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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

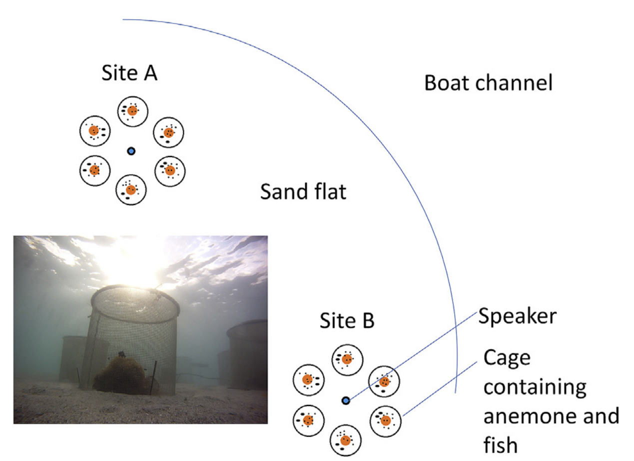

Fig. 1. Schematic diagram (not to scale) showing the layout of the sites used for sound playback and video recording, plus a photo of the cages at one site.

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