behaviour is affected by exposure to increased noise levels, an issue of growing global concern. In our laboratory study, we examined how exposure to playback of noise originally recorded from ships, a prevalent source of human-generated underwater noise, affects the feeding behaviour of two sympatric fish species: the three-spined stickleback, Gasterosteus aculeatus, and the European minnow, Phoxinus phoxinus. Both species consumed significantly fewer live Daphnia magna, and showed startle responses significantly more often during playback of additional noise than during control conditions. However, whereas minnows showed a qualitative shift in activity away from foraging behaviour (greater inactivity, more social behaviour) under increased noise conditions, consistent with a classic stress- or fear-related defence cascade, sticklebacks maintained foraging effort but made more mistakes, which may result from an impact of noise on cognition. These findings indicate that additional noise in the environment can lead to reduced food consumption, but that the effects of elevated noise are species specific. It remains to be tested whether these interspecific differences translate into different ultimate impacts, but differential disruptions to foraging may have potential consequences for relative individual fitness and community structure.

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Animals must minimize the risk of starvation if they are to survive and reproduce successfully. A wide range of morphological adaptations and behavioural techniques have therefore evolved to aid in the detection, acquisition and processing of food (Stephens, Brown, & Ydenberg, 2007). Foraging also involves constant decision making about when, where and on what to feed (Galef & Giraldeau, 2001), and how to optimize time allocation with other behaviours, such as reproduction and the avoidance of predators (Lima & Dill, 1990). Consequently, events that compromise any of these facets of foraging may have detrimental consequences for individual fitness.

It has long been established that foraging is affected by a range of internal and external factors, such as hunger level, health, quality and quantity of food sources, intra- and interspecific competition, and predation risk (Lima & Dill, 1990; Stephens et al., 2007). More

recently, we have begun to realize the extent to which human activities such as habitat fragmentation, climate change, species introductions and the use of fertilizers and pesticides can affect food availability, predator–prey interactions and foraging behaviour (Blumstein & Fernández-Juricic, 2010; Candolin & Wong, 2012). In the last few decades, there has been increasing concern about how anthropogenic (man-made) noise, from such sources as urban development, resource extraction and transport, might affect individual species and community ecology (Blickley & Patricelli, 2010; Popper & Hastings, 2009; Slabbekoorn et al., 2010). However, while there is a growing literature demonstrating that anthropogenic noise can affect the behaviour of animals in a wide range of taxonomic groups, the primary focus has been on movement patterns and vocal communication (see Morley, Jones, & Radford, 2014); relatively few studies have experimentally considered foraging behaviour (for exceptions see Schaub, Ostwald, & Siemers, 2008; Siemers & Schaub, 2011; Wale, Simpson, & Radford, 2013a).

Elevated sound levels could affect foraging behaviour in three main ways, which are not mutually exclusive. First, noise could act as a stressor (Wright et al., 2007), decreasing feeding behaviour

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directly through reduced appetite (Charmandari, Tsigos, & Chrousos, 2005), or indirectly through a reduction in activity and locomotion (Mendl, 1999) or alterations to the cognitive processes involved in food detection, classification and decision making (De Kloet, Oitzl, & Joëls, 1999; Lupien & McEwen, 1997). Second, noise could act as a distracting stimulus, diverting an individual's limited amount of attention from their primary tasks to the noise stimuli that have been added to the environment (Chan & Blumstein, 2011; Mendl, 1999). This could impair foraging success if, for instance, suitable food sources are detected less often or more slowly, are assessed less accurately, or if prey items are mishandled (Purser & Radford, 2011). Third, noise could mask crucial acoustic cues (Brumm & Slabbekoorn, 2005). If cues produced by prey are masked, feeding opportunities may be missed (Schaub et al., 2008; Siemers & Schaub, 2011). If acoustic predator cues are masked and animals compensate by relying on visual information to a greater extent (Quinn, Whittingham, Butler, & Cresswell, 2006), then visually guided food searching and acquisition might be compromised.

Not only has there been a limited amount of research investigating the impact of anthropogenic noise on foraging behaviour, but noise studies in general also tend to consider the responses of only a single species in isolation (but for exceptions see Francis, Ortega, & Cruz, 2011a, 2011b; Ríos-Chelén, Salaberria, Barbosa, Macías Garcia, & Gil, 2012). However, it is likely that there will be stable interspecific differences in susceptibility and responses to elevated noise levels depending on variation in, for example, hearing ability (Fay, Popper, & Webb, 2008) and mechanisms of physiological stress response (Hofer & East, 1998). In sympatry, and particularly if there is overlap in ecological niches, these differences may alter the relative success of each species under scenarios of disturbance, and so potentially affect community composition and structure.

In this study we investigated how exposure to additional noise affected the feeding behaviour of two sympatric fish species. Numerous fishes use and produce sounds for a variety of reasons (Popper, Fay, Platt, & Sand, 2003), and there is increasing evidence that at least some species are susceptible to anthropogenic noise (see Popper & Hastings, 2009; Radford, Kerridge, & Simpson, 2014; Slabbekoorn et al., 2010). Hearing in fishes varies greatly (Fay et al., 2008; Fay & Popper, 2012), resulting in interspecific differences in vulnerability to anthropogenic noise. For instance, comparative studies have shown different masking effects of noise on a range of Mediterranean fish species (Codarin, Wysocki, Ladich, & Picciulin, 2009) and different behavioural thresholds for startle responses to pure tones in eight marine fish species (Kastelein et al., 2008). Fishes also differ greatly in their sensitivity to stress (Pottinger, 2010) and to risk in general. For instance, species with body armour remain longer in potentially dangerous feeding locations, initiate escape behaviour later at shorter flight distances and hide less than fish without such defensive adaptations (Abrahams, 1995; Krause, Cheng, Kirkman, & Ruxton, 2000; McLean & Godin, 1989); such relatively risk-tolerant species may conceivably also be more tolerant of other stressors such as novel anthropogenic noise.

In our laboratory-based experiments, we compared the foraging behaviour of three-spined sticklebacks, *Gasterosteus aculeatus*, and European minnows, *Phoxinus phoxinus*, when exposed either to silent-playback controls or to playback of noise originally derived from recordings of ships. With over 50 000 merchant ships carrying 90% of world trade around the globe (International Chamber of Shipping, 2013), shipping is a major contributor to marine anthropogenic noise (Hawkins & Popper, 2012). If increased noise induces a stress response, acts as a distraction or masks important cues, we predicted that fish might suffer a reduction in food intake

arising from decreases in appetite, and thus in foraging effort and/or foraging performance (e.g. increased errors in detection, classification and handling). If noise acts as a stressor, we also expected increases in startle behaviour and/or inactivity during playback of additional noise. Interspecific differences in responses could arise if the species differ in their hearing capabilities and because minnows lack the morphological antipredator adaptations (bony plates and dorsal spines) of sticklebacks, and consequently show less bold behavioural patterns (Hoogland, Morris, & Tinbergen, 1957; Mathis & Chivers, 2003); they may therefore be more risk averse and show more stress-related behaviour in response to noise, at the expense of feeding activities.

METHODS

Ethical Note

All procedures were approved by the University of Bristol Ethical Committee (University Investigator Number: UB/10/034) and followed Association for the Study of Animal Behaviour and Animal Behavior Society Guidelines for the Use of Animals in Research. Fish were tested only once they were acclimated to the test set-up (i.e. when they did not hide or freeze in the test tank prior to trials). Data collected on stress-related behaviour during control conditions showed that fish were not disturbed to an unacceptable level by the test procedure. Moreover, fish showed only mild stress responses (such as brief startle responses) to playbacks of additional noise, and those responding to noise by decreasing their activity resumed pretrial activities within minutes of the playback stopping. All fish resumed normal pre-experimental behaviour (including feeding) in their holding tanks at the end of each test and training day. All fish used in this study were kept for future research.

Study Species and Holding Conditions

Three-spined sticklebacks and European minnows often coexist in freshwater habitats, such as ponds, streams, rivers and lakes, and brackish seashore and estuarine areas (Froese & Pauly, 2011; Joint Nature Conservation Committee and Centre for Ecology and Hydrolology, 2011). As a consequence, they can be exposed to a wide range of anthropogenic noise, from recreational boat traffic in lakes to shipping, pile driving and other industrial noise in major rivers and estuaries.

Thirty-six adult three-spined sticklebacks (30 for use as focal fish and six to act as familiar companions during experimental procedures to maintain normal behaviour) were caught using hand-held nets from a freshwater pond in southwest U.K. (51°30′4″ N, 2°38′13″ W; online stillwater associated with Hazel Brook/River Trym) with appropriate Environmental Agency permission. Fish were transported to the University of Bristol Aquarium Facility by car (journey time: 15 min) within 2 h after catching. For transport, a maximum of three fish were placed in transparent plastic bags (3 litres) that were filled with one-third pond water and two-thirds air; bags were placed in opaque black 10-litre plastic buckets, half-filled with pond water. Water conditioner (API stress coat, Mars Fishcare North America, Inc., Chalfont, U.S.A.) was added to the water to neutralize ammonia. All fish survived transport and were checked on arrival by the University Veterinary Officer, who had approved the transport process. After gradual acclimatization to the aquarium water, groups of up to 20 sticklebacks were transferred to three 100-litre glass holding tanks (90 × 36.5 cm; water depth: 30 cm; wall thickness: 4 mm). Tanks contained artificial plants for shelter, an external power filter and an airstone kept at low airflow

rates to minimize noise generated by vibration and collapsing air bubbles. Fish were kept at 17 °C on a 12:12 h light dark cycle.

Thirty-two adult European minnows (28 for testing, four for use as companions) were provided by two suppliers: Blades Biological Ltd, Kent, U.K., who caught them from a river in Kent using hand-held nets, and Newlyn Pets, Bradford, U.K., who kept minnows of wild origin in outdoor ponds. Minnows were transported overnight to the laboratory by post using standard transport methods of the suppliers: in groups of up to 15 in 20-litre transparent double plastic bags (25% of the volume filled with water) in cardboard (Blades Biological Ltd.) or polystyrene (Newlyn Pets) boxes containing two cooling elements. Over 95% of fish survived transport and were checked on arrival by the University Veterinary Officer. Following gradual acclimatization to the aquarium water, groups of up to 12 minnows were transferred to four glass holding tanks of the same dimensions as those used for the sticklebacks. Minnows were housed at 10 °C on a 12:12 h light dark cycle. Minnow holding tanks had sand substrate, artificial plants and half flower pots for shelter, an external power filter with UV-filter and an upwelling outflow to ensure sufficient water aeration but minimize low-frequency noise from vibration caused by aeration. All holding tanks were placed on 5 cm polystyrene boards, to minimize vibrations from water filters and those generated within the laboratory building, and positioned on shelves along the laboratory room walls (to maximize distance to the laboratory entrance). External power filters were placed on the laboratory floor and UV-filters were padded with pipe insulation foam to minimize transmission of low-frequency vibration noise. Inflow and outflow pipes were submerged below the water surface to minimize surface disruption noise. The laboratory building was separated from the main University building, thus minimizing low-frequency noise transmission of vibrations originating there.

All fish were monitored daily after arrival. The water quality of all tanks for both species was maintained at safe levels (pH: 8.0–8.2; ammonia and nitrite: 0 mg/litre; nitrate: 40 mg/litre maximum by weekly 10–20% water changes). Both species were kept in nonbreeding condition in the aquatic facilities at the University of Bristol, and were fed with frozen bloodworms (chironomid larvae) and flakes (Aquarian Goldfish Flake, Masterfoods, Batley, U.K.) three times a week.

Assessment of Noise Levels in Holding Tanks

An illustrative assessment of acoustic conditions in holding tanks was made at two locations: in the tank centre, 5 cm below the water surface, and at the tank corner next to the water inflow 5 cm above the tank floor. Complementary work, taking measurements at 33 different tank positions, had established that these chosen locations represented areas of minimum and maximum sound pressure levels, respectively (Voellmy, Simpson, Purser, & Radford, 2014). At each location, sound levels were recorded for 1 min at a sampling frequency of 44.1 kHz and a sampling rate of 16 bits with an omnidirectional hydrophone with preamplifier (HTI 96-MIN; manufacturer-calibrated sensitivity −164.3 dB re 1 μPa; frequency range 2–30 000 Hz) and a solid-state recorder (Edirol R09HR, Roland Corporation; recording levels calibrated against a 1 kHz reference tone of known amplitude). Spectral levels from 30 s of recordings (fast Fourier transformation (FFT); spectrum level units normalized to 1 Hz, Hann window, FFT length 1024, 50% overlap) were generated in Avisoft SAS Lab Pro v4.53 (Avisoft Bioacoustics, Berlin, Germany) and averaged from the three stickleback holding tanks and the four minnow holding tanks for each of the two chosen tank locations (Fig. 1).

While both study species are likely to be sensitive to particle motion (Fay et al., 2008; Popper & Fay, 2011), technical
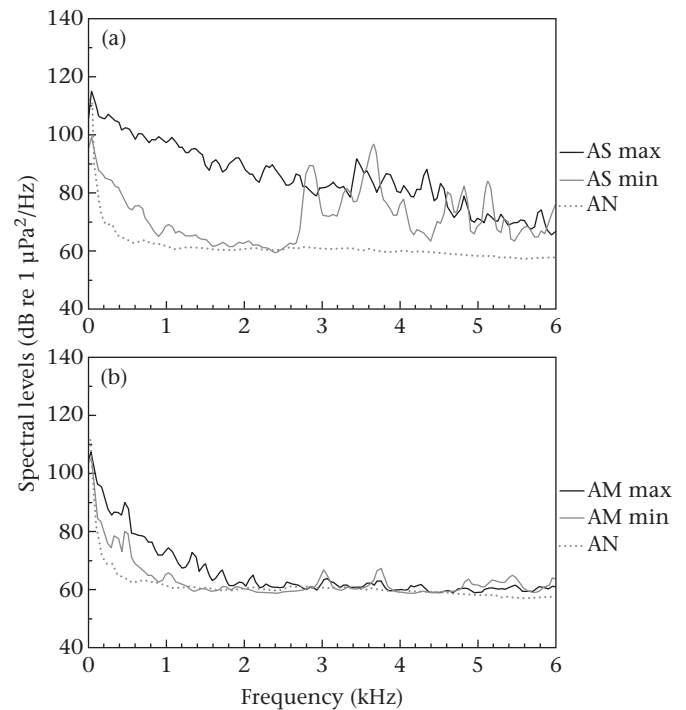
Figure 1. Average spectral levels of ambient sound from 30 s recordings of (a) three stickleback (S) and (b) four minnow (M) holding tanks (fast Fourier transformation FFT, spectrum level units normalized to 1 Hz, Hann, FFT length 1024, 50% overlap). Recordings were made at the loudest (next to the water inflow, 5 cm above the tank floor; AS max, AM max) and at the quietest (in the tank centre, 5 cm below the water surface; AS min, AM min) locations. Spectral levels of an example ambient condition in Gravesend, U.K. (AN) are included for comparison.

limitations currently prevent us from accurately measuring this component of the sound field in small tanks. Thus sound levels are given throughout in terms of sound pressure only. The aim of this study was not, however, to establish absolute values and sensitivity to the two components of sound, but rather explore behaviours that can potentially be affected by the addition of noise to the environment (see also Bruintjes & Radford, 2013; Purser & Radford, 2011; Wale et al., 2013a, 2013b) and to compare the responses of different species to the same potential disruption.

Preparation and Use of Playback Tracks

Recordings from seven different cargo ships at three different harbours (Plymouth, Portsmouth and Gravesend) were used to create playback tracks of additional noise (as per Wale et al., 2013a, 2013b). Recordings were made between 23 and 27 March 2010 between 0600 and 1800 hours, while ships were moving at a constant speed (up to 10 knots) according to port regulations. Ships varied in size (74–286 m length; 10–40 m breadth), unloaded weight (535–80 455 tonnes) and in their construction years (1975–2008). Ship recordings were made using the same hydrophone and solid-state recorder set-up as described above. The hydrophone was positioned at a depth of 1 m, 20 m from the shore with ships passing at a distance of 100–200 m at Plymouth (50°21′33.31″N, 4°7′25.57″W) and Portsmouth (50°47′20.82″N, 1°6′25.39″W), and 20–40 m from the shore with ships passing at a distance of 200–400 m at Gravesend (51°26′43.60″N, 0°22′0.07″E). Weather conditions during recordings ranged from 0 to 1 on the Douglas scale of sea state, with a still to moderate wind speed. No recordings during rainfall were used to form playback tracks.

Each recording was high-pass filtered using Avisoft-SASLab Pro v4.53 (Avisoft Bioacoustics) at 0.1 kHz, to play sounds only within the effective frequency range of the underwater loudspeaker. For minnows, frequencies above 5 kHz were also filtered out to minimize the build-up of sound resonances in the tank within the likely hearing range of the species. Each original recording was looped to form a continuous file of 5 min; each playback track contained noise generated from the passing of only one ship. The amplitudes of playback tracks were adjusted to spectral levels 5–10 dB re 1 μPa^2/Hz below the maximum levels in original ship noise recordings, such that they did not exceed those occurring naturally, at least in the sound pressure domain. Playback tracks made from different original recordings were also adjusted so that they all fell within approximately 5 dB re 1 μPa^2/Hz of one another. For control conditions, 5 min of silence was created in Avisoft-SASLab Pro v4.53.

During experimental trials, sounds were played back as wav files through an Aqua30 underwater loudspeaker (DNH; effective frequency range 80–20 000 Hz) using an amplifier (Kemo Electronic GmbH; 18 W; frequency response range: about 40–20 000 Hz), potentiometer (set to minimum resistance; Omeg Ltd; 10k logarithmic) and either an MP3 player (Logik 2GMP309, frequency range 20–20 000 Hz), for stickleback experiments, or a Toshiba laptop computer (TECRA M11-17V) and an audio interface (Focusrite Saffire Pro 14, High Wycombe, U.K.) for minnow trials. Noise and silence control tracks were played alternately and their order counterbalanced between days. Each playback track of additional noise was allocated to two or three different trials, with a maximum of once per day, and played at different times of day between trial days.

Experiments were conducted in an unfiltered 10-litre plastic tank (34 × 20 cm; water depth: 16 cm; wall thickness: 2 mm); the loudspeaker was positioned behind an opaque partition (width: 4 mm) 15.5 cm from one end wall of the tank. The test tank was placed on a 5 cm polystyrene board on a shelf along a laboratory side wall in a room separated from the main university building (see sound-reducing reasons given above). Acoustic conditions during playbacks were measured in the two feeding areas in the test tank (located 5 cm from the two test tank corners opposite the opaque tank partition; see next section for further details) at two tank depths (5 cm above tank floor and 5 cm below water surface) using the same recording equipment described above. For control conditions, spectral levels from 30 s recordings were calculated in Avisoft using an FFT analysis (spectrum level units normalized to 1 Hz, Hann window, FFT length 1024, 50% overlap) and averaged over the two feeding locations and measurement positions (Fig. 2). For playbacks of additional noise, spectral levels over the whole duration of a looped element were taken with a range of 77–84 s to account for power fluctuations within a recording of a moving ship and averaged over all playback samples and the two tank locations (Fig. 2).

Experimental Protocol

Experiments were conducted only when fish in the test tank showed sufficiently settled behaviour, defined as uninterrupted locomotor activity without adverse responses, such as startle responses, hiding or escape attempts, for at least 10 min. Sticklebacks were sufficiently settled for testing within 1 h of being introduced to the test tank for the first time. Minnows, however, required training sessions to overcome initial adverse responses to the test tank. Training sessions followed test procedures (see below), while playing a silent-control track, and took place on consecutive days until they reached the settlement criteria for testing.
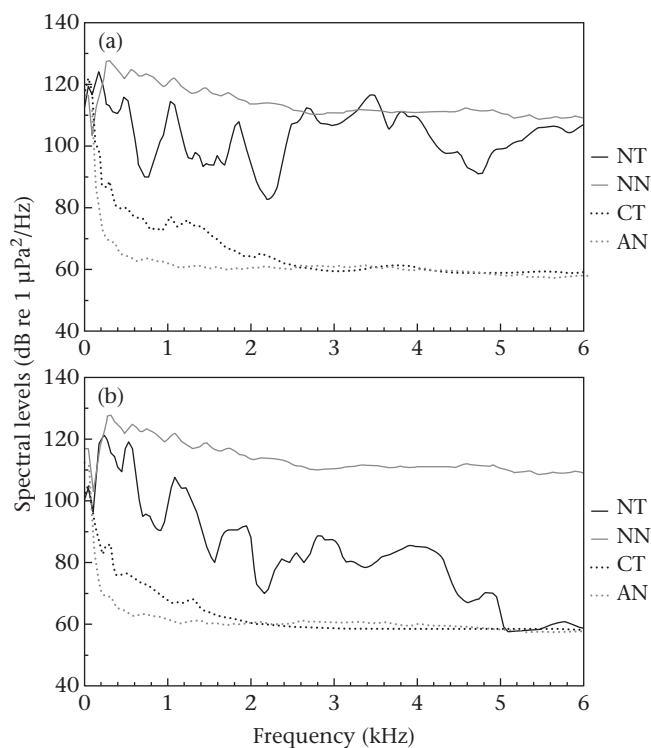
Figure 2. Average spectral levels (FFT analysis, spectrum level units normalized to 1 Hz, Hann, FFT length 1024, 50% overlap) of acoustic conditions at feeding areas in the experimental tank during playback of all additional-noise tracks (NT) and all silent-control tracks (CT). Recordings were made 5 cm above the test tank floor and 5 cm below the water surface. Spectral levels of an example original ship noise recording (NN) and an ambient-noise (AN) recording are provided to illustrate the respective maximum sound levels measured. Playbacks of additional noise were high-pass filtered at 0.1 kHz for (a) sticklebacks and band-pass filtered (0.1–5.0 kHz) for (b) minnows.
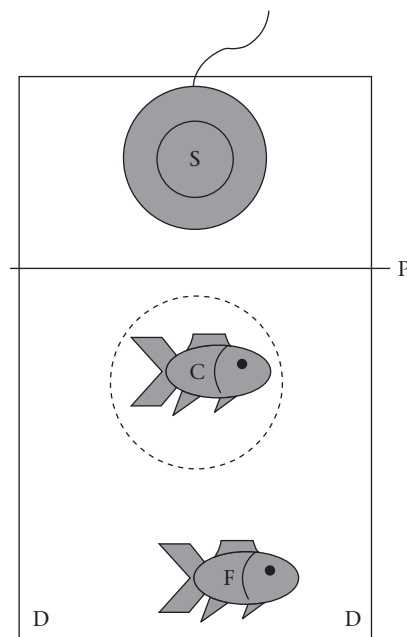
Figure 3. Overhead view of experimental tank set-up. Locations of the companion (C) and focal fish (F), the two feeding sites where single live *Daphnia* were fed alternately (D), and the positions of the loudspeaker (S) and opaque (P) partition are shown. During the experiment, companion sticklebacks were confined by a transparent plastic cylinder and companion minnows by a mesh cylinder (to ensure enough air circulation).

Prior to an experimental trial, a net and an opaque jug were used to transfer a companion/focal fish pair of the same species into the same section of the test tank (the section not containing the loudspeaker; Fig. 3). The companion fish was confined in a transparent cylinder (ca. 7 cm in diameter), while the focal fish was free to swim throughout that tank section (Fig. 3). Fish were left to settle until they resumed swimming and social interaction behaviour without adverse responses, such as hiding, startle responses, freezing or rapid escape attempts, to the presence and movements of the experimenter for 10 min.

Experimental trials for both species followed the protocol in Purser and Radford (2011). Ten seconds prior to the start of a playback, each trial was initiated by transferring a single live *Daphnia magna* to each of the two feeding sites on either side of the tank, using a plastic Pasteur pipette prefilled with several live *Daphnia*. During the 5 min playback, live *Daphnia* were delivered singly at 20 s intervals to the two feeding sites alternately. Between *Daphnia* deliveries, the pipette was lowered behind the opaque tank walls out of sight of the fish. Focal fish received only one trial (either additional noise or silent playback) in an independent-measures design.

Data Collection and Analysis

During each trial, the following data were recorded from the focal fish (definitions as per Purser & Radford, 2011). (1) Feeding behaviour: the number of strikes towards *Daphnia* and nonfood items (directed movement towards the relevant object with a concomitant expanding of the mouth; used as a measure of feeding effort), and the success (consumption, loss or failure to capture) of *Daphnia* strikes. The number of unsuccessful food attempts was calculated as the sum of (a) missed strikes at *Daphnia*, (b) occasions when a caught *Daphnia* was lost, and (c) strikes at nonfood items. The proportion of available *Daphnia* consumed (this varied slightly between trials as the manual delivery sometimes produced two *Daphnia* per event) was also calculated. (2) Stress-related behaviour: the number of startle responses (a sudden movement at high speed) and occasions the focal fish remained inactive (cessation of movements). During pretrial training (see above), it was noticeable that minnows interacted with their companion fish (sticklebacks rarely exhibit this behaviour; Purser & Radford, 2011; personal observation) and so data on instances of social behaviour (swimming behaviours along, or up and down the separating cylinder directed towards the companion fish) were also recorded for minnows.

For most statistical analyses, generalized linear models (GLMs) were used with noise treatment (silent, additional noise) and fish species (minnows, sticklebacks) as fixed factors (R version 2.15.2, core 'stats' package, 'lm' function, R Development Core Team, 2012). A quasibinomial distribution (data overdispersion) was used for the proportion of *Daphnia* eaten; a negative binomial distribution (data overdispersion) was used for the total number of feeding attempts and the number of unsuccessful attempts; a Poisson distribution was used for the number of startle responses; and a binomial distribution was used for occurrence or not of inactive behaviour. Stepwise backwards model simplification was used to identify the significance of model components (interaction: noise treatment*species; main effects: noise treatment, species) based on ANOVA likelihood ratio tests (LRT), except for models with quasibinomial error distributions, for which ANOVA *F* tests were used (R version 2.15.2, core 'stats' package, 'drop1' function). Wherever significant interactions or nonsignificant interaction trends ($P < 0.1$) were found, planned (a priori) post hoc comparisons of silent versus additional-noise effects were conducted for each species separately using permutation tests of independence (R version 2.15.2, 'coin' package, 'independence test' function with exact probability calculations, R package version 1.0-22; Hothorn, Hornik, van de Wiel, & Zeileis, 2006, 2008), except for binary inactivity data which were examined post hoc using Fisher's exact tests for 2×2 data for each species separately (R version 2.15.2, core 'stats' package, 'fisher.test' function). Since data for social interactions were only available for minnows, a two-sample *t* test was used to analyse the square root-transformed number of social interactions ($\mathrm{sqrt}(x + 0.5)$, to account for zeroes) exhibited in the two noise treatments.

RESULTS

Feeding Behaviour

The proportion of *Daphnia* eaten was significantly affected by noise treatment (GLM: $F_{1,54} = 7.09$, $P = 0.010$), with both species showing a similar decrease in food consumption during additional-noise playback compared to silent controls (nonsignificant interaction term: $F_{1,53} = 0.73$, $P = 0.397$; Fig. 4a). There was a strong tendency for minnows (mean + SE = 0.74 + 0.06, $N = 28$) to consume a lower proportion of *Daphnia* than sticklebacks (mean + SE = 0.86 + 0.03, $N = 29$; $F_{1,54} = 3.95$, $P = 0.052$).

The total number of strikes (against both food and nonfood items) was significantly affected by the interaction between
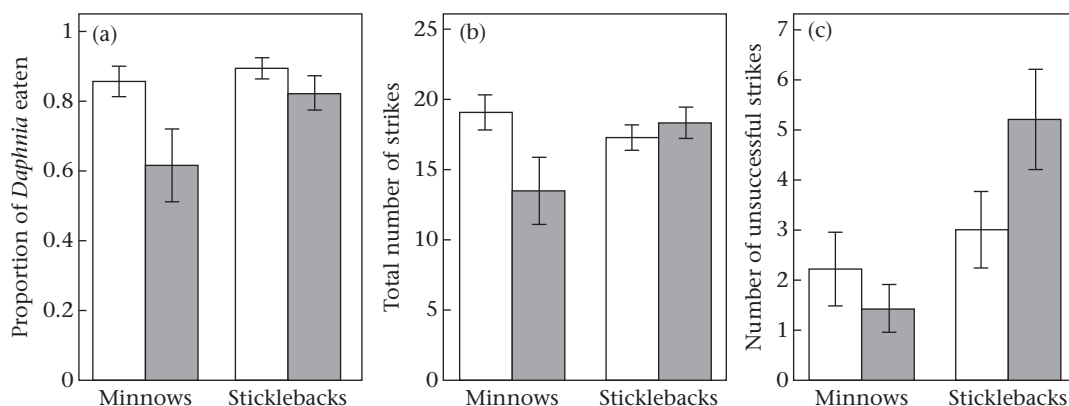
Figure 4. Mean ± SE (a) proportion of *Daphnia* eaten, (b) total number of strikes (against food and nonfood items), and (c) total number of unsuccessful strikes (*Daphnia* lost or missed after a strike or strikes towards nonfood items) by feeding minnows and sticklebacks during 5 min silent (white) and 5 min additional-noise (grey) playbacks. $N = 14$ minnows during both playback treatments, 14 sticklebacks during silent playback and 15 sticklebacks during additional-noise playback in an independent-measures design.

species and noise treatment (GLM: $LRT_{1,53} = 4.00$, $P = 0.045$; species: $LRT_{1,54} = 0.91$, $P = 0.340$; noise treatment: $LRT_{1,54} = 1.63$, $P = 0.201$). Post hoc comparisons revealed a tendency in minnows to make fewer strikes during additional-noise playbacks than in silent-control conditions (independence test: $P = 0.073$; Fig. 4b), whereas noise treatment did not significantly affect sticklebacks in this regard ($P = 0.278$).

The number of unsuccessful strikes (*Daphnia* lost or missed after a strike and strikes against nonfood items) was also affected, although not quite significantly, by the interaction between species and noise treatment (GLM: $LRT_{1,53} = 3.49$, $P = 0.062$; species: $LRT_{1,54} = 8.50$, $P = 0.004$; noise treatment: $LRT_{1,54} = 0.24$, $P = 0.623$). Post hoc comparisons revealed a tendency in sticklebacks to make more unsuccessful strikes during additional-noise playbacks than in silent-control conditions (independence test: $P = 0.066$; Fig. 4c), whereas noise treatment did not significantly affect minnows in this regard ($P = 0.554$). Strikes against nonfood items constituted the majority of unsuccessful strikes observed in sticklebacks (mean + SE overall: $80.8 + 6.3\%$, $N = 29$; control playback: $77.1 + 11.3\%$, $N = 14$; additional-noise playback: $84.5 + 5.8\%$, $N = 15$).

Stress-related and Social Behaviour

The number of startle responses was significantly affected by noise treatment (GLM: $LRT_{1,54} = 24.08$, $P < 0.0001$), with both species showing a similar increase during playback of additional noise compared to silent controls (nonsignificant interaction term: $LRT_{1,53} = 0.89$, $P = 0.347$; Fig. 5a). Minnows (mean + SE = $3.71 + 0.48$, $N = 28$) were startled significantly more often than sticklebacks (mean + SE = $1.62 + 0.37$, $N = 29$; $LRT_{1,54} = 24.91$, $P < 0.0001$).

Inactive behaviour was significantly affected by the interaction between species and noise treatment (GLM: $LRT_{1,53} = 3.91$, $P = 0.048$; species: $LRT_{1,54} = 21.12$, $P < 0.0001$; noise treatment: $LRT_{1,54} = 1.59$, $P = 0.208$; Fig. 5b). Post hoc comparisons revealed a tendency for more minnows to show inactivity during additional-noise playbacks than in silent-control conditions (Fisher's exact test: odds ratio = 8.99, $P = 0.077$; Fig. 5b), whereas noise treatment did not significantly affect sticklebacks (odds ratio = 0.58, $P = 0.651$).

Minnows tended to interact socially more often with their companion fish during additional-noise playbacks than during silent controls (two-sample *t* test: $t_{26} = -1.86$, P (exact) = 0.075; Fig. 5c).

DISCUSSION

During exposure to the playback of additional noise, both sticklebacks and minnows consumed fewer *Daphnia* and were startled more often than during control conditions. However, potential reasons for the reduced food consumption differed between the species: sticklebacks tended to make more foraging errors during playback of additional noise than control conditions, whereas minnows showed decreased foraging effort, tending to spend more time inactive or interacting socially and making fewer foraging strikes when exposed to playback of additional noise compared to silent-control trials. Our findings that increased noise levels can disrupt fish foraging behaviour support those of Purser and Radford (2011) and Bracciali, Campobello, Giacoma, and Sarà (2012), but the current work also indicates that different species can be affected in different ways by the same noise source.

Sustained decreases in food consumption could have long-term energetic impacts if compensation, by increasing food intake during less noisy time periods (Bracciali et al., 2012) or by allocating more overall time to foraging behaviour, is incomplete or impossible; reductions in growth, survival and breeding success may result. Moreover, compensatory feeding activities could increase predation risk by increasing time exposed to predators or by forcing animals to feed in less favourable conditions, such as in times or areas of higher predation pressure (Lima & Dill, 1990). Directing more strikes towards nonfood items, a component of the unsuccessful foraging strikes made by sticklebacks during playback of additional noise, also carries a potential cost, because there is an increased risk of swallowing poisonous or harmful objects (see also Purser & Radford, 2011).

In theory, the demonstrated effects of elevated noise could result from impacts on the behaviour of the invertebrate prey rather than the fish predators; there is growing evidence that invertebrates are able to detect sound (reviewed in Stocker, 2002) and that they are susceptible to anthropogenic noise (Wale et al., 2013a, 2013b). The *Daphnia* could, for instance, have become more alert, resulting in reduced catch rates. If it was prey activity but not predator activity that was driving the demonstrated effects, we would have expected to see fish missing or losing *Daphnia* more often during playback of additional noise than in silent-control conditions, whereas predator motivation (indicated by total number of strikes) would have remained unchanged or increased to compensate. However, even though sticklebacks made more foraging errors during playback of additional noise, these were mostly discrimination errors in which attacks were directed
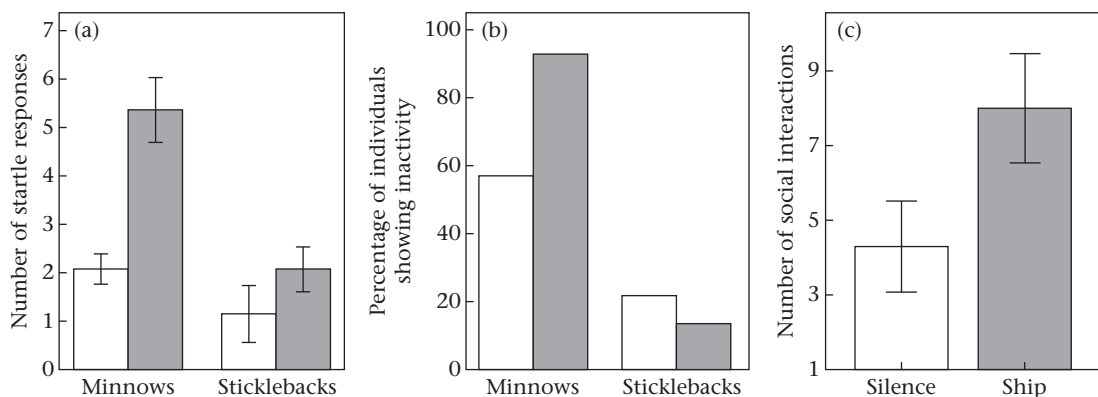
Figure 5. Mean ± SE (a) number of startle responses and (b) percentage of individuals showing any inactivity (hiding or stopping movements) during trials of silent (white) or additional-noise (grey) playbacks. $N = 14$ minnows during both playback treatments, 14 sticklebacks during silent playback and 15 sticklebacks during additional-noise playback in an independent-measures design. (c) Number of social interactions with companion fish by 14 minnows during both playback treatments in an independent-measures design.

towards nonfood items instead of *Daphnia*, rather than handling errors which might be expected if prey behaviour had changed (see also Purser & Radford, 2011), and minnows tended to decrease total number of attacks in favour of other, nonforaging behaviours. It seems likely, therefore, that our results are driven by the impacts of noise on the fish themselves.

Exposure to elevated noise levels could impair foraging behaviour in three, not mutually exclusive, ways: by triggering a stress- or fear-related response, by acting as a distraction or by masking acoustic information. The reduced foraging strikes and increased inactivity in minnows, and the increased startle responses in both species, are consistent with a classic defence cascade where an ongoing activity, such as foraging, is interrupted; such a response is typically associated with stressors and risky fear-inducing stimuli such as predators (Metcalfe, Huntingford, & Thorpe, 1987). Similarly, the decreased number of strikes in minnows is consistent with the reduction in appetite expected as a classic component of a physiological stress response (Wendelaar Bonga, 1997). Stress or fear under conditions of additional noise may also explain the increased association with companions in minnows, since social proximity offers defensive advantages and social support (e.g. Magurran, 1990). Stress may also impair cognitive performance (Mendl, 1999) and the ability to focus on a task (De Kloet et al., 1999), which might underpin the increase in foraging errors and increased startle responses (to a previously ignored stimulus) seen in sticklebacks. An increase in errors could also arise from a shift in attention by the sticklebacks if they were distracted by the playback of additional noise, thus preventing them focusing fully on the foraging task within the limits of their attention capacity (Chan & Blumstein, 2011). In addition, it is possible that the movement of the *Daphnia* provides some inadvertent acoustic cues used by the fish during foraging, and that these were masked by the additional noise, altering the detection of the prey. Further studies are needed to tease apart these potential underlying mechanisms. Nevertheless, it is apparent that minnows showed a qualitative shift in activity away from foraging behaviour under conditions of additional noise, consistent with a classic stress- or fear-related defence cascade, whereas sticklebacks maintained foraging effort but made more mistakes and startle responses, which may result from an impact of noise on cognition to the detriment of foraging performance.

Interspecific differences in antipredator strategies could underpin the different effects of noise exposure on behavioural responses and thus the different reasons for the reduced food consumption shown by sticklebacks and minnows. For instance, the lack of body armour in minnows could explain their increased tendency towards defensive and stress-related behaviour compared to armoured sticklebacks when responding to potentially threatening stimuli; sticklebacks may be adapted to remain in dangerous feeding locations for longer and more often, to flee at shorter distances and to hide less than minnows (Abrahams, 1995; Krause et al., 2000; McLean & Godin, 1989). Just as unarmoured fathead minnows, *Pimephales promelas*, have been shown to form closer associations with conspecifics in response to predator cues (Magurran & Pitcher, 1987), the increase in social interactions with companion fish shown by European minnows in our experiment may be indicative of their treating the playback of additional noise as a stimulus associated with potential danger. Any interspecific differences in hearing thresholds could also, in theory, help explain the differences in responses shown between the study species. Minnows have been shown to respond behaviourally to tones of up to 5000 Hz (Dijkgraaf & Verheijen, 1950), but there has been no assessment of the hearing of three-spined sticklebacks that we know about, and the measurements of the closely related nine-spined stickleback,

Pungitius pungitius, documenting electrophysiological responses up to 1600 Hz (Mann, Cott, Hanna, & Popper, 2007), were made using the auditory evoked potential technique which is now questioned (Fay & Popper, 2012). As such, while there are hints that minnows might have more sensitive hearing than sticklebacks, detailed and accurate comparative assessments of hearing thresholds are required before strong conclusions can be drawn in this regard.

The likely impacts of noise-induced behavioural effects may also be species specific, owing to the differences in underlying mechanisms. Sticklebacks, for example, did not seem to reduce feeding effort, and thus may be more likely to compensate by increasing feeding activity during less noisy conditions. The potential to resume foraging quickly after noise exposure and to compensate for missed foraging opportunities, as well as the possibility to take refuge from elevated noise levels, may play an important role in the resilience of species to impacts of noise on foraging performance (Magoulick & Kobza, 2003). However, the fact that sticklebacks showed more strikes against nonfood items may elevate the risk of consuming potentially poisonous or harmful items, with detrimental consequences for which there is no compensation possible.

Highly controlled laboratory studies are an important tool in our attempts to shed light on how acoustic noise affects organisms; they often allow more detailed and accurate data collection than field-based studies (Slabbekoorn, in press). However, care must be taken when extrapolating findings to real-world situations, because captive conditions represent a highly simplified and artificial environment. From a biological perspective, captive animals are usually more constrained than in the wild and individuals are receiving husbandry regimes that differ from natural conditions of resource availability. From an acoustics perspective, playbacks cannot fully replicate natural sound sources, partly because the speaker does not have a linear response and thus changes the spectral quality of the sounds played, and partly because the sound field in a tank is complex and results in a different balance between the sound pressure and particle velocity components of sound (Akamatsu, Okumura, Novarini, & Yan, 2002; Parvulescu, 1967). In addition, most animals in the wild will experience repeated and/or chronic exposure to noise, which could lead to changes in response as a consequence of such processes as habituation, sensitization and tolerance (Bejder, Samuels, Whitehead, Finn, & Allen, 2009; Wale et al., 2013b). Future studies utilizing real anthropogenic noise sources need to ascertain the scale of impact in natural conditions, and the implications of interspecific differences in the effect of anthropogenic noise for individual fitness and community structure; community interactions may be affected when sympatric species, particularly those occupying overlapping niches, are differentially affected by an introduced noise. For now, our study indicates that increased noise can potentially have a negative impact on foraging behaviour and food consumption, and that the risks and consequences of increased noise levels, as well as the potential for compensatory mechanisms, may differ between species.

References

Abrahams, M. V. (1995). The interaction between antipredator behaviour and antipredator morphology: experiments with fathead minnows and brook sticklebacks. *Canadian Journal of Zoology, 73*, 2209–2215.

Akamatsu, T., Okumura, T., Novarini, N., & Yan, H. Y. (2002). Empirical refinements applicable to the recording of fish sounds in small tanks. *The Journal of the Acoustical Society of America, 112*, 3073–3082.

Bejder, L., Samuels, A., Whitehead, H., Finn, H., & Allen, S. (2009). Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli. *Marine Ecology Progress Series, 395*, 177–185.

Blickley, J. L., & Patricelli, G. L. (2010). Impacts of anthropogenic noise on wildlife: research priorities for the development of standards and mitigation. *Journal of International Wildlife Law & Policy, 13*, 274–292.

Blumstein, D. T., & Fernández-Juricic, E. (2010). *A primer of conservation behavior.* Sunderland, MA: Sinauer Associates.

Bracciali, C., Campobello, D., Giacoma, C., & Sarà, G. (2012). Effects of nautical traffic and noise on foraging patterns of Mediterranean damselfish (*Chromis chromis*). *PLoS One, 7*, e40582.

Bruintjes, R., & Radford, A. N. (2013). Context-dependent impacts of anthropogenic noise on individual and social behaviour in a cooperatively breeding fish. *Animal Behaviour, 85*, 1343–1349.

Brumm, H., & Slabbekoorn, H. (2005). Acoustic communication in noise. *Advances in the Study of Behavior, 35*, 151–209.

Candolin, U., & Wong, B. B. M. (2012). *Behavioural responses to a changing world.* Oxford, U.K.: Oxford University Press.

Chan, A. A. Y.-H., & Blumstein, D. T. (2011). Attention, noise, and implications for wildlife conservation and management. *Applied Animal Behaviour Science, 131*, 1–7.

Charmandari, E., Tsigos, C., & Chrousos, G. (2005). Endocrinology of the stress response. *Annual Review of Physiology, 67*, 259–284.

Codarin, A., Wysocki, L. E., Ladich, F., & Picciulin, M. (2009). Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy). *Marine Pollution Bulletin, 58*, 1880–1887.

De Kloet, E. R., Oitzl, M. S., & Joëls, M. (1999). Stress and cognition: are corticosteroids good or bad guys? *Trends in Neurosciences, 22*, 422–426.

Dijkgraaf, S., & Verheijen, F. J. (1950). Neue Versuche über das Tonunterscheidungsvermögen der Elritze [New experiments on the ability of minnows to discriminate between tones]. *Zeitschrift für Vergleichende Physiologie, 32*, 248–256.

Fay, R. R., & Popper, A. N. (2012). Fish hearing: new perspectives from two 'senior' bioacousticians. *Brain, Behavior and Evolution, 79*, 215–217.

Fay, R. R., Popper, A. N., & Webb, J. F. (2008). Introduction to fish bioacoustics. In J. F. Webb, R. R. Fay, & A. N. Popper (Eds.), *Fish bioacoustics* (pp. 1–15). New York: Springer.

Francis, C. D., Ortega, C. P., & Cruz, A. (2011a). Different behavioural responses to anthropogenic noise by two closely related passerine birds. *Biology Letters, 7*, 850–852.

Francis, C. D., Ortega, C. P., & Cruz, A. (2011b). Vocal frequency change reflects different responses to anthropogenic noise in two suboscine tyrant flycatchers. *Proceedings of the Royal Society B, 278*, 2025–2031.

Froese, R., & Pauly, D. (2011). *Fishbase.* Retrieved from: www.fishbase.org.

Galef, B. G., Jr., & Giraldeau, L.-A. (2001). Social influences on foraging in vertebrates: causal mechanisms and adaptive functions. *Animal Behaviour, 61*, 3–15.

Hawkins, A. D., & Popper, A. N. (2012). *Effects of noise on fish, fisheries, and invertebrates in the U.S. Atlantic and Arctic from energy industry sound-generating activities. (US Department of the Interior, Bureau of Ocean Energy Management, Trans.).* Bedford, NH: Normandeau Associates, Inc.

Hofer, H., & East, M. L. (1998). Biological conservation and stress. *Advances in the Study of Behavior, 27*, 405–525.

Hoogland, R., Morris, D., & Tinbergen, N. (1957). The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as means of defence against predators (*Perca* and *Esox*). *Behaviour, 10*, 205–236.

Hothorn, T., Hornik, K., van de Wiel, M. A., & Zeileis, A. (2006). A lego system for conditional inference. *The American Statistician, 60*, 257–263.

Hothorn, T., Hornik, K., van de Wiel, M. A., & Zeileis, A. (2008). Implementing a class of permutation tests: the coin package. *Journal of Statistical Software, 28*, 1–23.

International Chamber of Shipping (2013). Retrieved from: www.ics-shipping.org.

Joint Nature Conservation Committee and Centre for Ecology and Hydrology. (2011). *National Biodiversity Networks Gateway Interactive Map.* Retrieved from: www.data.nbn.org.uk.

Kastelein, R. A., van der Heul, S., Verboom, W. C., Jennings, N., van der Veen, J., & de Haan, D. (2008). Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz. *Marine Environmental Research, 65*, 369–377.

Krause, J., Cheng, D. J. S., Kirkman, E., & Ruxton, G. D. (2000). Species-specific patterns of refuge use in fish: the role of metabolic expenditure and body length. *Behaviour, 137*, 1113–1127.

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology, 68*, 619–640.

Lupien, S. J., & McEwen, B. S. (1997). The acute effects of corticosteroids on cognition: integration of animal and human model studies. *Brain Research Reviews, 24*, 1–27.

Magoulick, D. D., & Kobza, R. M. (2003). The role of refugia for fishes during drought: a review and synthesis. *Freshwater Biology, 48*, 1186–1198.

Magurran, A. E. (1990). The adaptive significance of schooling as an anti-predator defense in fish. *Annales Zoologici Fennici, 27*, 51–66.

Magurran, A. E., & Pitcher, T. J. (1987). Provenance, shoal size and the sociobiology of predator-evasion behaviour in minnow shoals. *Proceedings of the Royal Society B, 229*(1257), 439–465.

Mann, D. A., Cott, P. A., Hanna, B. W., & Popper, A. N. (2007). Hearing in eight species of northern Canadian freshwater fishes. *Journal of Fish Biology, 70*, 109–120.

Mathis, A., & Chivers, D. P. (2003). Overriding the oddity effect in mixed-species aggregations: group choice by armored and nonarmored prey. *Behavioral Ecology, 14*, 334–339.

McLean, E. B., & Godin, J.-G. J. (1989). Distance to cover and fleeing from predators in fish with different amounts of defensive armour. *Oikos, 55*, 281–290.

Mendl, M. (1999). Performing under pressure: stress and cognitive function. *Applied Animal Behaviour Science, 65*, 221–244.

Metcalfe, N. B., Huntingford, F. A., & Thorpe, J. E. (1987). The influence of predation risk on the feeding motivation and foraging strategy of juvenile Atlantic salmon. *Animal Behaviour, 35*, 901–911.

Morley, E. L., Jones, G., & Radford, A. N. (2014). The importance of invertebrates when considering the impacts of anthropogenic noise. *Proceedings of the Royal Society B, 81*, 20132683.

Parvulescu, A. (1967). The acoustics of small tanks. In W. N. Tavolga (Ed.), *Marine bioacoustics* (pp. 7–14). Oxford, U.K.: Pergamon Press.

Popper, A. N., & Fay, R. R. (2011). Rethinking sound detection by fishes. *Hearing Research, 273*, 25–36.

Popper, A. N., Fay, R. R., Platt, C., & Sand, O. (2003). Sound detection mechanisms and capabilities of teleost fishes. In S. P. Collin, & N. J. Marshall (Eds.), *Sensory processing in aquatic environments* (pp. 3–38). New York: Springer.

Popper, A. N., & Hastings, M. C. (2009). The effects of anthropogenic sources of sound on fishes. *Journal of Fish Biology, 75*, 455–489.

Pottinger, T. G. (2010). A multivariate comparison of the stress response in three salmonid and three cyprinid species: evidence for inter-family differences. *Journal of Fish Biology, 76*, 601–621.

Purser, J., & Radford, A. N. (2011). Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). *PLoS One, 6*, e17478.

Quinn, J. L., Whittingham, M. J., Butler, S. J., & Cresswell, W. (2006). Noise, predation risk compensation and vigilance in the chaffinch *Fringilla coelebs. Journal of Avian Biology, 37*, 601–608.

Radford, A. N., Kerridge, E., & Simpson, S. D. (2014). Acoustic communication in a noisy world: can fish compete with anthropogenic noise? Manuscript submitted for publication.

R Development Core Team. (2012). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. Retrieved from: http://www.R-project.org.

Ríos-Chelén, A. A., Salaberria, C., Barbosa, I., Macías Garcia, C., & Gil, D. (2012). The learning advantage: bird species that learn their song show a tighter adjustment of song to noisy environments than those that do not learn. *Journal of Evolutionary Biology, 25*, 2171–2180.

Schaub, A., Ostwald, J., & Siemers, B. M. (2008). Foraging bats avoid noise. *Journal of Experimental Biology, 211*, 3174–3180.

Siemers, B. M., & Schaub, A. (2011). Hunting at the highway: traffic noise reduces foraging efficiency in acoustic predators. *Proceedings of the Royal Society B, 278*, 1646–1652.

Slabbekoorn, H. (2014). Aiming for progress in understanding underwater noise impact on fish: complementary need for indoor and outdoor studies. In A. N. Popper, & T. A. Hawkins (Eds.), *Effects of noise on aquatic life II.* New York: Springer. in press.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., & Popper, A. N. (2010). A noisy spring: the impact of globally rising underwater sound levels on fish. *Trends in Ecology & Evolution, 25*, 419–427.

Stephens, D. W., Brown, J. S., & Ydenberg, R. C. (2007). *Foraging: Behavior and ecology.* Chicago: University of Chicago Press.

Stocker, M. (2002). Fish, mollusks and other sea animals' use of sound, and the impact of anthropogenic noise in the marine acoustic environment. *Journal of the Acoustical Society of America, 112*, 2431.

Voellmy, I. K., Simpson, S. D., Purser, J., & Radford, A. N. (2014). Prior acoustic exposure affects fish responses to increased noise levels. Manuscript in preparation.

Wale, M. A., Simpson, S. D., & Radford, A. N. (2013a). Noise negatively affects foraging and antipredator behaviour in shore crabs. *Animal Behaviour, 86*, 111–118.

Wale, M. A., Simpson, S. D., & Radford, A. N. (2013b). Size-dependent physiological responses of shore crabs to single and repeated playback of ship noise. *Biology Letters, 9*, 20121194.

Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews, 77*, 591–625.

Wright, A. J., Soto, N. A., Baldwin, A. L., Bateson, M., Beale, C. M., Clark, C., et al. (2007). Anthropogenic noise as a stressor in animals: a multidisciplinary perspective. *International Journal of Comparative Psychology, 20*, 250–273.