

**PROCEEDINGS OF
THE ROYAL SOCIETY B**
BIOLOGICAL SCIENCES

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Journal:	<i>Proceedings B</i>
Manuscript ID	RSPB-2018-0033
Article Type:	Research
Date Submitted by the Author:	05-Jan-2018
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Subject:	Cognition < BIOLOGY, Ecology < BIOLOGY, Environmental Science < BIOLOGY
Keywords:	sound pollution, anthropogenic noise, predator recognition, behaviour, alarm cues
Proceedings B category:	Behaviour

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School is out on noisy reefs: the effect of boat noise on predator learning and survival of juvenile coral reef fishes.

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Keywords: sound pollution, anthropogenic noise, predator recognition, behaviour, alarm cue.

ABSTRACT

20 Noise produced by anthropogenic activities is increasing in many marine ecosystems. Here, we investigated the effect of playback of boat noise on fish cognition. We focused on noise from small motorboats, since its occurrence can dominate soundscapes in coastal communities, the number of noise-producing vessels is increasing rapidly and their proximity to marine life has the potential to cause deleterious effects. Cognition – or the ability of individuals to learn and

25 remember information – is crucial, given that most species rely on learning to achieve fitness-promoting tasks, such as finding food, choosing mates and recognizing predators. The caveat with cognition is its latent effect: the individual that fails to learn an important piece of information will live normally until the moment where it needs the information to make a fitness-related decision. Such latent effects can easily be overlooked by traditional risk

30 assessment methods. Here, we conducted three experiments to assess the effect of boat noise playbacks on the ability of fish to learn to recognize predation threats, using a common, conserved learning paradigm. We found that fish that were trained to recognize a novel predator while being exposed to ‘reef+boat noise’ playbacks failed to subsequently respond to the predator, while their ‘reef noise’ counterparts responded appropriately. We repeated the training,

35 giving the fish three opportunities to learn three common reef predators, and released the fish in the wild. Those trained in the presence of ‘reef+boat noise’ playbacks survived 40% less than the ‘reef noise’ controls over our 72-h monitoring period, a performance equal to that of predator-naïve fish. Our last experiment indicated that these results were likely due to failed learning, as opposed to stress effects from the sound exposure. Neither playbacks nor real boat noise affected

40 survival in the absence of predator training. Our results indicate that boat noise has the potential to cause latent effects on learning long after the stressor has gone.

INTRODUCTION

Noise produced by human activities is increasing in many marine ecosystems [1] and is a pollutant for which impact assessment data are sparse [2]. With the increasing numbers of sources of noise [3] and the increased power of noise-producing equipment and vessels, anthropogenic noise can now dominate ambient sounds in some habitats [4]. Given the low attenuation of sounds in water, some low frequency sounds can be detected thousands of kilometers away from their source [1]. Due to the wide spatial and temporal distribution of anthropogenic noise, aquatic species may lack refuge from this modern-day stressor. The magnitude of the direct effects are proportional to the proximity of the animals to the source. In close proximity (metres), mortality and injury can be observed [5], while further away, in the zone of responsiveness (metres to kilometres depending on species), sub-lethal physiological stress and behavioural alterations can be seen [6, 7]. However, some impacts of noise pollution may be subtle, or latent. Many sources of anthropogenic noise occupy the same frequencies as biological sounds, and as a result, can lead to informational masking [8], which interferes with animal communication [9]. Latent effects are difficult to assess, because their impacts can occur after noise has abated and thus may be confounded with other phenomena. As with many other pollutants, the consequences of sub-lethal effects of noise on the integrity of communities have yet to be thoroughly investigated.

In many coastal environments, transportation, fishing and recreational activities involving boating are the most prevalent sources of anthropogenic noise, with more than 12.5 million registered motorboats in the US in 2013 [10] and more than half a million motorboats predicted to cruise on the Great Barrier Reef by 2040 [11]. While small motorboats produce neither the amplitude nor the low frequency sounds of large ships such as bulk carriers, they can be present

65 in high numbers and travel in shallow water close to aquatic life, two characteristics that have the potential to impact the ecology, behaviour and physiology of organisms in coastal ecosystems. For example, playbacks of small boat noise can reduce the rate of development and increase mortality of seahare (*Stylocheilus striatus*) embryos [12], modify the behaviour and induce stress responses in spiny lobsters (*Palinurus elephas*) [13] and alter orientation behaviour of coral reef cardinalfish (*Apogon doryssa*) [14]. In the presence of noise produced by actual boats (as 70 opposed to playback), some species alter their swimming speed and distribution [15] and a coral reef damselfish (*Pomacentrus amboinensis*) is more susceptible to predation [16]. Simpson et al. [16] also provide evidence that boat-noise playbacks and real boat noise have comparable effects on physiology, behaviour and predation-induced mortality endpoints.

75 Although these studies suggest that boat noise could have widespread impacts on the behaviour and physiology of fishes in shallow water habitats, no study has thus far addressed the effects of boat noise on cognition in fishes. The ability of animals to maintain proper cognitive skills is crucial, as many fitness-promoting behaviours, such as finding food, choosing a suitable mate, recognizing predators and identifying migration routes rely on learning and memory [17]. To 80 address the issue of the effects of noise on cognition, we chose a highly conserved and efficient learning mechanism of fishes termed ‘alarm cue conditioning’ [18, 19]. Alarm cues, or injured conspecific cues, are chemical compounds present in the skin or tissues of aquatic species, and are known to elicit overt antipredator responses when detected by nearby conspecifics. They represent a reliable indicator of risk, since the cues are only released in the water column upon 85 mechanical damage, typically following an attempt at predation. The response to alarm cues is innate, and present in a wide variety of species, including corals, molluscs, crustaceans, fishes and aquatic amphibians [19 for a review]. The learning mechanism associated with these cues

involves the simultaneous pairing of alarm cues with cues of a novel predator, such as its smell, sight or sound, to which predator-naïve prey do not innately respond. A single pairing results in a learned association between fear and the novel predator cue, a phenomenon observed in both
90 invertebrates and vertebrate prey [19 for a review]. Exposed prey then display antipredator responses upon subsequent detection of the predator cue. The only reports of failed alarm cue conditioning involved the deactivation of alarm cues via aquatic pollutants (acid rain, pesticides, metals) [20, 21] or via alteration in the animal's neurotransmission pathways [22, 23].

95 The ability of prey to learn rapidly to distinguish predators from non-predators is crucial to their survival [24]. Most newborn prey are predator-naïve and rely on experience to acquire predation-related information. For mammals and birds, this information can be transmitted socially via parents or experienced members of their groups [25]. However, many aquatic species lack parental care or cross-generational social groupings. In addition, the complex life history of
100 many species results in ontogenetic habitat changes, where animals are confronted to a novel suite of predators in the new habitat to which they migrate. Many coral reef fishes, for instance, have a bipartite life history, whereby pelagic larvae recruit back to the reef, after weeks to months in open waters [26]. This life history transition is associated with a major mortality bottleneck, where 60–90% of juveniles are consumed by predators within the first 48 h following
105 settlement [27]. The speed at which these prey can gather information about local predation threats is thus under strong selection. The impact of learning is illustrated by the fact that young fish trained to recognize three common reef predators have 40% better survivorship than naïve individuals over the first few days of life on the reef [28].

We used a series of laboratory and field experiments to examine the effect of boat noise
110 playbacks on the ability of juvenile Ambon damselfish (*Pomacentrus amboinensis*) to learn to

recognize a novel predator. In the first experiment, we trained naïve fish to recognize the odour of the predatory dusky dottyback (*Pseudochromis fuscus*) as a threat, in the presence of playback of either ‘reef noise’ or ‘reef+boat noise’, and compared the ability of the two groups to respond to the predator cues the following day. To provide ecological relevance to experiment 1, we trained fish to recognize three common predators, in the presence of playback of either ‘reef noise’ or ‘reef+boat noise’, and monitored their *in situ* behaviour and 72-h survival (experiment 2). We used a field experiment to disentangle the effects of stress from those of cognitive impairment (experiment 3), by comparing the survival of fish exposed to ‘reef noise’ vs. ‘reef+boat noise’ playbacks in the laboratory with that of fish exposed to real-world ‘reef’ or ‘reef+boat’ noise *in situ*. This also allowed us to compare the impact of playbacks vs. realistic acoustic conditions. We hypothesized that if stress was the main driver behind impaired learning in the presence of noise, fish exposed to boat noise (real or playbacks) should suffer higher mortality than those that were exposed only to reef noise.

METHODS

125 *Fish collection and maintenance*

Fish were collected at dawn from pelagic light traps deployed overnight in nearshore waters off the reefs of Lizard Island, QLD, Australia (14°40’S, 145°28’E). Because the fish were caught prior to their settlement onto the reef, they were naïve to the suite of benthic predators that would await them upon settlement [28, 29]. Later that day, the fish to be used in the field experiments were tagged using a fluorescent elastomer and returned to their holding tanks. Tagging does not impair behaviour or survival [30]. The fish were maintained in the laboratory for at least 48 h before being used in the experiments [31] (for more information, see online supplement).

Cue preparation

Fish odours: Four dusky dottybacks (range: 7.05–7.72 cm Standard Length, SL), eight
135 lizard fish, *Synodus dermatogenys* (7.36–8.97 cm SL), and eight moonwrasses, *Thalassoma*
lunare (9.90–13.67 cm SL) were collected using hand nets and clove oil one week prior to the
start of the experiment. Species were housed separately in 50-l flow-through tanks. The tank
housing the dottybacks was only filled halfway to account for the lower density of fish. All
fishes were fed juvenile apogonids (which lack alarm cues recognized by damselfish), which
140 constitute a normal diet for these fish at this time of year [32]. We turned off the flow-through
system 3 h prior to using the tank water containing the fish odour in the experiments.

Alarm cues: Conspecific alarm cues were used fresh and obtained by euthanizing nine *P.*
amboinensis and making four cuts on each side of each fish. The sides were then rinsed with 20
ml of seawater, and the solution used as a source of alarm cues. This yielded enough for four
145 injections of alarm cues.

Noise parameters

Playbacks: Playback treatments were identical to those used in Simpson *et al.* (2015).
Laboratory exposures to ‘reef noise’ or ‘reef+boat noise’ were achieved by exposing fish to the
150 playback of recordings of subsets of five of the Lizard Island Research Station dinghies (5 m
long aluminium hull with 30 hp 2-stroke Suzuki outboard engines, model DT30) or subsets of
five recordings from different reefs within 1 km of the field test site (control treatment). All
recordings were taken during daytime, from a small kayak moored using an anchor without chain
to avoid unwanted noise (e.g. waves on the hull of a boat), and were made 1-2 m above the
seabed for 5 min (for details on noise measurements, see online supplement).

155 For the playback experiments, a unique compilation of three of the five reef recordings (made
using Audacity v2.0.2, <http://audacity.sourceforge.net>) was used for each ‘reef noise’ track, and
compilations of three of the five boat-noise recordings made at the same five reefs were used for
the ‘reef+boat noise’ tracks. The sound systems used for playback of ‘reef’ and ‘reef+boat’
recordings consisted of a battery (12V 7.2 Ah sealed lead-acid), WAV/MP3 player (GoGEAR
160 Vibe, frequency response 0.04–20 kHz; Philips, Netherlands), amplifier (M033N, 18W,
frequency response 0.04–20 kHz; Kemo Electronic GmbH, Germany) and speaker (University
Sound UW-30; maximal output 156 dB re 1 μ Pa at 1 m, frequency response 0.1–10 kHz; Lubell
Labs, Columbus OH). While acoustic analysis (see supplementary figure 1) indicates that the
boat playbacks were not an exact match to real boat noise (~ 20 dB *lower*), this point only
165 emphasizing the conservative nature of the results obtained from playback experiments.

Field exposures to boat noise: At each location, a recording was also made with one of
five of the research station boats motoring at various speeds 10–200 m from the hydrophone and
accelerometer, replicating the kinds of boat operations common in coral reefs and similar to that
during the recordings used for playbacks. Full acoustic analysis is presented as online
170 supplement.

Experiment 1: Laboratory test of learning

The goal of this experiment was to investigate the effect of boat noise on the ability of fish to
learn to recognize a predator. We used a well-established one-time training protocol, which has
previously been shown to be effective in many aquatic species, including our test subjects [31].
175 We compared the learning outcomes of fish learning under ‘reef noise’ (positive control) and
under ‘reef+boat noise’ (experimental group). We also added a no-learning group to act as our

negative control. Due to the logistical constraints associated with performing training in the field, this experiment took place under controlled laboratory conditions, using playback recordings.

Experimental overview: The experiment consisted of two phases: (1) a conditioning phase, where the predator-naïve fish were trained (or not) to recognize the predator under either ‘reef noise’ or ‘reef+boat noise’; and (2) a testing phase, taking place the following day, where we observed the response of the fish to the predator odour or a water control. Learning was considered successful if the fish displayed a significant antipredator response (reduction in foraging and activity levels, details below) to a cue that was previously unknown (predator odour). The experiment followed a 3 x 2 design, with three conditioning groups crossed with two testing cues.

Conditioning phase: Four 15-l plastic tanks (32 x 25 x 16 cm), equipped with a field-collected sand substrate and 1.5 m long injection tube used to introduce stimuli into the tank, were placed on 20-cm high plastic stands, in a semi-circle in a large food-grade plastic pool (111 cm diameter, 45 cm high, 368 l). These tanks were covered in black plastic to prevent visual information being transmitted between individuals from different tanks. The pool and the tanks were filled with fresh seawater, pumped directly from the ocean. In each pool, one underwater speaker was placed on the opposite side of the semi-circle, facing the tanks. Two *P. amboinensis* were placed in each tank while reef noise was playing. The fish were left to acclimate for 30 min. After the acclimation period, the track was changed to either another ‘reef noise’ recording or ‘reef+boat noise’ recording. One min later, the fish were taught to recognize the odour of a dottyback, via the simultaneous injection of 15 ml of dottyback odour and 5 ml of injured conspecific cues. Our negative control fish (no learning) were exposed to reef noise while we injected 15 ml of water paired with 5 ml of injured conspecific cues. Each injection was followed

200 by a 60-ml injection of seawater to ensure the stimuli were completely flushed into the tank. Three min after the end of the injection period, the fish were transferred individually into similar 15-l flow-through tanks equipped with a sand substrate, a small piece of dead coral, and a 1.5 m long injection hose. Each day, we conditioned eight fish in each of the three treatments.

Testing phase: The testing phase took place 24 h after the conditioning phase and followed an established protocol, described in detail in the supplementary information. The fish were 205 observed 4 min before and 4 min after the injection of 20 ml of seawater (control) or dottyback odour. We quantified changes in feeding strikes (all strikes were counted, regardless of whether or not the fish was successful at striking a food item) and activity (using a grid drawn on the side), as per Ferrari *et al.* [33]. Typical antipredator responses are manifested via a decrease in 210 both foraging and activity in the presence of predator threats. We tested 83 fish (n=13–15 per treatment). The observer was blind to the treatment and the order of the treatments was randomized. To account to temporal biases, the same number of replicates from each treatment was tested each day. Fish were 1.39 ± 0.03 (mean \pm SD) cm SL.

215 ***Experiment 2: Ecological consequences of learning with boat noise***

This experiment was designed to test the survival consequences of learning in the presence of ‘reef+boat noise’ playbacks. We followed the methodology from experiment 1 and conditioned three groups of fish: fish learning predators with ‘reef noise’ (positive control), fish not learning predator with ‘reef noise’ (negative control) and fish learning predator with ‘reef+boat noise’ 220 (experimental group). For this experiment, the fish were trained three times, to recognize three common predators (dottyback, lizardfish and moonwrasse) both visually and chemically or

alternatively, they would undergo the same experimental manipulation, but would not learn any information about the predators (naive, untrained controls). We used the triple training procedure and the multi-sensory approach to increase their opportunity to learn, following previous
225 published learning protocols and survival outcomes [28]. The predators were chosen as they are often seen striking at newly-settled damselfish at our locations [28]. After the conditioning took place, the fish were bagged and released in the field onto small patches of coral. Their behaviour was assessed and their survival in the field was monitored for 72 h post-release, and followed established procedures [16, 34]

230 *Conditioning phase:* This phase was similar to the conditioning phase of experiment 1. After the 30-min reef-noise acclimation period, soundtracks were changed to either ‘reef noise’ or ‘reef+boat noise’. The training protocol consisted of three exposure blocks (one for each predator), each separated by 1 min. At the start of each block, 5 ml of injured conspecific cues were injected into the tank. Seconds later, a clear, sealed, 1-l plastic bag filled with seawater and
235 a live predator was gently lowered at one end of the tank, and 15 ml of odour from that same predator was injected into the tank (followed by 60 ml of seawater to flush the stimulus into the tank). This provided the fish with a reliable indicator of risk (injured conspecific cues) paired with both visual and chemical cues from the predator. The predator was removed from the tank after one min, and we waited another min before starting the next block. The order of
240 presentation of the three predators was randomized across trials. The untrained control fish underwent the three-block procedure, with the exception that the bags contained no predator (empty bags), and that the injections of predator odour were replaced by blank water controls; they still received the injured conspecific cue solution, but were not provided with any information about predators. Three minutes after the end of the conditioning phase, fish were

245 photographed and placed in labelled 1-l plastic bags filled with seawater. The bags were kept in a water bath of flowing seawater until deployment in the field. To reduce transport and handling stress, fish in bags were transported to the field site in a 60-l bin of seawater (to reduce temperature fluctuations) under subdued light conditions.

Behaviour and survival: Reef patches were cleared of all resident fish, and a single randomly
250 selected *P. amboinensis*, was released onto the reef and immediately protected by a mesh cage for 30 min. After this acclimation period, the cage was removed and after a few minutes, the fish were observed for 3 min. A scuba diver, located ~2 m away from the patch reef, recorded the behaviour of each individual for a period of 3 min (an observation time found to obtain a representative quantification of behaviour for *P. amboinensis*; [35]). Four aspects of activity and
255 behaviour were assessed: 1) bite rate, 2) total distance moved; 3) maximum distance ventured from the habitat patch; and 4) boldness (see online supplementary information for more details). Following the end of the observation period, the fish were monitored twice daily for survival. The observer was blind to the treatment during observations and survival surveys. We released and observed 96 fish (n=32 per treatment).

260 ***Experiment 3: Test of latent effects of noise exposure***

The goal of this experiment was to further advance our understanding of the mechanisms behind the survival results observed in experiment 2. Evidence clearly suggests that fish exposed to ‘reef+boat noise’ survived poorly in comparison to fish exposed to ‘reef noise’. Their poor survival could be due to a failure of learning, or could be a temporary carry-over effect of boat
265 noise exposure, in the form of an acute stress response. Testing this hypothesis would have required us to monitor fish survival after varying post-boat exposure delays (hours to days, post-

boat exposure). Given the field constraints, we opted to design a new experiment to test the potential survival costs associated with boat noise exposure *per se*. We hypothesized that if fish exposed to boat noise survived similarly to those exposed to reef noise only, then the stress associated with noise exposure would not be the proximate cause from the results from experiment 2. We used this opportunity to not only compare ‘reef noise’ vs ‘reef+boat noise’ playbacks, but to also test if the fish subjected to playback would have the same survival as fish receiving exposure to real boats *in situ*. We exposed four groups of fish in a 2 x 2 design, testing the effect of noise (reef noise vs. reef+boat noise) and noise origin (real, *in situ* boat exposure vs. laboratory playback).

Sound exposure phase: For the fish receiving this treatment, the playback exposures were identical to the ones described above, with a 30-min ‘reef-noise’ playback, followed by a 3-min playback of either another ‘reef noise’ track or a ‘reef+boat noise’ track. Fish were photographed, bagged and released onto patch reefs.

For *in situ* exposure, the fish were photographed and tagged. Some fish were first released onto a reef and placed under a protective cage. Those ‘field boat noise fish’ were then exposed to 15 min of real boat noise (30 hp 2-stroke Suzuki engine model DT30, 20–100 m loops). After this, the ‘field reef noise’ and the laboratory-treated fish (‘reef’ and ‘reef+boat’) were released and placed under protective cages. After 30 min, all the cages were removed and survival was monitored twice a day for up to 72 h post-release. We monitored 120 fish (N=28–31 per treatment).

Statistical analysis

Experiment 1: Behavioural data were computed into proportion change in behaviour from the pre-stimulus baseline ($[\text{post-pre}]/\text{pre}$) and these data were used in our analysis. Because the two
290 fish present in each conditioning tank were not considered independent, we introduced “conditioning tank” as a nested factor in our analysis (tanks nested within sound treatment, type I SS). We used a 3-way nested design, testing the effect of treatment (learning reef vs learning reef+boat vs no learning) and testing cue (water vs predator odour) on the behaviour of fish. Pre-stimulus baselines were compared to ensure no difference in behaviour occurred prior to the
295 stimulus presentation (results presented in the online supplement).

Experiment 2: Behavioural data from fish from the three groups were compared using a 2-way nested ANOVA, testing the effect of training and introducing ‘tank’ as a nest (type I SS). Tukey post-hoc tests were used to assess differences among groups. Survival data was analyzed using a Kaplan-Meier survival analysis.

300 *Experiment 3:* A Kaplan-Meier survival analysis was used to compare the survival of the four groups of fish.

RESULTS

Experiment 1: The responses of the fish was affected by a significant interaction between training and test cue (activity: $F_{2,41.5}=5.30$, $P=0.009$; feeding: $F_{2,44.5}=17.42$, $P<0.001$, figure 1),
305 although no effect of tank was detected (activity: $F_{45,33}=1.04$, $P=0.46$; feeding: $F_{45,33}=1.42$, $P=0.15$). The interaction stemmed from a differential response to the predator odour by fish receiving different trainings. As predicted, no-learning control fish did not respond differently to water and dottyback odour, indicative of predator naivety (activity: $F_{1,12.6}=0.10$, $P=0.76$; feeding: $F_{1,12.6}=0.57$, $P=0.47$); fish trained under reef noise playback showed a reduction in line crosses

310 and feeding strikes when exposed to the predator odour (activity: $F_{1,20.6}=41.8$, $P<0.001$; feeding:
 $F_{1,20.6}=40.1$, $P<0.001$), consistent with expected antipredator responses. Our experimental fish,
that were trained with ‘reef+boat noise’ did not respond differently to water and dottyback odour
(activity: $F_{1,12.7}=1.99$, $P=0.18$; feeding: $F_{1,13.3}=1.9$, $P=0.19$), indicating a failure to respond to the
predator odour, despite receiving identical predator training. Tank had no effect on the above
315 mentioned tests (all $P>0.2$).

Experiment 2:

Behavioural assessment: In the wild, fish from different training groups differed in their boldness
scores ($F_{2,54.3}=50.17$, $P<0.001$) and maximum distance ventured ($F_{2,55.6}=3.74$, $P=0.030$, figure 2),
but not on bite rate ($F_{2,51.3}=0.55$, $P=0.58$) or distance moved ($F_{2,52.9}=2.46$, $P=0.095$). Tank had no
320 effect (all $P>0.2$). Specifically, fish trained under reef noise were significantly shyer and stayed
closer to their coral shelter than fish from the two other groups, suggestive of predator-wary
behaviour (Tukey post-hoc comparisons: all $P<0.001$). Untrained fish and fish trained under
‘reef+boat noise’ treatments did not differ in their behaviour ($P=0.19$ and $P=0.99$ for boldness
and distance ventured respectively).

325 *Survival:* Fish from different training groups differed in their survival rates (Kaplan-Meier: $\chi^2_2=$
24.96, $P<0.001$, figure 3). Specifically, fish trained under ‘reef noise’ survived 3.5 times better
than fish that were untrained ($P<0.001$) or those that were trained under ‘reef+boat noise’
($P<0.001$). These latter two groups did not differ in their respective survival ($P=0.56$).

Experiment 3:

330 *Survival:* Fish from the four groups did not differ in their survival pattern, with an average
survival of 50% over 3 days (Kaplan-Meier : $\chi^2_3= 1.92$, $P=0.59$, supplementary figure 2).

DISCUSSION

The results of our experiments provide insights into the effect of boat noise exposure on fish cognition. Our first experiment suggested that when fish were presented with a novel predator odour paired with alarm cues in the presence of boat noise playback, they failed to subsequently display the antipredator response typically observed in fish that successfully learned to associate the predator cue with risk. Indeed, the ‘reef+boat noise’ group displayed behaviours that were not different from our ‘no learning’ controls. To ensure these results were not simply reflecting a lack of motivation, we repeated the procedure and tested both behaviour and survival in the field, following a triple-conditioning procedure. Our results were consistent, suggesting that the presence of boat noise at the time of learning reduced the subsequent antipredator response and survival compared to individuals that were trained with playbacks of reef noise. Just as we observed in the previous experiment, the behaviour and survival of the fish trained in the presence of boat noise did not differ from those of untrained fish.

The results of experiment 3 provide some insights into the mechanisms responsible for these results. One could argue that the lack of behavioural response observed in the boat noise group was not due to a learning failure *per se*, but instead, to a temporary, stress-induced decrease in performance. In other words, with enough delay between the learning and the subsequent predator exposure, those fish might have been able to display full antipredator responses and increased survival. This point is important from a mechanistic perspective but moot from an ecological one: if exposure to boat noise renders fish functionally naïve for hours or days that follow, the benefits of learning would be altered nonetheless, as demonstrated by experiment 2. Our last experiment, however, indicates that exposure to boat noise *per se*, does not induce reduced performance, as measured by *in situ* survival, our best ecological proxy of fitness. Given

355 this information, we feel confident that the observed results from experiments 1 and 2 can be attributed to a failure in learning.

Our study manipulated noise via playbacks. We took this approach because reproduction of our learning manipulations *in situ*, in the presence of real boats would be onerous in terms of time, money and effort. From a logistical perspective, a major problem also arises in the need to segregate replicates. The spatial scale needed to produce true independent replicates would compromise the experiment because predator density and predator types would likely not be comparable across a large area of reef. We fully acknowledge the potentially artificial nature of the noise exposure, given the unique soundscape that would be created by playing boat noise playbacks in our arenas. However, results from experiment 3 indicate that playback exposures, whether reef or boat noise, did not cause a measurable decline in survival compared to fish exposed to real reef noise or real boat noise. This indicates that our noise playbacks were not creating artificial mortality. We also argue that it is not the soundscape of boat *per se*, that is causing the reported effects on wildlife, but rather the elevated noise levels in the environment. Finally, Simpson *et al.* [16] compared physiological, behavioural and survival endpoints of young reef fish in the presence or absence of boat noise using both techniques, that is real boat noise exposure in the field and laboratory playbacks of boat noise. They reported similar patterns of impairments under both conditions, indicating that laboratory playbacks do not create artificial effects, as compared to real boat noise exposure. Such evidence for the validity of laboratory manipulations is important, since many ecological endpoints are not easily measurable *in situ*.

375 The detrimental effects of some pollutants stem from long-term, sub-chronic exposure to a particular stressor, via increased stress levels, while small, short-term exposures to that same stressor might not have any immediately measurable short-term consequences [36]. Cognition,

unfortunately, is one of those endpoints with latent effects: learning is only beneficial when the information learned is used in the future to make fitness-promoting decisions. These decisions
380 may come hours, days or years after the information was learned. Loss of homing behaviour, for instance, is only visible when the adult does not know where to go. Similarly, a lack of imprinting that occurred during the early life stages does not have any visible consequences until that individual needs that information; such effects of information loss via ‘sensory pollution’ are many [37]. Sound pollution, either by distracting or by reducing the efficacy of learning, has the
385 potential to cause changes to the ecological landscape of the community by interfering with information acquisition. In some contexts, such as finding foraging patches, individuals may have more than one chance to learn, relying on the redundancy of the information within their social groups. However, in some other contexts, the loss of information may have dramatic fitness consequence [38]. In our study system, for instance, prey are under severe selection
390 pressure to quickly learn and avoid predation threats, given the predation-driven bottleneck associated with their particular life history transition. We encourage future research to consider latent effects when assessing the sublethal effects of their pollutant.

Coral reef communities are already affected by a multitude of stressors, including global warming, ocean acidification, chemical pollution, overfishing and habitat degradation.
395 Communities are becoming less resilient to additional stressors [39, 40]. Noise pollution, contrary to some other stressors, can more easily be detected and controlled, both spatially and temporally. Being able to identify species and/or life stages that are particularly sensitive to noise pollution could help mitigate some of the reported effects on our marine communities.

400 **ACKNOWLEDGEMENTS:** We thank the staff from Lizard Island Research Station. Funding
for this study was provided by the Natural Sciences and Engineering Research Council of
Canada (MF, DC), the Australian Research Council (MIM, MF, DC, MGM), the ARC Center of
Excellence for Coral Reef Studies (MIM) and the UK Natural Environment Research Council
(SS).

405 **ETHICS:** All work carried herein followed animal care ethics and was approved by James Cook
University protocols A1720 and A2080.

AUTHORS' CONTRIBUTIONS: MF, DC, MIM, MGM designed the project; MF, DC, MIM,
MGM collected data, MGM, SS, SN obtained the soundtracks, MF, SN analyzed the data, MF
wrote the first draft, all authors contributed to the final draft.

410 **COMPETING INTERESTS:** We have no competing interests.

DATA AVAILABILITY: The data will be uploaded as supplementary information if the ms is
accepted.

REFERENCES

- 415 1. Hildebrand J.A. 2009 Anthropogenic and natural sources of ambient noise in the ocean.
Marine Ecology Progress Series **395**(5).
2. Hawkins A.D., Pembroke A.E., Popper A.N. 2015 Information gaps in understanding the
effects of noise on fishes and invertebrates. *Reviews in Fish Biology and Fisheries* **25**(1), 39-64.
3. Shannon G., McKenna M.F., Angeloni L.M., Crooks K.R., Fristrup K.M., Brown E.,
420 Warner K.A., Nelson M.D., White C., Briggs J. 2015 A synthesis of two decades of research
documenting the effects of noise on wildlife. *Biological Reviews*.

4. Kikuchi R. 2010 Risk formulation for the sonic effects of offshore wind farms on fish in the EU region. *Mar Pollut Bull* **60**(2), 172-177.
5. Kight C.R., Swaddle J.P. 2011 How and why environmental noise impacts animals: an integrative, mechanistic review. *Ecology Letters* **14**(10), 1052-1061.
6. Popper A.N., Halvorsen M.B., Kane A., Miller D.L., Smith M.E., Song J., Stein P., Wysocki L.E. 2007 The effects of high-intensity, low-frequency active sonar on rainbow trout. *The Journal of the Acoustical Society of America* **122**(1), 623-635.
7. Popper A.N., Hastings M.C. 2009 The effects of human-generated sound on fish. *Integrative Zoology* **4**(1), 43-52.
8. Pollack I. 1975 Auditory informational masking. *The Journal of the Acoustical Society of America* **57**(S1), S5-S5.
9. 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). *Mar Pollut Bull* **58**(12), 1880-1887.
10. N.M.M.A. 2013 Recreational Boating Statistical Abstract. In *National Marine Manufacturers Association, Industry Statistics and Research* (
11. Great Barrier Reef Marine Park Authority. 2014 Great Barrier Reef Outlook Report In *2014 GBRMPA, Townsville* (
12. Nedelec S.L., Radford A.N., Simpson S.D., Nedelec B., Lecchini D., Mills S.C. 2014 Anthropogenic noise playback impairs embryonic development and increases mortality in a marine invertebrate. *Scientific reports* **4**.
13. Filiciotto F., Vazzana M., Celi M., Maccarrone V., Ceraulo M., Buffa G., Di Stefano V., Mazzola S., Buscaino G. 2014 Behavioural and biochemical stress responses of *Palinurus elephas* after exposure to boat noise pollution in tank. *Mar Pollut Bull* **84**(1), 104-114.

14. Holles S., Simpson S.D., Radford A.N., Berten L., Lecchini D. 2013 Boat noise disrupts orientation behaviour in a coral reef fish. *Mar Ecol Prog Ser* **485**, 295-300.
15. Jacobsen L., Baktoft H., Jepsen N., Aarestrup K., Berg S., Skov C. 2014 Effect of boat noise and angling on lake fish behaviour. *Journal of Fish Biology* **84**(6), 1768-1780.
- 450 16. Simpson S.D., Radford A.N., Nedelec S.L., Ferrari M.C., Chivers D.P., McCormick M.I., Meekan M.G. 2016 Anthropogenic noise increases fish mortality by predation. *Nature communications* **7**.
17. Shettleworth S.J. 2001 Animal cognition and animal behaviour. *Animal Behaviour* **61**, 277-286. (doi:10.1006/anbe.2000.1606).
- 455 18. Brown G.E., Chivers D.P. 2005 Learning as an adaptive response to predation. In *Ecology of predator-prey interactions* (eds. Barbosa P., Castellanos I.), pp. 34-54. Oxford, Oxford University Press.
19. Ferrari M.C.O., Wisenden B.D., Chivers D.P. 2010 Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology* **88**(7),
- 460 698-724.
20. Kusch R.C., Krone P.H., Chivers D.P. 2008 Chronic exposure to low concentrations of waterborne cadmium during embryonic and larval development results in the long-term hindrance of antipredator behavior in zebrafish. *Environmental Toxicology and Chemistry* **27**(3), 705-710. (doi:10.1897/07-273.1).
- 465 21. Brown G.E., Elvidge C.K., Ferrari M.C., Chivers D.P. 2012 Understanding the importance of episodic acidification on fish predator-prey interactions: Does weak acidification impair predator recognition? *Science of The Total Environment* **439**, 62-66.

22. Ferrari M.C.O., Manassa R.P., Dixson D.L., Munday P.L., McCormick M.I., Meekan M.G., Sih A., Chivers D.P. 2012 Effects of ocean acidification on learning in coral reef fishes. *Plos One* **7**(2), e31478.
- 470
23. Chivers D.P., McCormick M.I., Nilsson G.E., Munday P.L., Watson S.-A., Meekan M.G., Mitchell M.D., Corkill K.C., Ferrari M.C.O. 2014 Impaired learning of predators and lower prey survival under elevated CO₂: a consequence of neurotransmitter interference. *Global Change Biology* **20**, 515-522.
- 475
24. Chivers D.P., McCormick M.I., Mitchell M.D., Ramasamy R.A., Ferrari M.C.O. 2014 Background level of risk determines how prey categorize predators and non-predators. *Proceedings of the Royal Society B: Biological Sciences* **281**(1787), 20140355.
25. Crane A.L., Ferrari M.C.O. 2013 Social learning of predation risk: a review and prospectus. In *Social learning theory: phylogenetic considerations across animal, plant, and microbial taxa* (ed. Clark K.B.), pp. 53-82, Nova Science Publisher. N-Y, USA.
- 480
26. Cowen R.K., Sponaugle S. 1997 Relationships between early life history traits and recruitment among coral reef fishes. In *Early life history and recruitment in fish populations* (pp. 423-449, Springer.
27. Almany G.R., Webster M.S. 2006 The predation gauntlet: early post-settlement mortality in reef fishes. *Coral Reefs* **25**(1), 19-22. (doi:10.1007/s00338-005-0044-y).
- 485
28. Ferrari M.C., McCormick M.I., Meekan M.G., Chivers D.P. 2015 Background level of risk and the survival of predator-naive prey: can neophobia compensate for predator naivety in juvenile coral reef fishes? *Proceedings of the Royal Society of London B: Biological Sciences* **282**(1799), 20142197.

- 490 29. Lönnstedt O.M., McCormick M.I., Meekan M.G., Ferrari M.C.O., Chivers D.P. 2012
Learn and live: predator experience and feeding history determines prey behaviour and survival.
P Roy Soc B-Biol Sci **279**(1736), 2091-2098. (doi:10.1098/rspb.2011.2516).
30. Hoey A.S., McCormick M.I. 2006 Effects of subcutaneous fluorescent tags on the growth
and survival of a newly settled coral reef fish, *Pomacentrus amboinensis* (Pomacentridae). In
495 *Proceedings of the 10th international coral reef symposium* (pp. 420-424).
31. Ferrari M.C.O., McCormick M.I., Allan B.J.M., Choi R., Ramasamy R.A., Johansen J.L.,
Mitchell M.D., Chivers D.P. 2015 Living in a risky world: the onset and ontogeny of an
integrated antipredator phenotype in a coral reef fish. *Scientific reports* **5**.
32. Feeney W., Lönnstedt O., Bosiger Y., Martin J., Jones G., Rowe R., McCormick M.I.
500 2012 High rate of prey consumption in a small predatory fish on coral reefs. *Coral Reefs* **31**(3),
909-918.
33. Ferrari M.C.O., Dixon D.L., Munday P.L., McCormick M.I., Meekan M.G., Sih A.,
Chivers D.P. 2011 Intrageneric variation in antipredator responses of coral reef fishes affected by
ocean acidification: implications for climate change projections on marine communities. *Global*
505 *Change Biology* **17**(9), 2980-2986.
34. Munday P.L., Dixon D.L., McCormick M.I., Meekan M., Ferrari M.C.O., Chivers D.P.
2010 Replenishment of fish populations is threatened by ocean acidification. *Proceedings of the*
National Academy of Sciences of the United States of America **107**, 12930-12934.
(doi:10.1073/pnas.1004519107).
- 510 35. McCormick M.I. 2009 Behaviourally mediated phenotypic selection in a disturbed coral
reef environment. *Plos One* **4**(9), e7096.
36. Klaassen C.D. 2013 *Casarett and Doull's toxicology: the basic science of poisons*,
McGraw-Hill New York (NY).

37. Zakon H.H. 2015 Human impact on fish sensory systems in the long term: An
515 evolutionary perspective. *Integrative zoology* **10**(1), 83-90.
38. Lönnstedt O.M., McCormick M.I., Chivers D.P., Ferrari M.C.O. 2014 Habitat
degradation is threatening reef replenishment by making fish fearless. *Journal of Animal Ecology*
83, 1178-1185.
39. Crain C.M., Kroeker K., Halpern B.S. 2008 Interactive and cumulative effects of multiple
520 human stressors in marine systems. *Ecology Letters* **11**(12), 1304-1315.
40. Darling E.S., McClanahan T.R., Côté I.M. 2013 Life histories predict coral community
disassembly under multiple stressors. *Global change biology* **19**(6), 1930-1940.

FIGURE CAPTIONS:

- 525 Figure 1: Mean (\pm SE) proportion change in foraging (a) and activity (b) for juvenile
Pomacentrus amboinensis exposed to water (empty bars) or predator odour (solid bars). Fish
underwent a false conditioning with reef playback (no learning with reef playback), or were
trained to recognize a novel predator odour in the presence of reef noise (learning with reef
playback) or in the presence of reef+boat noise (learning with reef+boat playback).
- 530 Figure 2: Mean (\pm SE) boldness score (a) and maximum distance ventured (b) for juvenile
Pomacentrus amboinensis released in the wild on small patch reefs. Prior to release, fish
underwent a false conditioning with reef playback (no learning with reef playback), or were
trained to recognize a novel predator (sight and smell) in the presence of reef noise (learning
with reef playback) or in the presence of reef+boat noise (learning with reef+boat playback).
- 535 Figure 3: Survival plot (72 h) of juvenile *Pomacentrus amboinensis* released in situ on small
patch reefs. Prior to release, fish underwent a false conditioning with reef playback (no learning
with reef playback), or were trained to recognize a novel predator (sight and smell) in the
presence of reef noise (learning with reef playback) or in the presence of reef+boat noise
(learning with reef+boat playback).

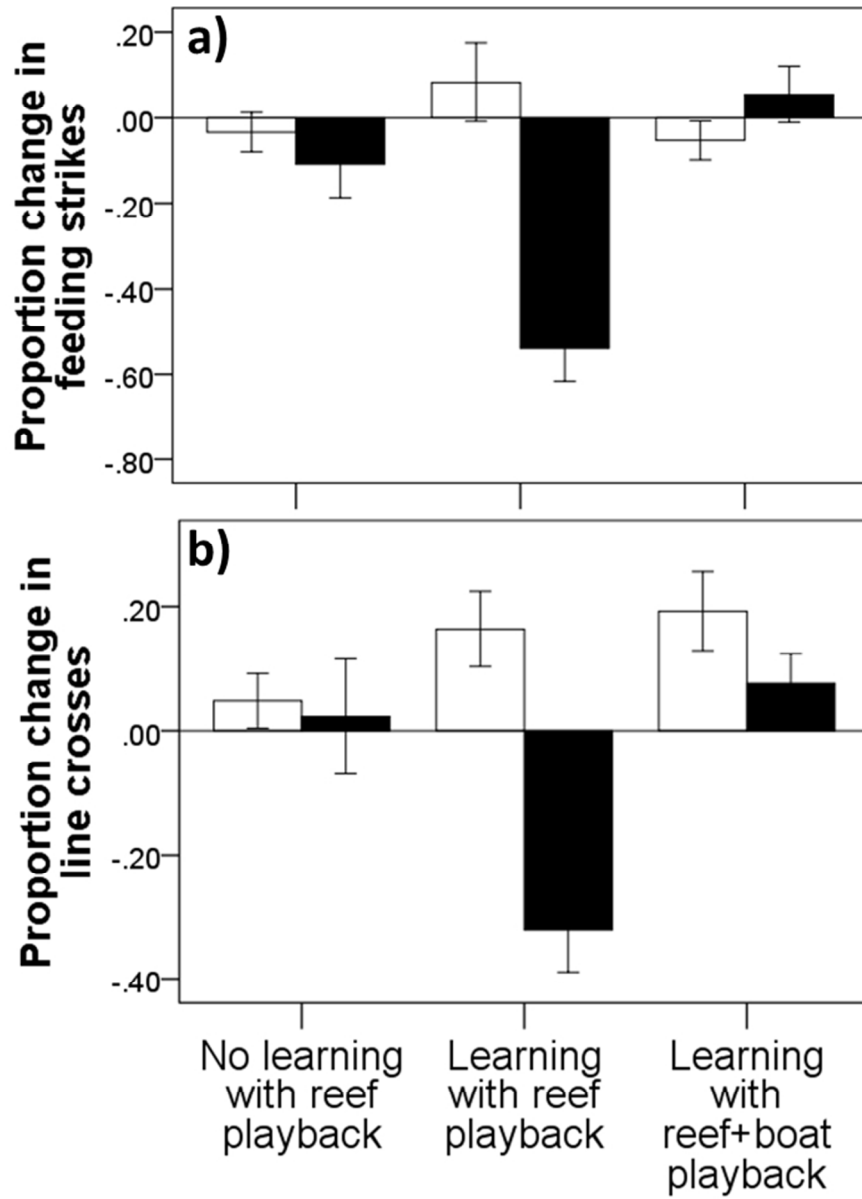


Figure 1

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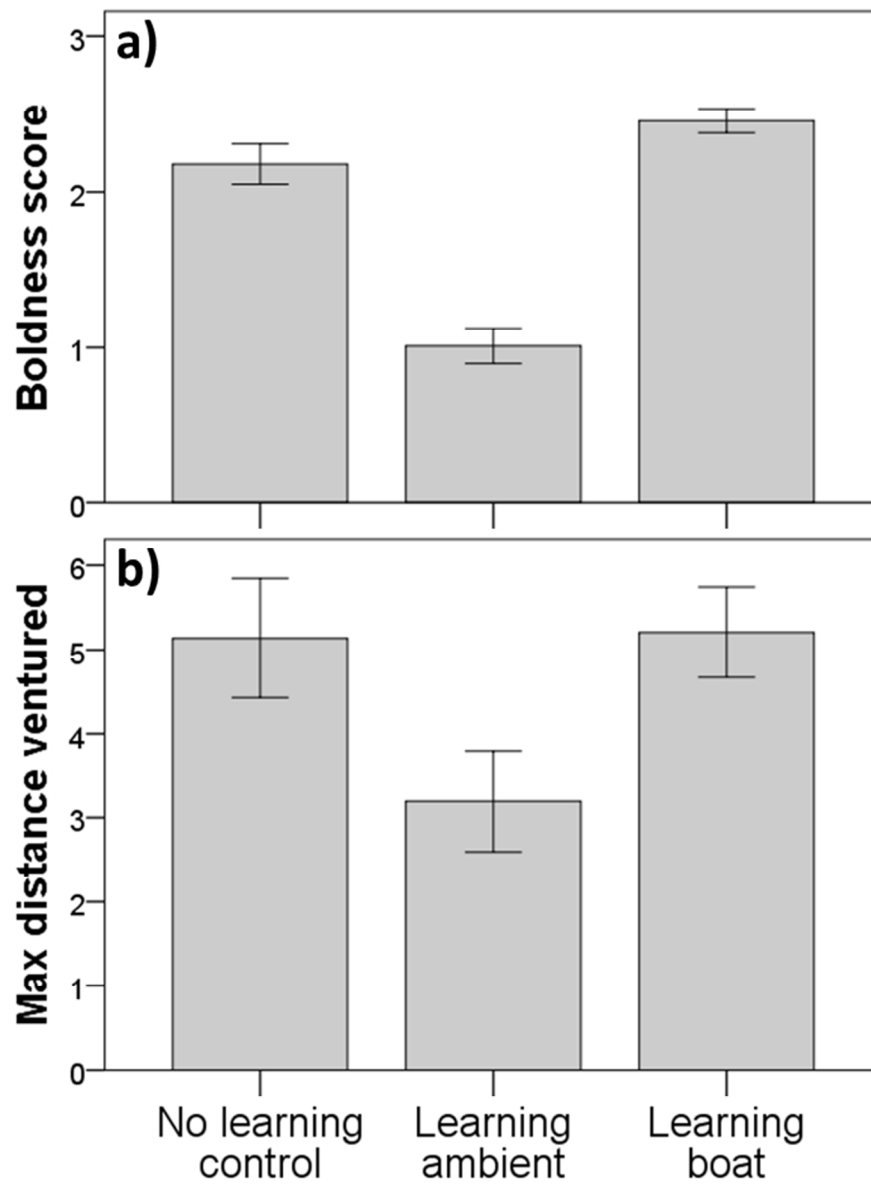


Figure 2

190x251mm (96 x 96 DPI)

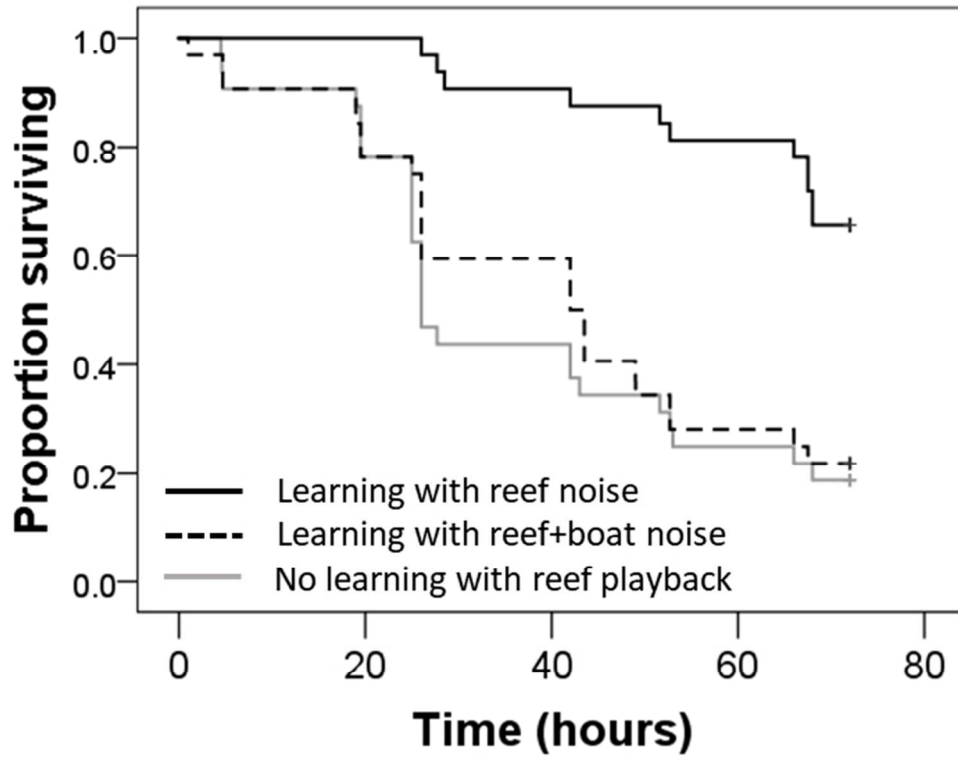


Figure 3

190x157mm (96 x 96 DPI)