

Response of Marine Invertebrate Larvae to Natural and Anthropogenic Sound: A Pilot Study

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Abstract: Many vertebrates and invertebrates in the marine environment create and respond to sound. Due to increasing use of waterways, levels of anthropogenic sound are greater than ever. We examined the responses of larvae of temperate invertebrates to three sound treatments: natural ambient sound (shallow rocky reef), anthropogenic sound (an outboard motor) and no sound (control). Sound recordings were played to molluscan, echinoderm and bryozoan larvae in Petri dishes in the laboratory and the movement of swimming larvae was filmed and quantified in two-dimensional space. Larvae of the gastropod *Bembicium nanum* increased their swimming activity in response to both natural and anthropogenic sound, while larvae of the bryozoan *Bugula neritina* decreased swimming activity when exposed to boat sounds, but not recordings from the natural reef. Considerable variation was observed in the swimming behavior of larvae of the echinoid *Heliocidaris erythrogramma* and we did not observe any differences in response among the treatments. The behavior of the oyster *Crassostrea gigas* was dependent on its nutritional status. Unfed larvae did not respond to sound, whereas fed larvae increased swimming activity, but only in response to natural sound. Hence effects were highly species-specific, with three of the four species showing some response to sound and apparently distinguishing among different sound frequencies. This study adds to the growing body of evidence that sound may be an important behavioral cue. It may justify further research into the use of sound as an antifouling agent or a tool in the restoration of reef species.

Keywords: Marine invertebrate larvae, noise, recruitment, sound, settlement, swimming behavior.

INTRODUCTION

Human impacts on coastal ecosystems have received considerable attention, with the effects of pollution, marine species harvesting, invasive species and physical changes to the marine habitat as focal areas of research [1, 2]. In contrast, the auditory impacts of coastal activities on marine organisms have been generally ignored or overlooked, despite significant increases in recreational boating and the growth of port facilities and associated industrial shipping [3-5]. It is now well established that anthropogenic activities have contributed to significant increases in the level and changes in the character of underwater ambient noise [3, 6]. The impacts on cetaceans and other mammals have been the focus of such work [7], but it is clear that a large number of non-mammalian marine organisms, including fish and marine invertebrates, also respond to sound [8-11].

Ambient noise in shallow temperate and tropical waters shows large temporal and spatial variability [3]. This is particularly evident in the 500Hz to 5kHz frequency range as a result of biological choruses, particularly after sunset [3]. In temperate regions, snapping shrimp and urchins are significant contributors to the underwater chorus and show dramatic diurnal changes associated with the crepuscular feeding habits of reef associated urchins [12, 13]. In addition

to ambient noise, high levels of shipping noise are evident near ports and shipping routes in the 50-300 Hz frequency range [6]. The main form of sound associated with shipping is hull vibration [14]. Fishing activity, including sounds of motors, is a further source of anthropogenic noise in the marine environment, generating sound across a range of frequencies [6]. Any significant increase in these levels of underwater ambient noise may impact animals that use acoustics to locate prey or communicate.

A variety of post-larval marine invertebrates, particularly crustaceans, have been demonstrated to respond to or use sound for communication or defensive purposes [11, 15-19]. Similarly, larval fishes on tropical reefs use sound to locate suitable sites for settlement [20, 21]. It is likely that the functions and effects of sound are much more pervasive than we currently believe in marine systems, but the stage of invertebrate development at which sound can be detected and used as a cue is poorly studied.

Here we determine whether larvae from a variety of invertebrate phyla other than crustaceans respond to sound. We examined in the laboratory two dimensional changes in the movement of larvae in response to anthropogenic and natural sound, testing the null hypothesis that sound had no effect on swimming activity.

MATERIALS AND METHODS

Collection of Larvae

Larvae of four invertebrate species, representing three phyla, were exposed to sound in the laboratory. Larvae of the

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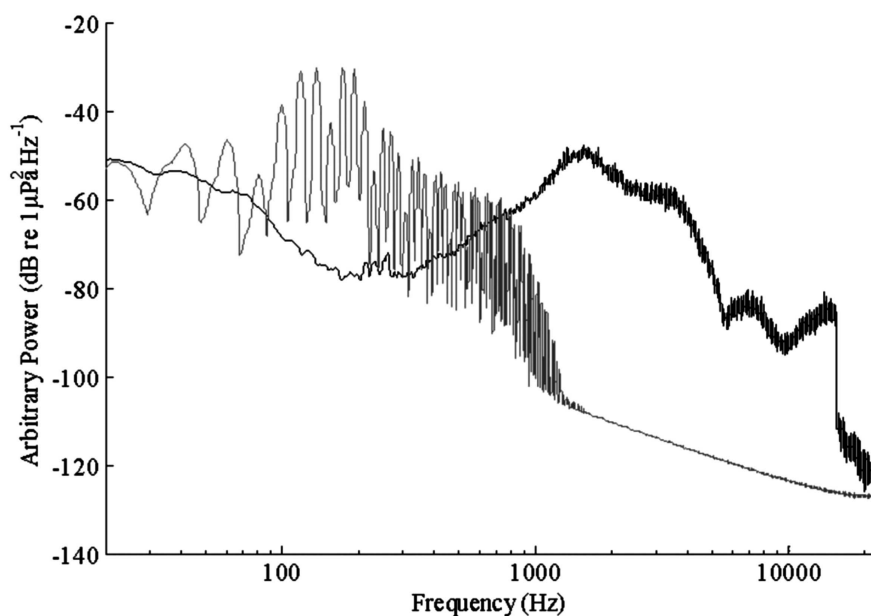


Fig. (1). Spectral composition of the two sound treatments: natural (reef) sound (black) and anthropogenic (outboard motor) sound (grey).

arborescent bryozoan *Bugula neritina* were obtained by light-shocking reproductive colonies in the laboratory [22]. Adults were collected from pier pilings in Port Kembla Harbor, (34°27'48.63"S, 150°54'14.70"E) and were held in aerated seawater overnight. Egg masses of the common intertidal gastropod *Bembicium nanum* were collected from the rock platform at North Wollongong (34°25'9.29"S, 150°54'19.37"E). Egg masses were held in aerated seawater, changed daily, until the veligers hatched (approximately 6 days). Larvae of the direct-developing sea urchin *Heliocidaris erythrogramma* were reared in the laboratory by removing reproductive products from mature adults collected at Little Bay Sydney (33°58'48"S, 151°15'08"E). Following fertilization of this urchin [23], larvae were approximately 2 days post fertilization when used in our experiments. Finally, 5-day-old veliger larvae of the Pacific oyster *Crassostrea gigas* were obtained from the Port Stephens Fisheries Centre. Larvae were fed twice daily on a mix of approximately 50% (based on cell number) *Chaetoceros calcitrans* (CS-178, CSIRO culture accession No), 25% Tahitian *Isochrysis* aff. *galbana* (CS-177) and 25% *Pavlova lutheri* (CS-182). Five hours prior to the investigation ~ 3000 veligers were removed from grow out tanks prior to feeding so that the response of unfed individuals could be compared to fed larvae. At the time of the experiments they had not been fed for 12 hours.

Exposure to Sound

Larvae were exposed to three sound treatments: (i) natural ambient sound (recording of waves striking a shallow rocky reef), (ii) anthropogenic sound (recording of an outboard motor) and (iii) no sound (control). These natural and anthropogenic sounds were chosen because of their striking differences in frequency and their overlap with known natural and anthropogenic sounds [3, 6] (Fig. 1). Sounds were played on a portable CD player (Panasonic) mounted on a separate bench 35 cm from dishes containing larvae to prevent potential effects of vibration. The speaker was directed toward the petri dish. The laboratory was vacated and ambient noise kept to a minimum during all trials.

Larvae were pipetted into 85mm diameter Petri dishes with six ml of filtered seawater in the following numbers: 500 *C.gigas* per dish or 300 *B. neritina*, *B. nanum* or *H. erythrogramma* per dish. Following pipetting into the Petri dishes larvae were allowed to stand for 3 minutes before recording commenced. The movement of larvae was recorded with a hard disk camcorder (JVC Everio) mounted 10cm directly above the Petri dish and each recording (replicate) lasted 5 minutes. A blue background was used to improve contrast for recordings of *C. gigas* and *H. erythrogramma* larvae and the other two species were recorded over a white background. Cardboard screens were used to minimise directional light and minimize reflection.

Swimming activity was assessed by quantifying the movement of larvae into and out of circles drawn onto a television screen. Four 8.5 cm circles were randomly positioned with no overlap on the screen. A pilot study confirmed that circles of this size yielded the lowest standard deviation relative to the number of movements across the boundary. The number of larvae that moved into or out of the circle for each 5 minute recording were combined for the four circles and divided by four, yielding a single movement count for each replicate. Larvae that were spinning in close proximity to the circle edge, with a typical spinning diameter of approximately 10 times the body length of the species being examined, were excluded from counts because it was feared they might bias the outcome.

Statistical Analysis

A one-factor ANOVA was used to compare movements across the three sound treatments (fixed factor) for *B. neritina*, *B. nanum* and *H. erythrogramma*. Groups of larvae were exposed to a single sound treatment and these were conducted in random sequence, so that they remained independent of one another. Levels of replication; *B. nanum* and *B. neritina* n=8, *H. erythrogramma* n=6. A two-factor ANOVA was used to examine movements in *C. gigas* for the sound (fixed) and nutritional status (fixed) treatments. Levels of replication were n=6 for fed *C. gigas* and n=4 for unfed *C.*

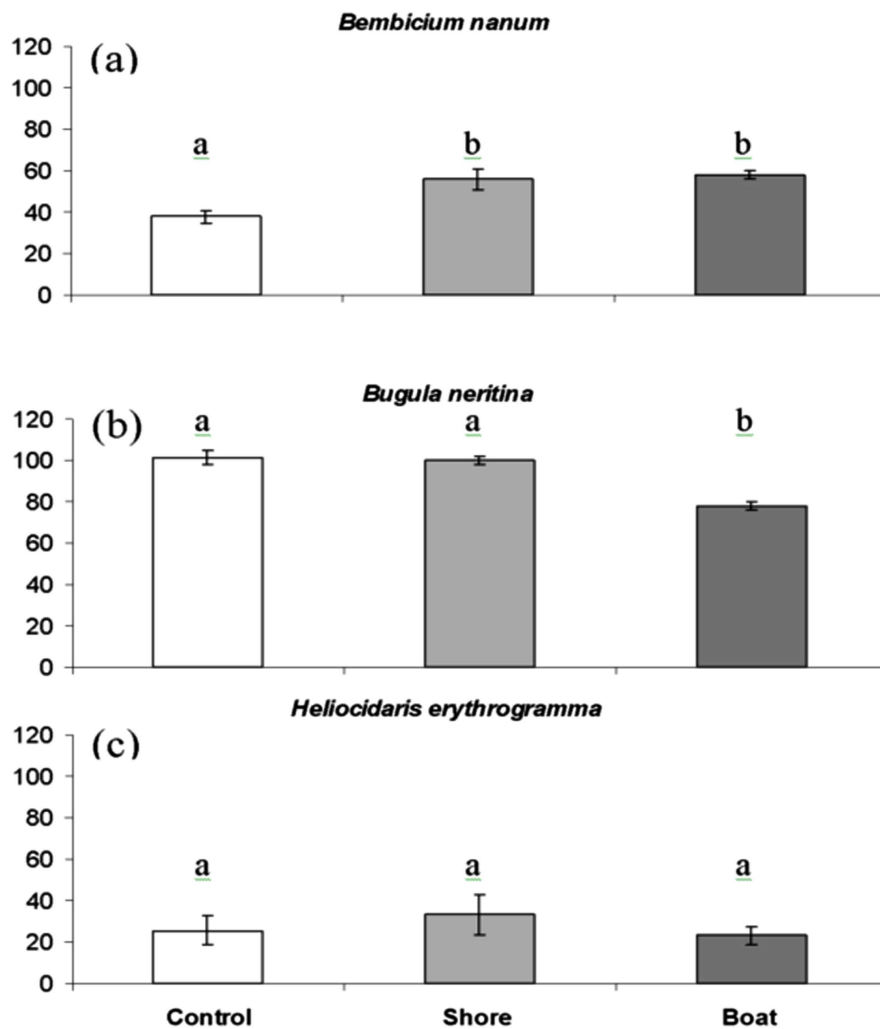


Fig. (2). Mean movement (\pm SE) of larvae of (a) *Bembicium nanum* (n=8) (b) *Bugula neritina* (n=8) and (c) *Heliocidaris erythrogramma* (n=6) in response to sound treatments over a 5 minute period. See text for a description of movement. Sound treatments were: no sound, natural reef sound and anthropogenic sound. Number of larvae in each replicate=300. Bars with the same letters were not significantly different (SNK tests).

Table 1. ANOVA Results for Movement of Three Species of Invertebrate Larvae (*Bembicium nanum*; Mollusca, *Bugula neritina*; Bryozoa and *Heliocidaris erythrogramma*; Echinodermata) in Response to Three Sound Treatments (Fixed Factor): Natural Reef Sound, Anthropogenic Sound (Outboard Boat Motor) and no Sound (Control)

Source	<i>B. nanum</i>				<i>B. neritina</i>				<i>H. erythrogramma</i>			
	df	MS	F	P	df	MS	F	P	df	MS	F	P
Sound	2	977.75	9.02	0.0015	2	1369.6	27.02	<0.001	2	137.3	0.52	0.609
RES	21	108.38			21	50.7			12	265.8		

Cochran's C: *B.nanum* = 0.5995 (NS); *B.neritina* = 0.5995 (NS); *H.erythrogramma* =0.5816 (NS).

gigas. Assumptions of these analyses were examined before proceeding; normality was examined visually and homogeneity among the variances determined with Cochran's C test. We used SNK tests for post hoc comparisons. We used GMAV5 (University of Sydney) and JMP (5.1) for the analyses.

RESULTS

Bryozoan and molluscan larvae responded to sound treatments, but the patterns were not consistent between

species. Larvae of *Bembicium nanum* were almost twice as active in response to sound, irrespective of whether it was natural or anthropogenic in origin (Fig. 2a, Table 1). In contrast, larvae of *Bugula neritina* appeared to discriminate among sound frequencies, reducing their swimming activity by 20% in response to anthropogenic sound, while we did not detect differences in activity between reef sound and control treatments (Fig. 2b, Table 1). Movement of *H. erythrogramma* larvae was much lower than for the other species, highly variable and not significantly different among the treatments (Fig. 2c; Table 1).

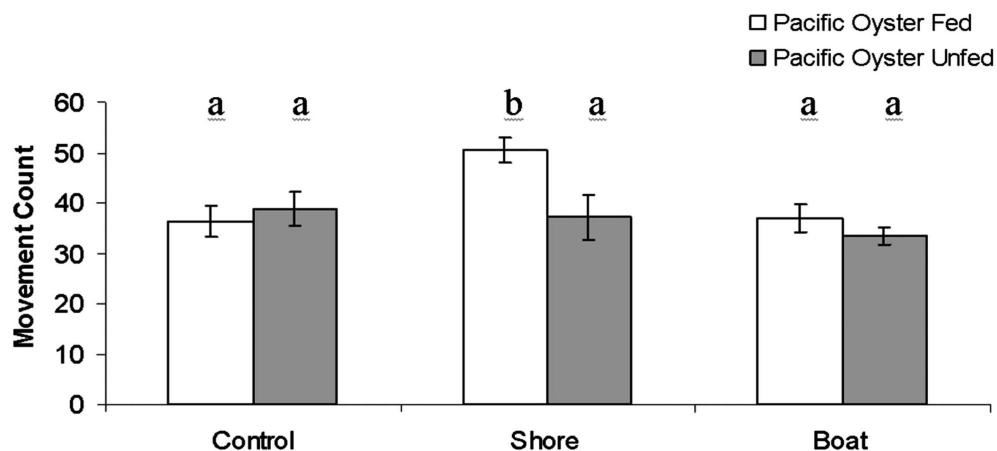


Fig. (3). Mean movement (\pm SE) of fed and unfed *Crassostrea gigas* larvae in response to sound treatments over a 5 minute period. Sound treatments as for Fig. 1. Number of larvae in each replicate=500; replication for fed and unfed = 6 and 4, respectively. Bars with the same letters were not significantly different (SNK tests).

Table 2. Two-factor ANOVA Results for Patterns of Movement in Oyster Larvae (*Crassostrea gigas*) in Response to Three Sound Treatments (Fixed Factor); Natural Reef Sound, Anthropogenic Sound (Outboard Boat Motor) and no Sound (Control) When Fed and Unfed (Nutritional Status – Fixed Factor)

Source	d.f.	MS	F	P
Feeding Status	1	167.81	3.7	0.066
Sound	2	190.40	4.2	0.027
FS * S	2	156.31	3.4	0.048
RES	24	43.35		

Cochran's C= 0.5816 (NS)

The response of the *C. gigas* larvae to the sound treatments was a function of their nutritional status and type of sound. There was no change in swimming activity in response to sound when larvae were unfed (Fig. 3). In contrast, fed larvae showed an increase in activity of >25% in response to the natural reef sound, but no increase in control and boat motor sounds. We detected a significant interaction between these factors (Table 2, SNK tests).

DISCUSSION

Larvae of three of the four invertebrate species examined responded to the treatments and the responses were strongly species specific. While larvae of *Bembicium nanum* increased their swimming activity on exposure to sound, irrespective of the source, larvae of *Bugula neritina* reduced their swimming activity when exposed to the anthropogenic sound. No response was detected for larvae of *Heliocidaris erythrogramma* or unfed *Crassostrea gigas* larvae to any sound treatment, but fed oyster larvae significantly increased their swimming activity in response to natural sound.

There is considerable evidence that adult invertebrates use sound in defense, communication and orientation [15, 17, 18, 19]. Our work adds to the body of evidence that invertebrate larvae in the temperate zone also respond to sound [11, 24] potentially modifying their time to settlement with increased swimming activity [25]. Importantly, all species in our study, with the exception of the echinoid, appear to be capable of distinguishing among sound frequencies. It remains unclear how these larvae detect sound

and whether the behavioral changes we have observed in the laboratory are sufficient to alter the distribution of weakly-swimming invertebrate larvae in the plankton or the likelihood of their settlement. It is clear that noise associated with surf and wave action on rock platforms can radiate out to at least 9 km offshore [26] with a 10 Hz to 500 kHz frequency range [6], corresponding to reef sound frequencies used here.

Examination of shallow water ambient sound in temperate and tropical waters around Australia confirms high levels of traffic noise near ports and shipping routes [3]. There is evidence that sound can have detrimental impacts on invertebrates; for example, exposing brown shrimp (*Crangon crangon*) to high sound levels in the laboratory stunted growth, compromised reproduction and induced aggressive cannibalistic behavior [9]. Although it is not clear whether anthropogenic noise in the marine environment will adversely affect larvae, our findings indicate that larvae are capable of detecting and responding to sound. The potential impacts of anthropogenically generated sounds are thus deserving of much closer attention, and field experiments are required, but it is premature to suggest that anthropogenic sound has a negative impact. A further implication of the present research is that the utility of noise as a benign antifouling or species restoration tool is also worthy of closer scrutiny [27].

CONFLICT OF INTEREST

None declared.

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