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Repeated exposure to noise increases tolerance in a coral reef fish[☆]

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ABSTRACT

Some anthropogenic noise is now considered pollution, with evidence building that noise from human activities such as transportation, construction and exploration can impact behaviour and physiology in a broad range of taxa. However, relatively little research has considered the effects of repeated or chronic noise; extended exposures may result in habituation or sensitisation, and thus changes in response. We conducted a field-based experiment at Moorea Island to investigate how repeated exposure to playback of motorboat noise affected a coral reef fish (*Dascyllus trimaculatus*). We found that juvenile *D. trimaculatus* increased hiding behaviour during motorboat noise after two days of repeated exposure, but no longer did so after one and two weeks of exposure. We also found that naïve individuals responded to playback of motorboat noise with elevated ventilation rates, but that this response was diminished after one and two weeks of repeated exposure. We found no strong evidence that baseline blood cortisol levels, growth or body condition were affected by three weeks of repeated motorboat-noise playback. Our study reveals the importance of considering how tolerance levels may change over time, rather than simply extrapolating from results of short-term studies, if we are to make decisions about regulation and mitigation.

1. Introduction

Some anthropogenic noise is now considered a global pollutant. As well as featuring in national and international legislation (e.g. the European Commission Marine Strategy Framework Directive and the United States National Environmental Policy Act), mounting evidence shows that anthropogenic noise can impact behaviour (e.g. vocal communication, anti-predator defence, foraging) and physiology (e.g. ventilation rate, metabolic rate, heart rate) in at least some species from a broad range of taxa (Shannon et al., 2015; Morley et al., 2014; Slabbekoorn et al., 2010). However, response variables in the majority of experimental studies are only measured once and only after relatively short-term noise exposure (e.g. (McLaughlin and Kunc, 2013; Simpson et al., 2015). There is some evidence that on-going exposure to anthropogenic noise can impact animals (Barber et al., 2010; Crino et al., 2013; Wale et al., 2013), yet there are few experimental studies that investigate how responses may change over time (for an exception, see (Wale et al., 2013)). This is an important consideration in the context of regulation, because human disturbance of natural habitats is becoming more frequent and the pervasive nature of anthropogenic noise means that animals are likely to be exposed multiple times during their lifetime.

Research in other fields reveals that animal responses to various stimuli can change over time with repeat exposures (Bejder et al., 2009). Responses may be heightened (reduced tolerance), one explanation for which could be sensitisation (Richardson et al., 1995). For example, yellow-eyed penguins (*Megadyptes antipodes*) from areas of greater human disturbance show higher baseline corticosterone levels than those from less disturbed areas (Ellenberg et al., 2007). Alternatively, responses could be attenuated (increased tolerance), one explanation for which could be habituation (Thorpe, 1963). For example, male white-crowned sparrows (*Zonotrichia leucophrys*) in breeding pairs showed decreases in several behavioural responses (song and flight) with repetition of playbacks of conspecifics (Petrinovich and Patterson,

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1979). If animals continue to respond to stimuli, they could become chronically stressed (Cyr and Romero, 2009), with potential downstream effects on growth and condition (Anderson et al., 2011). If an animal habituates fully to a stressor, baseline cortisol concentration, behaviour and health will be the same as unstressed animals (Cyr and Romero, 2009). Experimental data with repeat measures from the same individuals over time are lacking in field studies of anthropogenic noise, so whether animals are able to habituate to this stressor is unknown.

We used a field-based experiment on a coral reef fish to investigate the effects of repeated exposure to playback of motorboat noise over three weeks. Fish are socio-economically important, yet many species are vulnerable to anthropogenic pressures such as overfishing and ocean acidification (Harley et al., 2006; Simpson et al., 2011). Moreover, wherever humans inhabit coastal waters, including coral reefs, small boats provide a ubiquitous source of anthropogenic disturbance, including generation of additional noise (Whitfield and Becker, 2014). All fish detect sound, often possessing specialised auditory apparatus, and are exposed to underwater noise across the globe (Bleckmann, 2004; Popper, 2003). There is increasing evidence that at least some fish species can be affected by anthropogenic noise, including behavioural changes such as foraging, nest caring and predator avoidance (e.g. (Bruintjes and Radford, 2013; Picciulin et al., 2010; Simpson et al., 2015)), physiological changes such as increases in plasma cortisol concentrations, oxygen consumption and ventilation (opercular beat rate) (e.g. (Debusschere et al., 2016; Simpson et al., 2015; Wysocki et al., 2006)), and fitness consequences (Simpson et al., 2016). However, the majority of studies on the impacts of noise have focused on short-term responses. The few that have conducted longer term experiments have been conducted in tanks (Anderson et al., 2011; Bruintjes and Radford, 2014; Davidson et al., 2009; Filiciotto et al., 2013; Nedelec et al., 2015). Tanks offer certain benefits, including greater control over environmental variables such as extraneous noise, temperature and water quality, the acoustics of small tanks mean that relevant sound exposure levels are very difficult to measure and control (Parvelescu, 1967). However, field studies offer greater ecological relevance.

In this study, we exposed juvenile coral reef fish in their natural habitat to playbacks of motorboat noise. *Dascyllus trimaculatus* is a site-attached damselfish which is easily observed in shallow waters with high visibility (Bernardi et al., 2012). Juvenile *D. trimaculatus* associate closely with anemones, and schools can be relocated successfully to different anemones to create independent experimental units. We relocated 24 schools of *D. trimaculatus* to anemones that surrounded loudspeakers playing either motorboat noise or ambient noise in the lagoon of Moorea, French Polynesia to investigate whether: 1) there was a short-term response to motorboat noise; 2) tolerance of motorboat noise changed over several days of exposure; and 3) repeated exposure to motorboat noise resulted in chronic stress. Specifically, we tested whether hiding behaviour and ventilation rate responses to motorboat-noise playback differed after repeat exposure. We predicted that these responses would be heightened if fish tolerance to playbacks decreased, while these responses would attenuate if tolerance increased. We also measured fish size, condition and baseline plasma cortisol concentrations to test the longer term consequences of any change in tolerance to repeated playback of motorboat noise.

2. Materials and methods

2.1. Ethical approval

Approval was granted from our institutional animal ethics committees, le Centre National de la Recherche Scientifique (CNRS), for sacrificing and subsequently dissecting fish (Permit Number: 006725). *Dascyllus trimaculatus* is not on the endangered species list and no specific authorization was required from the French Polynesian government for collection.

2.1.1. Experimental set-up

Work was conducted from the CRIOBE research station, Moorea, French Polynesia. Juvenile *D. trimaculatus* (threespot dascyllus) were collected using clove oil and hand nets from anemones around the north coast of Moorea and introduced to one of 12 experimental anemones relocated to two sites on a natural sand flat. The two sites were on a sand flat close to the research station, with similar depth (1.3–1.8 m), water turbidity, prevailing currents, and proximity to reef (>10 m) and nearest boat channel (>60 m). Anemones were 20–40 cm in diameter and were attached to dead coral which rested on the sand. Cages surrounding anemones to exclude predators were 50 cm diameter, 1 m high cylinders made from 6 mm-square metal mesh, fixed to the sandy bottom of the lagoon flat using 1 m metal pegs hammered into the sand. Fig. 1 shows a schematic of the layout of the sites used.

Each anemone was 1 m from a loudspeaker (UW-30, frequency response 0.1–10 kHz, University Sound, Columbus OH). Loudspeakers were fixed to the sandy bottom facing upwards by a custom-made mount pegged into the sand. Loudspeakers were used to play one of two sound treatments (Ambient or Boat; as per (Nedelec et al., 2014)). Original recordings for use in playback tracks were as in (Nedelec et al., 2014). We made boat recordings during the day (on 4/11/2010 and 5/11/10) at 2 m depth in a deep bay in the lagoon on the east coast of Moorea using a hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier; sensitivity 165 dB re 1 V/mPa; frequency range 2 Hz–30 kHz; High Tech Inc., Gulfport MS) and a solid-state recorder (Edirol R-09HR 16-bit recorder; sampling rate 44.1 kHz; Roland Systems Group, Bellingham WA). The recorder was fully calibrated using pure sine wave signals generated in SAS Lab (Avisoft, Germany), played on an mp3 player, measured in line with an oscilloscope. To reduce pseudoreplication of playbacks, we used 36 recordings of two different boats (5 m long aluminium outboard motorboats with 25 horse power Suzuki engines, one boat used per recording) making passes of the hydrophone (boats started 50 m from the hydrophone and drove past in a straight line for 100 m; passing the hydrophone at a closest distance of 10 m), and 12 recordings of ambient noise.

Sound samples were combined and looped into 12 h long

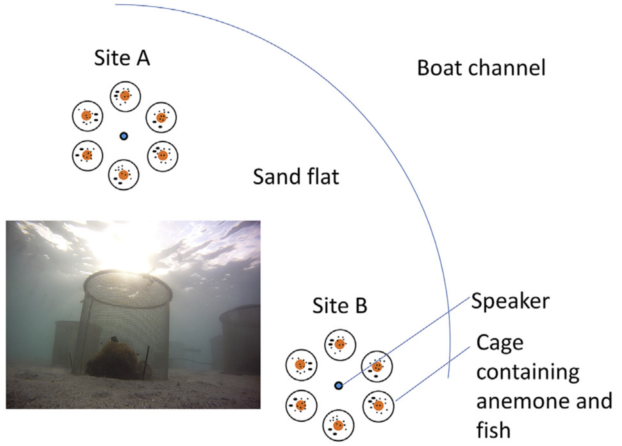

Fig. 1. Schematic diagram (not to scale) showing the layout of the sites used for sound playback and video recording, plus a photo of the cages at one site.

playbacks such that there were two different replicate playbacks for each treatment. Sound pressure levels (measured using the hydrophone set-up described above) and particle acceleration levels (measured using an M30 accelerometer, sensitivity 0–3 kHz, manufactured and calibrated by GeoSpectrum Technologies, Dartmouth, Canada; recorded on a laptop via a USB soundcard, MAYA44, ESI Audiotechnik GmbH, Leonberg, Germany) of playbacks was recorded and compared with recordings of real boats at a nearby location in the lagoon (Figs. 2 and 3). Playbacks were recorded at 1 m from the speaker, at the location of the experiment with experimental apparatus in place. The two experimental sites were 100 m apart and playbacks at one site could not be heard above local ambient noise levels from the other (verified with sound pressure and particle acceleration recordings). Sound travel between the two sites may have been limited due to the sandy bottom with occasional coral heads and coral rubble and the fact that between the two sites there is a large area where the depth reduces to ca. 40 cm, cutting off low frequencies.

All fish received ambient sound from the environment (e.g. from the nearby reef), in addition to that included in the playback of recordings taken from another location. Fish in the Boat treatment also received boat-noise playback for 45 s every 5 min, totaling 144 boat passes per day between the hours of 06:00 and 18:00. Playbacks played throughout the experiment during daylight hours so that sound was already playing when fish were introduced and when they left cages.

D. trimaculatus took shelter in the anemone within seconds of being introduced. Each anemone received a school of 12 fish; 10 fish with standard length 10–20 mm ('focal fish') and two fish with standard length 35–45 mm (for aiding settlement and measuring blood cortisol concentration). The smaller fish were small enough to leave the cage, but the larger fish were not. Thus we included the larger fish for two reasons: firstly because the smaller fish did not leave the cage when the larger fish were present and secondly because the smaller fish were too small to bleed for a blood cortisol measurement. Fish on the same anemone were introduced on the same day; fish on different anemones could be introduced on different days. Allocation of fish to anemones was random within treatment, and cages were filled in an alternating pattern between treatments (Ambient, Boat, Ambient, Boat … etc.) to avoid temporal bias between treatments. During a given experimental replicate, each site was allocated to one of the two sound treatments; two temporal replicates were performed with sound treatment reversed between sites on the second occasion.

2.1.2. *Hiding behaviour*

A video camera (GoPro Hero 2) was placed on the top of each cage to film down through an opening for 20 min on the second day of playback exposure (during the period of four motorboat passes in the Boat treatment), between 15:00 and 18:00. We also filmed for

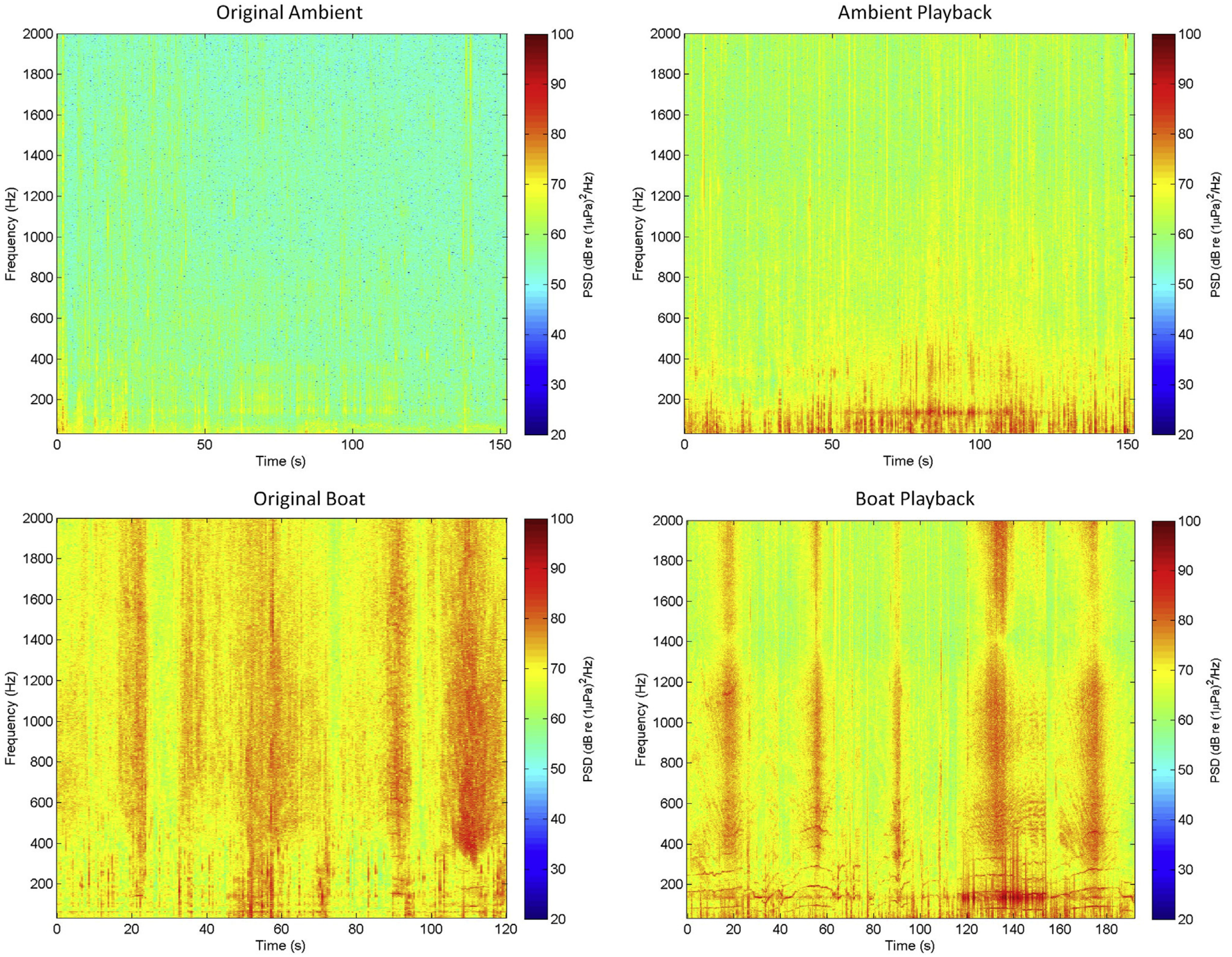

Fig. 2. Spectrograms of noise types for sound pressure. Window length = sample rate = 44.1 kHz, window = Hamming, overlap = 50%.

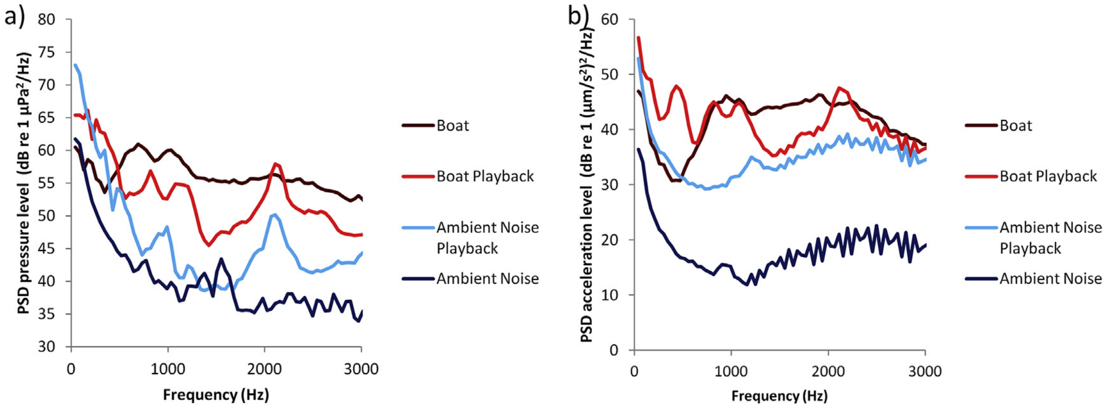

Fig. 3. Power spectral densities (PSD) of (a) sound pressure level and (b) monoaxial (horizontal axis) particle acceleration level of original recordings of motorboats and motorboat-noise playbacks at experimental site along with ambient noise and ambient-noise playbacks. Window length = 1024. Mean of five boat passes or 3 min ambient noise. Playback of ambient noise matched local ambient noise relatively well in sound pressure. As the experiment was conducted on a sand flat, most ambient sound sources were not nearby, particle acceleration ambient noise levels were thus lower as the particle motion component of sound drops off with distance from the sound source faster than the pressure component. Playbacks were affected by near-field effects and speaker performance meaning some frequencies were louder and others quieter, but motorboats were louder than ambient noise and motorboat-noise playbacks were louder than ambient-noise playbacks. Some recordings contained harmonic noise at 50 Hz intervals; this was an artefact of recording via a laptop.

10 min after 1 week (7–9 days) and for 10 min after 2 weeks (14–18 days) of playback exposure between 06:00 and 10:00 (we were unable to film during the afternoon due to logistical constraints). The first 5 min of each video recording were discarded for analysis as preliminary observations revealed that behaviour stabilised 5 min after the start of the video (when schools were disturbed by the presence of someone setting up the camera). Videos were watched in a random order without sound by an observer that was blind to experimental treatment. The same observer was used for all videos. We focused on the 50 s prior to a motorboat pass (‘pre’), the 45 s of the motorboat pass (‘during’) and the 50 s following a motorboat pass (‘post’) in Boat replicates. Scan samples of fish behaviour were performed every 10 s during each period. Scans in matched periods were also made of Ambient replicates. In each scan, each focal fish was recorded as hiding in the protection of the anemone or not hiding. Fish were defined as hiding in the protection of the anemone if all or part of their body was within anemone tentacles or if they were within one body length of the rim of the anemone (the underside of the anemone where there are no tentacles). In each video, the mean number of focal fish hiding in pre-, during- or post-exposure periods were used for statistical analysis. Since the mean number of fish hiding in each assessment period was used for statistical analysis, the sample size was determined by the number of schools.

2.1.3. Ventilation rate

Ventilation rate (measured as opercular beat rate; OBR) is a recognised secondary indicator of stress (Barton, 2002), is a robust measure allowing control for the baseline OBR of individual fish in a matched design, is easily measured by an observer who is blind to the acoustic experience of each fish, and has previously been shown to be affected by anthropogenic noise (Bruintjes et al., 2016; Purser et al., 2016; Simpson et al., 2015). Four randomly selected focal fish were caught from each anemone after 1 week and 2 weeks of playback exposure between 6:00 and 9:00. Fish were introduced one at a time to the experimental arena (a 20 × 20 × 15 cm plastic tub suspended mid-water on the same sand flat, 100 m from experimental cages), situated 1 m from a loudspeaker. The associated loudspeaker playing one of two sound treatments was placed 1 m away on the sandy bottom facing upwards. Motorboat-noise playbacks were composed of loops of the loudest 2 s of motorboat passes with a 10 s ramp-up. Four replicate playbacks of each sound treatment were used.

Fish were observed for 1 min settling time, followed by 1 min during playback of ambient noise where OBR was counted to establish a ‘baseline’, followed by 1 min during playback of either a different ambient-noise track or motorboat-noise track while OBR was counted. Fish were randomly allocated to short-term sound treatment and the observer was blind to the long-term treatment when possible (dependent on whether fish from both Boat and Ambient schools were available on the same day; ca. 50% of the time). After the experiment, fish were taken back to the CRIOBE research station.

2.1.4. Size, mass and body condition

The standard length of each fish was measured to the nearest 1 mm using a ruler before entering the experiment. The standard lengths of fish allocated randomly to experimental anemones did not differ significantly between sound treatments (independent samples *t*-test: $t_{282.7} = 0.07$, $p = 0.944$). Fish that were taken back to the research station after week 1 and week 2 were sacrificed using an overdose of MS222 before standard length (measured in the same way) and wet mass (measured using a balance, to the nearest 0.001 g). were measured. These were used to calculate condition factor using the following formula:

$$K = \frac{10^5 M}{L^3}$$

where:

K is the Condition Factor, **M** is the wet mass of the fish in grams (g) and **L** is the standard length of the fish in millimetres (mm) (Nash et al., 2006).

2.1.5. Blood cortisol concentration

After 18–21 days (during which playbacks continued), the remaining fish in each cage were caught and a blood sample was taken to investigate the impact of long-term motorboat-noise playback on baseline plasma cortisol levels. Fish were decapitated and bled from the caudal vein within 0:22–4:23 min (mean = 1:40 min) of the start of capture attempts. Time to bleed (independent samples *t*-test: $t_{23.7} = 0.05$, $p = 0.960$), standard length of fish bled ($t_{23.9} = 0.53$, $p = 0.599$) and number of days fish had spent in the cage ($t_{23.9} = 0.54$, $p = 0.596$) did not differ significantly between sound treatments. Blood was collected in a heparinised 75 µl haematocrit capillary tube primed with 2 µl of enzyme immunoassay (EIA) buffer (to assist the entry of small amounts of blood into the capillary tube). After the sample was taken, a further 18 µl of the EIA buffer was added to the capillary tube to achieve the desired dilution. Samples were kept on ice until they were centrifuged for 10 min at 10,000 rpm, to separate the plasma.

Samples were obtained from 12 fish across eight different anemones in the Ambient treatment and 14 fish across nine different anemones in the Boat treatment. Plasma cortisol concentrations were measured using a Cortisol EIA Kit (No. 500360, Cayman Chemicals, SPI BIO, France) as described in (Mills et al., 2010) and validated for this species using a pool from 25 individuals using both parallel displacement of serially diluted plasma to the standard curve and precision from intra- and inter-assay variabilities.

With respect to validation of the cortisol assessment procedure, the dose-response curves (11 dilution ratios: 1:3, 1:7, 1:20, 1:53, 1:143, and 1:387; as well as 1:11, 1:28, 1:69, 1:172, and 1:430) were parallel to the cortisol EIA assay kit standards (ANCOVA of homogeneity of slopes: $F_{1,46} = 0.542$, $p = 0.466$; kit standards: $y = -33.153\,x - 28.014$, $R^2 = 0.98$, N = 25, $p < 0.001$; samples: $y = -33.906\,x - 8.385$, $R^2 = 0.96$, N = 22, $p < 0.001$). The dilution factor for 50% of antibody bound determined from a regression analysis was 1:53 (a dilution of 0.019). A high degree of accuracy and precision was achieved with samples from *D. trimaculatus* using the cortisol kit, as determined from intra- (4.4%; n = 14) and inter-assay (7.6%; n = 4) variability respectively.

2.1.6. Statistics

Where there were sufficient data, we used general linear mixed effects models to test for impacts of motorboat-noise playback. Linear mixed-effects models with normal errors were used to analyse the effect of short and long-term playback exposure on the change in OBR from baseline and the effect of long-term noise treatment on baseline OBR, size, mass and condition. Number of days exposure was included in the models as a fixed effect and school was included as a random effect which was specific to temporal replicate.

For mixed models, the minimal model was obtained by sequential deletion of fixed effects and their interactions where they were found to be non-significant. Significance was tested by likelihood ratio model comparisons of the maximal model with the nested model where an effect in question was dropped. Chi-squared statistics and p-values for fixed effects were obtained by likelihood ratio tests comparing the minimal model with a model excluding the effect where it was included in the minimal model, or including the effect where it was not. The degrees of freedom given are the difference in degrees of freedom for the two models compared and the degrees of freedom for the minimal model. All potential interactions of fixed effects were examined and are only presented where their exclusion from the model made the model significantly worse at explaining the data at the level $p < 0.10$. In the case where interaction terms were included in the best model, planned contrasts were conducted using Markov Chain Monte Carlo methods. Z tests were used for post-hoc tests where the sample size was large (>20); t-tests were used where the sample size was small (<20). The variance and standard deviation for the random effect of school and the size of any effects with standard error (se) are given.

Elsewhere, we used paired t-tests or Wilcoxon signed-ranks tests on the mean per school (selected after checking relevant assumptions of normality and heterogeneity of variances). To establish whether the noise of boat passes affected hiding behaviour, the mean number of fish hiding in schools was compared for pre–during, during–post and pre–post comparisons in a repeated-measures design (within-schools comparison). Mean cortisol concentration for each Boat school was compared with mean cortisol concentrations in Ambient schools in an independent-measures design (between-schools comparison).

3. Results

3.1. Hiding behaviour

On day 2, a significantly greater number of focal fish in Boat schools were found hiding in the anemone during the 45 s period of motorboat-noise playback compared with the 50 s period pre-noise (paired *t*-test: $t_7 = 2.38$, $p = 0.049$). On average, 0.83 more fish were found hiding during motorboat noise compared to pre-motorboat noise (95% CIs: 0.01–1.66; Fig. 4). In the 50 s post-motorboat-noise exposure, the number of fish hiding declined slightly but was not significantly different to the number during motorboat noise ($t_7 = 0.63$, $p = 0.546$, mean difference = 0.71, 95% CIs = −1.94–3.36). The post-exposure number of focal fish hiding did not differ significantly from the pre-exposure number ($t_7 = 0.43$, $p = 0.679$, mean difference = 0.11, 95% CIs = −0.52–0.76). There were no significant differences in the number of fish hiding in the anemone between any pair of equal time points in Ambient schools (N = 5, *t*-test p-values > 0.1). Nor were there significant differences in the number of fish hiding in the anemone when comparing pre–during, during–post and pre–post periods at weeks 1 and 2 (N Boat week 1 = 9, N Boat week 2 = 11, N Ambient week 1 = 10, N Ambient week 2 = 7, paired t-tests or Wilcoxon signed ranks tests p-values > 0.1; Fig. 4).

3.2. Opercular beat rate

The interaction between long-term and short-term noise exposure affected the change in OBR from baseline (LMM: $\chi^2_3 = 81.80$, $p < 0.001$; long-term noise: $\chi^2_1 = 6.84$, $p = 0.009$; short-term noise: $\chi^2_1 = 48.41$, $p < 0.001$; school variance = 27.57, standard deviation = 5.25), with qualitatively the same result found after 1 and 2 weeks of noise exposure ($\chi^2_1 = 1.39$, $p = 0.239$). While short-term exposure to motorboat-noise playback resulted in a significantly greater increase in OBR compared to ambient-noise playback, that effect was significantly lessened by long-term exposure to playback of motorboat noise (Table 1; Fig. 5).

3.3. Size, mass and body condition

Although there was a significant positive effect of days in the cage (i.e. age), there was no significant effect of long-term noise exposure on standard length (LMM: $\chi^2_1 = 0.11$, $p = 0.745$; days in cage: $\chi^2_1 = 20.26$, $p < 0.001$; school variance = 0.71, standard deviation = 0.84), wet mass ($\chi^2_1 = 0.16$, $p = 0.694$; days in cage: $\chi^2_1 = 15.79$, $p < 0.001$; school variance = 0.03, standard deviation = 0.16) or body condition ($\chi^2_1 = 0.30$, $p = 0.582$; days in cage: $\chi^2_1 = 5.90$, $p = 0.015$; school variance = 0.00, standard deviation = 0.06) (N Ambient = 82; Boat = 93).

3.4. Blood cortisol concentration

Long-term noise-exposure treatment had no significant effect on the baseline cortisol concentration (independent samples *t*-test: $t_{15} = 1.8$, $p = 0.091$; Fig. 6).

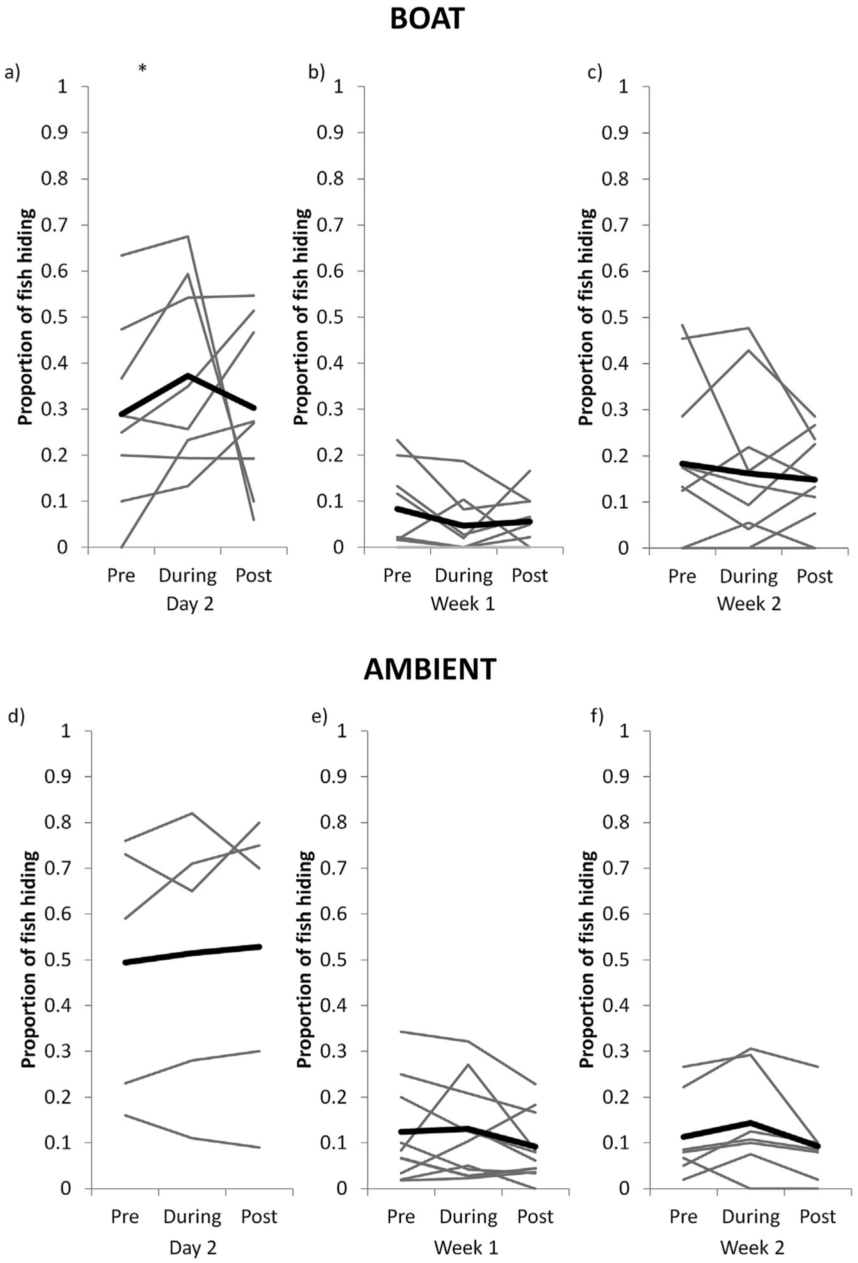

Fig. 4. Proportion of the total number of focal fish in the school hiding during three periods of playback (pre-, during and post-motorboat-noise playback periods; in Ambient schools, 'during' refers to the matching time points in videos when Boat schools received motorboat-noise playbacks, when Ambient schools continued to receive ambient-noise playback). Grey lines represent the mean proportion within schools; thick black lines represent means across all schools. a) Boat day 2; b) Boat week 1; c) Boat week 2; d) Ambient day 2; e) Ambient week 1; f) Ambient week 2.

Table 1
Planned contrasts for post-hoc testing of the effect of the interaction between long- and short-term playbacks on opercular beat rate. A = Ambient, B = Boat. Significant results are shown in bold. N Ambient:Ambient = 39; Ambient:Boat = 43; Boat:Ambient = 43; Boat:Boat = 45.

Long-term: Short-term treatment combination	Effect size estimate	Standard error of effect size estimate	t value	Degrees of freedom	p
A:A × A:B	31.42	3.66	8.59	141	<0.001
A:A × B:B	−21.72	5.07	−4.28	141	<0.001
A:B × B:B	19.84	4.27	4.65	141	<0.001
A:B × B:A	21.72	5.07	4.28	141	<0.001
B:B × B:A	−9.69	3.52	−2.76	141	0.007
A:A × B:A	−1.88	4.39	−0.43	141	0.668

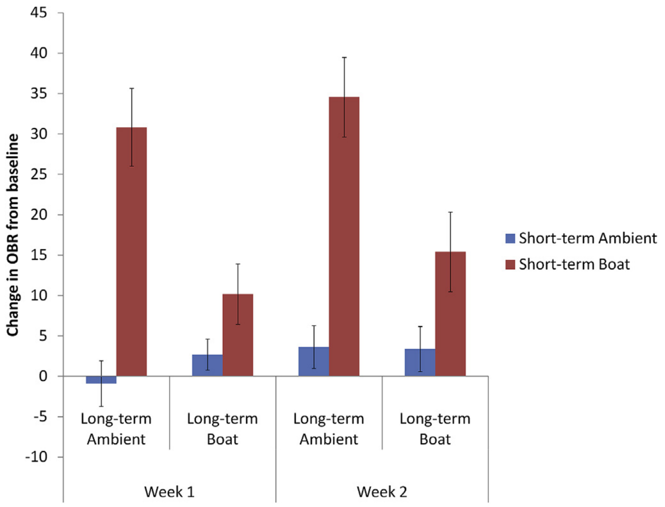

Fig. 5. Mean ± 1 se change in ventilation rate (opercular beat rate, OBR) from baseline (mean baseline OBR = 249) when fish that had been exposed to long-term ambient or motorboat-noise playback were played a short-term ambient or motorboat-noise track. Long-term: 1 or 2 weeks, Short-term: 1 min. Long-term Ambient Short-term Ambient: N = 40; Long-term Ambient Short-term Boat: N = 43; Long-term Boat Short-term Ambient: N = 44; Long-term Boat Short-term Ambient N = 45.

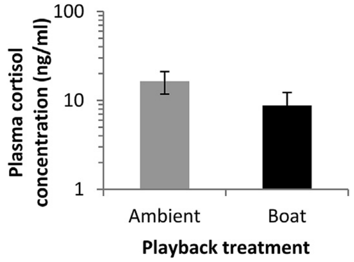

Fig. 6. Mean ± 1 se baseline plasma cortisol concentration in fish exposed to 18–21 days of either ambient- or motorboat-noise playback.

4. Discussion

We found a behavioural and a physiological response to motorboat-noise playback in the short term: after two days of exposure, juvenile *Dascyllus trimaculatus* were more likely to hide during the period of a motorboat-pass playback than in the period immediately before, and naïve fish (those that had not experienced motorboat-noise playback before) also showed an increased ventilation rate (opercular beat rate, OBR) in response to noise in the short term (1 min exposure). Our results concur with other studies that have found short-term behavioural and physiological effects of anthropogenic noise in fish (Bruintjes and Radford, 2013; Buscaino et al., 2010; Nedelec et al., 2015; Picciulin et al., 2010; Simpson et al., 2015; Williams et al., 2015). However, we also found evidence for behavioural and physiological attenuation: after 1 week of motorboat-noise exposure, hiding responses were no longer observed during motorboat passes in repeat measures of the same fish, and OBR increased less in response to motorboat-noise playback. Perhaps as a consequence, we found that motorboat-noise playback did not cause chronic stress responses: size, mass, condition and baseline cortisol levels were not significantly different from ambient-noise exposed controls after up to 21 days. We would expect these measures to be affect had habituation not occurred (Cyr and Romero, 2009).

Typical interpretations of how increases in hiding behaviour and OBR could impact fitness are that less time is available for foraging and/or that the animal was exhibiting a stress response. Reduced resource acquisition could in turn lead to reduced growth, body condition and ultimately either starvation, reduced ability to escape predators, or fewer or poorer quality offspring (e.g. Picciulin et al., 2010). Stress responses are associated with increases in cortisol which can have ‘detrimental effects on growth, sexual maturation and reproduction, immunological function and survival’ (Dickens and Romero, 2013; Wysocki et al., 2006 and references therein). However, our data show that after 1 week of exposure, hiding and OBR responses are attenuated, calling into question such extrapolations from short-term responses (see also (Bejder et al., 2006)). Attenuated responses remained consistent into the second week of noise exposure and were accompanied by no significant differences in size, mass, condition or baseline plasma cortisol concentration between fish exposed to ambient- or motorboat-noise playback. Thus, the fish in our experiment did not appear to be under chronic stress as a consequence of repeated exposure to motorboat-noise playback.

Possible explanations for the increased tolerance that we observed are hearing threshold shifts and habituation. While current opinion is that measures made in the acoustic near field in the lab are not easily translated to open-water conditions (Fay and Popper, 2012), we designed our noise exposure so that sound levels were raised from ambient conditions within the frequency range that other damselfishes have been shown to hear (up to 1200 Hz (Myrberg and Spires, 1980), yet to be below the level likely to cause temporary hearing loss based on the limited knowledge we have from auditory abilities in other species (Amoser and Ladich, 2003; Ramcharitar and Popper, 2004; Smith et al., 2004). As we caged fish, and thus emigration of more sensitive individuals could not explain the increased tolerance either, habituation is the most likely explanation for our results. Habituation entails learning by animals that a stimulus does not represent a threat; in order to show habituation, the same individuals must be tested over time and a diminished response must be observed (Bejder et al., 2009). Habituation has previously been studied in other contexts (e.g. the siphon withdrawal reflex to a jet of seawater wanes with repeat stimulation in *Aplysia* (Carew and Kandel, 1973), and the mobbing of predators by chaffinches (*Fringilla coelebs*) if the stimulus is prolonged or repeated (Hinde, 1954)). We provide the first evidence of this kind from a field-based experimental manipulation involving anthropogenic noise.

The regime of sound exposure in our experiment was highly regular; one motorboat playback every 5 min during daylight hours. Although areas of regular disturbance exist, in many cases exposure to motorboat noise might be less regular. Nedelec et al. (2015) showed that in Atlantic cod (*Gadus morhua*), chronic regular noise led to reduction in body condition when compared to random noise; however different species may respond differently. We also caged fish to exclude predators, which may have reduced some potential impact. For instance, recent work has found that predator avoidance behaviour in fish can be negatively impacted by exposure to anthropogenic noise (Simpson et al., 2015, 2016), thus there is the possibility that our experimental subjects were cognitively impaired but the exclusion of predators protected them. Also, stressors can permanently alter the stress-induced cortisol response of coral reef fish (which we did not measure) and can impact their response to other stressors (such as predation) (Mills et al., 2015). Finally, while we do believe that all our evidence points to fish becoming habituated to motorboat-noise playback, it should be considered that habituation does not necessarily link with better welfare or chances of survival. Fish that are habituated to motorboat noise may be more likely to be exposed to predation risk (from fishing) or exposure to disease (Bejder et al., 2009).

Further work is therefore warranted, ideally examining the impact of real-world noise sources on uncaged fish in natural conditions.

Data accessibility

Data are available at the following DOI: 10.5523/bris.uvjg014dbwqd1j705jsfnm6av (DOIs can be resolved at https://dx.doi.org/).

Author contributions

SN, DL, SS & AR conceived the original idea; SN, SM, DL, SS & AR planned the project; SN & BN designed and deployed equipment; SN, SM & BN collected the data; SN & AR analysed the data; SN, SM, DL, SS & AR interpreted the data; SN wrote the initial manuscript with comments from all other authors.

Competing interests

The authors declare no competing interests.

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