

Sound detection *in situ* by the larvae of a coral-reef damselfish (Pomacentridae)

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ABSTRACT: Settlement-stage pelagic larvae of the coral-reef damselfish *Chromis atripectoralis* consistently swam to the south at 24 to 25 cm s⁻¹ in day-time ambient conditions off Lizard Island, Great Barrier Reef. This was true on both the windward and leeward sides of the island, 100 to 1000 m from the nearest reef. Larvae released during the day 25 to 100 (mean 58) m from an underwater speaker broadcasting nocturnal reef sounds had no overall swimming direction. This was true on both windward and leeward sides of the island, 500 to 1000 m from the nearest reef. The broadcast sounds resulted in an alteration of behavior indicating that the larvae heard them. In the presence of the nocturnal reef sounds, swimming speed increased about 5 cm s⁻¹ off the leeward side but not the windward side. Larvae released 50 to 150 (mean 78) m from a speaker broadcasting artificial sound (pure tones) at the leeward location swam to the south at 30 cm s⁻¹. This shows that larvae of *C. atripectoralis* can distinguish between a sound with potential biological significance and one devoid of biological significance. Larvae did not swim in any particular direction relative to the speaker when nocturnal reef sounds were broadcast; therefore, we have no evidence that the larvae can localize these sounds. We conclude that settlement-stage larvae of this damselfish can hear reef sounds, and can distinguish between reef sounds and an artificial sound, but we have no indication that they can localize the sound. We speculate on the reasons for altered swimming behavior in the presence of reef sounds.

KEY WORDS: Fish · Dispersal · Settlement · Larva · Behavior · Hearing

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INTRODUCTION

Nearly all teleost reef fishes have a pelagic larval stage (Leis 1991). At the end of this stage, the larva must find a suitable reef habitat upon which to settle, and little is known about how this is accomplished. Considerable uncertainty exists about the sensory abilities of larval fishes, especially their abilities to detect and respond to sensory cues that might be useful for locating settlement habitat. Recent reviews have concluded that although a number of possible senses could be used for navigation to settlement sites, only 3—vision, olfaction and hearing—are known to be functional during the pelagic larval stage of reef fishes, and the latter 2 in only a few species (Leis &

McCormick 2002, Myrberg & Fuiman 2002, Kingsford et al. in press). Of these, none has been shown to be useful over scales of more than a few meters, although it is likely that all may play a role in navigation to settlement sites (Kingsford et al. in press). The literature contains persistent assertions that sound should be useful to larval fishes attempting to find settlement sites (e.g. Leis et al. 1996, Doherty & Carleton 1997, Wolanski et al. 1997, Stobutzki & Bellwood 1998). The reasons are obvious: reefs are noisy places, sound travels well under water and at least the otoliths of larval fishes, if not functional auditory senses, are present at a very early stage in larval development (Cato 1978, 1992, Leis et al. 1996, Leis & McCormick 2002). However, on the basis of recently settled damselfishes, Myrberg & Fuiman (2002) conclude that reef-fish larvae are unlikely to hear well (i.e. they have a high-

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threshold hearing level). Only 1 study has shown that larvae of any reef fish can hear and use sound to navigate (Tolimieri et al. 2000): a single species of temperate blenny (Tripterygiidae) was more abundant in light traps that broadcast a reef sound than in light traps that were silent.

Determination of the hearing abilities of fish larvae is difficult. First, work with sound in the laboratory is fraught with complications (Kenyon 1996). Second, the standard method of measuring hearing abilities in fishes involves conditioning or training the animal to respond to stimuli (e.g. Mann et al. 1997). This indirect behavioral method is extremely challenging when the object of study is a larva that is developing and, indeed, metamorphosing at a rapid rate. By the time the training is complete the larva will probably no longer be a larva, but a juvenile, quite possibly with different hearing abilities. A more direct method, measurement of the auditory brainstem response (Kenyon et al. 1998), is becoming more widely used but is very difficult to apply to very small fishes such as settlement-stage reef-fish larvae (D. Higgs pers. comm.). We used a different indirect behavioral method in the field to measure the hearing abilities of reef-fish larvae.

In a field experiment, we attempted to determine whether the larvae of a common coral-reef damselfish, *Chromis atripectoralis*, could do the following: (1) detect sounds we broadcast underwater; (2) distinguish between natural reef sounds that are potentially biologically meaningful and meaningless artificial sounds; and (3) locate the source of the sound.

In each case, the null hypothesis was that the larvae could not do these things.

We used differences in swimming behavior—speed and direction *in situ*—under experimental and ambient conditions as the criteria that the sound we broadcast underwater had been heard. The consistent swimming behavior of larvae of *Chromis atripectoralis* in ambient open water conditions provided the opportunity to clearly identify alterations in behavior due to the sounds we broadcast. In short, at least during the morning and early afternoon in the vicinity of Lizard Island on the Great Barrier Reef, larvae of this species swim to the south at about 24 cm s^{-1} in ambient conditions. Alterations to this behavior under experimental sonic conditions show that the sounds were detected, although not all the null hypotheses could be rejected.

We used sounds recorded over local coral reefs at night as the sound cue in our experiments. There is good evidence for a few species of pomacentrids that the majority of (although not all) settlement takes place at night (reviewed in Leis & Carson-Ewart 1999). Further, Stobutzki & Bellwood (1998) found that damselfish larvae (an unspecified mix of species) swam toward reefs at night and concluded that sound

was the cue most likely to have been used by the larvae to achieve this. The highest noise levels in tropical waters tend to occur at night in the vicinity of reefs due to activity of biological sources (Cato 1978, McCauley & Cato 2000). Therefore, it is reasonable to hypothesize that sounds emanating from reefs at night provide cues that settlement-stage pomacentrid larvae can use for navigation. Diurnal reef sounds might be used in a similar manner, but they are present in ambient conditions during the day, which was the only time we could conduct our experiments. We reasoned that this decreased the possibility that we could detect any effect on behavior that diurnal reef sounds might have stimulated.

MATERIALS AND METHODS

The study took place off Lizard Island, on Australia's Great Barrier Reef (14.5° S , 146° E , Fig. 1). A sound system consisting of a cassette player, a car-stereo amplifier, a 12 V battery and an underwater speaker (University Sound UW30) with a 5 m cable was placed in an anchored 2.3 m inflatable boat. Larvae were released near the anchored speaker boat, distributed in all directions so that there was no significant directionality to the release location relative to the sound source ($r = 0.10$ to 0.32 , $p = 0.17$ to 0.79 , Rayleigh test; see below). In the first experiment, sounds recorded during the night of 12 December 1998 over the reefs at Lizard

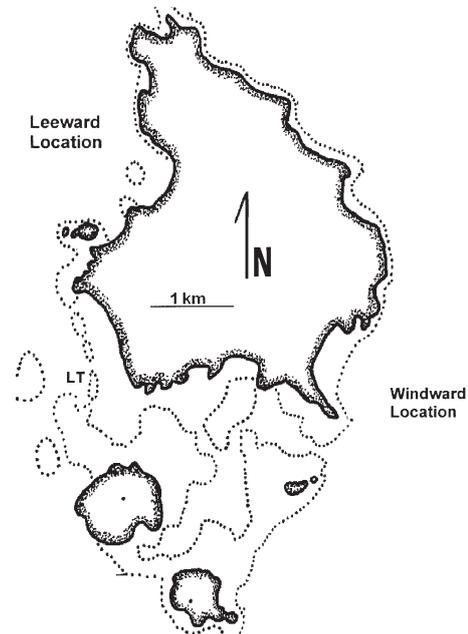


Fig. 1. Lizard Island, Great Barrier Reef. Study areas were the leeward location off the NW side of the island and the windward location off the SE side of the island. LT is location of the light traps. Dotted lines are coral reefs

Island were broadcast, and larvae were released 25 to 100 (mean 58) m from the speaker. A 45 min tape of nocturnal reef sounds was constantly repeated (Fig. 2a–c). In the second experiment, a constant artificial sound consisting of a mixture of 4 pure tones (100, 500, 1000 and 2000 Hz) was broadcast, and larvae were released 50 to 150 (mean 78) m from the speaker (Fig. 2d). The range between 100 and 2000 Hz covers the frequencies over which fishes are reported to be able to hear well (Fay & Simmons 1999) but includes neither the infrasound nor ultrasound frequencies recently reported to be detectable by a few species (e.g. Mann et al. 1997). Most tones of the artificial sound were detectable above background to distances of more than 200 m, and the strongest was still evident at 500 m. The inflatable boat was anchored 500 to 1000 m off the nearest reefs at Lizard Island on either the windward (SE) or leeward (NW) side of the island (Fig. 1). Larvae were also released in ambient conditions 100 to 1000 m off the nearest reef in the same locations. In this case, no inflatable boat was used, and no sounds were broadcast.

The nocturnal reef sounds were recorded onto a Sony WM-D6 audio cassette recorder using an Edmund Scientific Corporation hydrophone with a cus-

tom-built preamplifier, with a system frequency response 30 Hz to 6 kHz. During broadcast of both nocturnal reef sounds and the artificial sounds, the resulting sound field was recorded as a function of distance from the loudspeaker using a HighTech HTI-96-MIN hydrophone with built-in preamplifier and Sony WM-D6C recorder, with a frequency response 30 Hz to 15 kHz. The recordings' acoustic data were analyzed using a Hewlett Packard type 3567A Dynamic Signal Analyzer and sound spectrograms produced using Spectrogram software version 5.1.7 (R. S. Horne) after editing using Cool Edit Pro.

A sound spectrogram of the nocturnal reef sounds used in the broadcast is shown in Fig. 2a. It is rich in biological sounds, mainly very short-duration clicks and snaps evident as vertical lines on the spectrogram. The sources were mainly snapping shrimp (Johnson et al. 1947), with some contribution from other invertebrates and fish. The reef sounds were broadcast during daylight hours at a position 500 to 1000 m from the nearest reef, so that the background ambient noise was considerably lower than the sound that prevailed

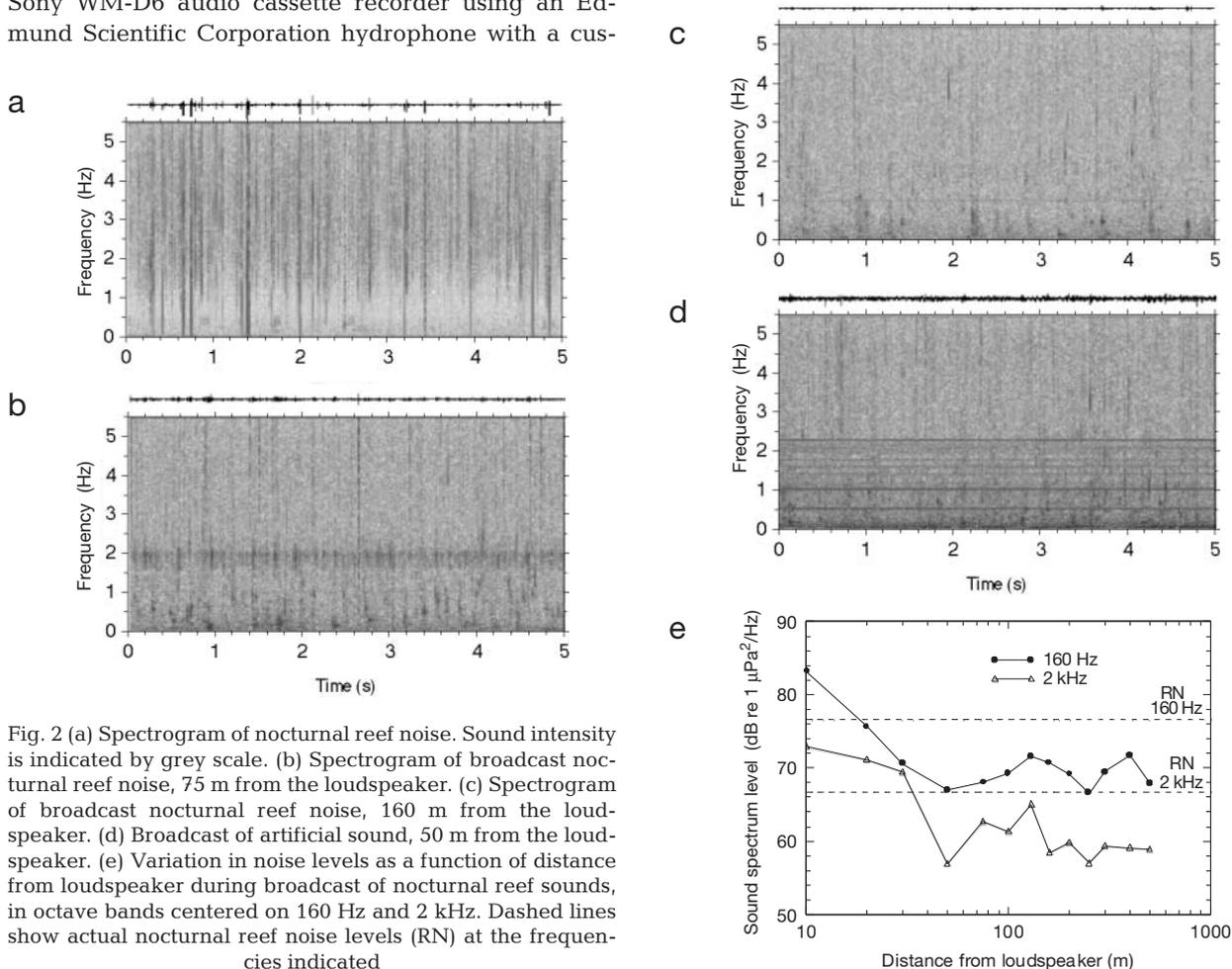


Fig. 2 (a) Spectrogram of nocturnal reef noise. Sound intensity is indicated by grey scale. (b) Spectrogram of broadcast nocturnal reef noise, 75 m from the loudspeaker. (c) Spectrogram of broadcast nocturnal reef noise, 160 m from the loudspeaker. (d) Broadcast of artificial sound, 50 m from the loudspeaker. (e) Variation in noise levels as a function of distance from loudspeaker during broadcast of nocturnal reef sounds, in octave bands centered on 160 Hz and 2 kHz. Dashed lines show actual nocturnal reef noise levels (RN) at the frequencies indicated

during the reef noise recordings, partly because ambient noise tends to be lower in Australian tropical waters during daylight hours (Cato 1978, 1992) and partly because of a lower concentration of potential biological sources at the playback position.

Examples of the received noise field during broadcast of the nocturnal reef sounds are shown in the spectrograms of Fig. 2b,c recorded at distances of 75 m and 160 m, respectively, from the loudspeaker. At 75 m, some of the clicks of the broadcast sounds are evident above the background noise. At 160 m, only an occasional click is evident above the background. The dark band at about 2 kHz in Fig. 2a results from the response of the loudspeaker. An example of the sound field during broadcast of the artificial sounds is shown in Fig. 2d. The tones broadcast are evident as horizontal lines of constant frequency. The character is quite different from that of the nocturnal reef sounds.

Fig. 2e shows the variation in received noise spectrum level as a function of distance from the loudspeaker during broadcast of the nocturnal reef sounds, for 2 representative frequency bands: the octave centered on 160 Hz (112 to 224 Hz) and that centered on 2 kHz (1.4 to 2.8 kHz). The noise levels received during the recording of the nocturnal reef sounds (i.e. over the reef) are also shown (dashed lines) for these frequency bands. It is apparent that the received levels of the broadcast noise were lower than the nocturnal reef noise for distances beyond about 20 m at 160 Hz and beyond about 35 m for 2 kHz. Since larvae were released at distances between 25 and 100 m, they generally experienced broadcast noise levels that were no higher than those they would have experienced at the position and time of our nocturnal reef noise recording. On the other hand, the broadcast noise levels were higher than ambient background noise at significant distances from the loudspeaker. As distance increases, the noise level can be expected to approach the ambient background noise level as the broadcast signal weakens through propagation loss. This is complicated by some waxing and waning of the signal as a result of interference of sound arriving by different paths, reflected from the surface and bottom in the shallow water. However, at the largest distances, there is little variation with distance, indicating that the broadcast signal has fallen below the background noise.

Late-stage larvae (6 to 9 mm, standard length) of the pomacentrid *Chromis atripectoralis* (Fig. 3) were obtained from light traps off the west side of the island and released by divers in the field, who then observed their behavior (Leis et al. 1996). Larvae were released individually at 5 m depth and followed by divers for 10 min, if possible, and each larva was used only once. Every 30 s, a compass bearing of the swimming direction was recorded (Leis et al. 1996). All bearings are

presented as degrees magnetic, which in the study area differs from degrees true by 7°. Flowmeter readings enabled estimates of speed (Leis & Carson-Ewart 1997). From these data we estimated swimming direction and speed relative to the water (which may be moving). The divers were followed by a boat, and a global positioning system (GPS) reading was made from the surface at the start and end of each fish trajectory. This enabled an estimate of net swimming direction and speed (relative to the bottom), although the accuracy and precision of this estimate varied depending on the ascent time of the divers once they ceased following the larva and on the inaccuracy of the GPS readings, which varied during the course of the study. In some cases, larvae were either lost or descended too deep (>18 m) for the divers to follow, so the trajectory consisted of fewer than the 21 bearings over 10 min normally obtained. When sounds were being broadcast, the divers were followed by a surface observer on a surf ski. Ambient conditions included the sound of an out-board powered boat at idle speed as it circled the divers at a radius of 20 to 50 m.

A drogue with a 1 × 1 m cruciform underwater 'sail' located at 5 m depth was used to measure current. GPS measurements were made at the start and end of each drogue trajectory, thus enabling estimation of current velocity.

Data were gathered over 3 summers (Table 1). Because of limitations imposed by the availability of larvae and weather conditions, and the need to analyze the results of each part of the study before proceeding further, temporal separation of the portions of the study was inevitable. The data were gathered in the morning, normally between about 08:00 and 12:00 h local time, but on a few occasions extending until 14:00 h.

The experiments involving broadcast sound were conducted only on days when wind speed was less than 10 knot (18 km h⁻¹) and it was not raining, to avoid the high background noise levels resulting from the interaction of these atmospheric phenomena with the sea-surface (Cato 1978, 1992, Urick 1983).

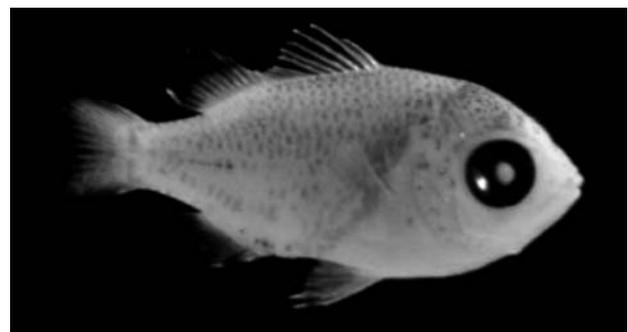


Fig. 3. *Chromis atripectoralis* settlement-stage pelagic larva (8 mm standard length)

Table 1. Dates on which data were gathered under experimental and ambient conditions

Location and conditions	Dates
Leeward ambient	5 to 10, 16 December 1998; 3 February 1999; 25 January 2000
Leeward nocturnal reef sounds	10, 11 December 1999
Leeward artificial sounds	18, 21 to 23 January 2001
Windward ambient	18 to 20, 23, 24 December 1998; 5, 8 to 10, 17 to 21 February 1999
Windward nocturnal reef sounds	12 December 1999; 28, 29 January 2000

Circular statistical procedures followed Batschelet (1981) and Zar (1996). Mean vector length (r) is a measure of angular dispersion ranging from 0 (maximum dispersion) to 1 (lack of dispersion). The Rayleigh test was used for single-sample hypotheses about directional swimming. Watson's F test was used for hypotheses about directional swimming involving 2 samples. The Wallraff test was used to test for differences in angular dispersion between 2 samples. The non-parametric angular-angular correlation procedure was used to test for correlations between directions. The Moore test was used for 1-sample second-order analysis of directional data (i.e. means of means). Second-order directional statistical tests, like the Moore test, require that the number of observations per individual be equal (Batschelet 1981). Our data did not meet this requirement, although they approached it. An alternative is to use only the mean angles for each individual, but this ignores the variation associated with that mean (Batschelet 1981). We used the mean angles and the 1-sample Rayleigh test, but in order to take some account of within-individual variation in trajectory, we excluded individual trajectories that prior Rayleigh testing had shown were not directional. We also applied the Moore test to the full data sets, including non-directional trajectories. In all cases, both statistical methods gave the same results. We report here only the first.

For evaluation of swimming direction relative to the location of the sound source, the bearing to the sound source at the start location was subtracted from the average swimming direction of the larva. Neither the inflatable boat, its anchor line nor the underwater speaker were visible from any release location.

RESULTS

Swimming direction

Overall, 92% of 143 larvae released had directional trajectories. In ambient conditions, at the leeward location 42 of 48 (87.5%) larvae had directional trajectories (median $r = 0.83$), whereas at the windward location the values were 40 of 41 (97.6%) (median $r = 0.91$). Of

22 larvae released at the leeward location in the presence of broadcast nocturnal reef sounds, 19 (86.4%) had directional trajectories (median $r = 0.87$). At the windward location 15 of 17 (88.2%) larvae had directional trajectories (median $r = 0.89$). All 23 of the larvae released near the artificial sound had directional trajectories. Therefore, there is no indication that the proportion of larvae with directional trajectories varied among locations or sound treatments. The only possible exception was that the ambient measurements at the windward location and the artificial sound measurements might have had a higher proportion of directional trajectories (98 to 100%) than did the other treatments (86 to 88%). However, even if real, this difference was small.

Swimming directions in ambient conditions were primarily to the southeast at both locations, although they were somewhat more variable at the windward than at the leeward location (Figs. 4a & 5a, Table 2). Current directions at both locations were bimodal (NNE and SSW) during these measurements.

In the presence of broadcast nocturnal reef sounds, the distribution of average swimming directions was non-directional at both locations (Figs. 4b & 5b, Table 2). In neither location did mean direction differ between ambient conditions and the nocturnal reef sound treatment. At both locations, as expected based on the Rayleigh test results, the distribution of average swimming directions was more variable when nocturnal reef sounds were broadcast than under ambient conditions, although only at the leeward site was this difference significant. If all ambient and nocturnal reef sound data were pooled without regard to location, swimming was directional in ambient conditions and not directional with broadcast nocturnal reef sounds (Table 2). Further, pooled swimming direction was significantly more variable when nocturnal reef sounds were present than under ambient conditions (Table 2).

The difference in swimming directionality between ambient sound and nocturnal reef sound treatments and the significantly more variable direction for nocturnal reef sound treatments led us to reject the hypothesis that *Chromis atripectoralis* cannot hear.

Net swimming directions were consistent with an interaction of the currents experienced during the

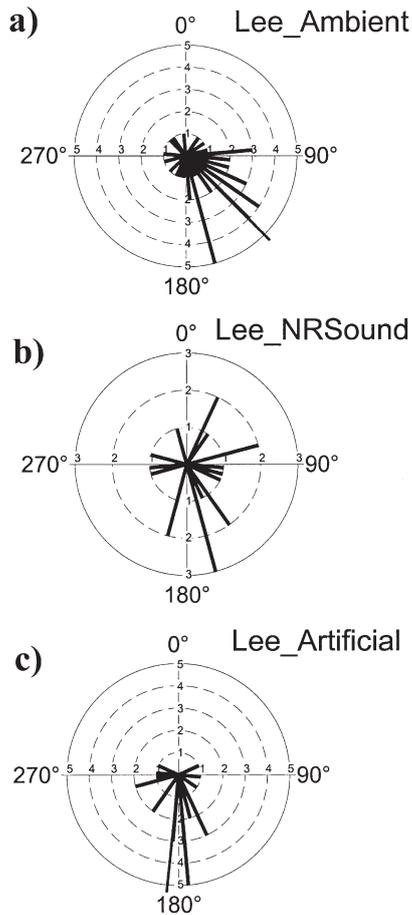


Fig. 4. Leeward (Lee) location. Frequency distribution of the mean swimming directions of *Chromis tripteralis* larvae. Only larvae with a non-random swimming trajectory are included. Frequencies are grouped in 10° intervals. Mean direction of the population of larvae is shown as a slightly thinner line that pierces the outer circle. This is shown only if swimming is directional (Table 2). (a) Ambient conditions (n = 42); (b) nocturnal reef sounds (n = 19); (c) artificial sounds (n = 23)

experiment and a random distribution of swimming directions. At the windward location, there was no directionality to net swimming directions when reef sounds were played ($r = 0.26$, $p = 0.33$, Rayleigh test).

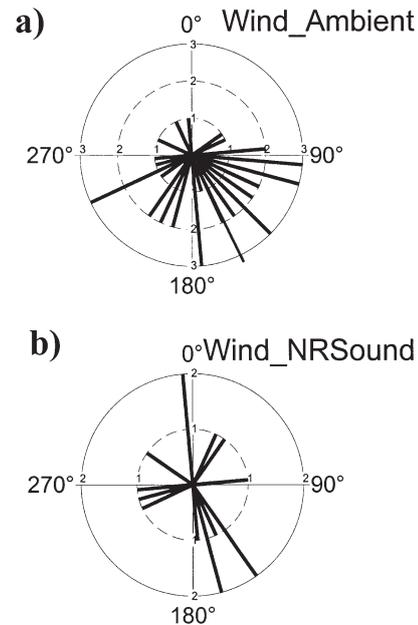


Fig. 5. Windward (Wind) location. Constructed as in Fig. 4. (a) Ambient conditions (n = 40); (b) nocturnal reef sounds (n = 15)

At the leeward location, the net swimming direction was significantly, although weakly, to the east ($r = 0.41$, $p = 0.02$, Rayleigh test).

The artificial sound was broadcast only at the leeward location. Average swimming direction was strongly to the south when the artificial sound was present (Fig. 4c, Table 2). Mean direction differed significantly between artificial sound and leeward ambient conditions by about 50°. Mean swimming direction was also more variable under ambient conditions than when the artificial sound was being played. Further, the pooled nocturnal reef sound swimming directions were significantly more variable than were artificial sound swimming directions (Table 2).

The differences in swimming directionality and variability between nocturnal reef sound and artificial broadcasts led us to reject the null hypothesis that *Chromis tripteralis* larvae cannot distinguish among sounds.

Table 2. Swimming direction of *Chromis tripteralis* larvae in different sound conditions. Directions are degrees magnetic. p is for the Rayleigh test. r: mean vector length; LA: leeward ambient; LNS: leeward nocturnal reef sounds; LAS: leeward artificial sound; WA: windward ambient; WNS: windward nocturnal reef sound; L: leeward; NS: not significant

	LA (Fig. 4a)	LNS (Fig. 4b)	LAS (Fig. 4c)	WA (Fig. 5a)	WNS (Fig. 5b)	Pooled ambient (L+WA)	Pooled nocturnal reef sounds (L+WNS)
Number	42	19	23	40	15	82	34
Mean direction	135.3	130.6	185.8	154.2	173.2	142.2	140.2
p	< 0.0001	0.13; NS	< 0.0001	0.0001	0.78; NS	< 0.0001	0.16; NS
r	0.580	0.320	0.640	0.465	0.132	0.524	0.227

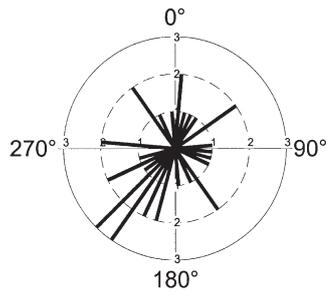


Fig. 6. Frequency distribution of mean swimming directions of *Chromis tripteralis* larvae in the presence of nocturnal reef sounds standardized relative to the position of the speaker (the speaker is located at 0°). Windward and leeward measurements were pooled. This distribution is not directional (Rayleigh test $p = 0.33$).

Larvae swam in no particular direction in relation to the position of the sound source. For nocturnal reef sounds, the Rayleigh test showed no deviation from random directionality relative to the position of the sound source at either leeward or windward locations, or when the 2 were pooled for either swimming direction ($r = 0.15$ to 0.29 , $p = 0.19$ to 0.72 ; Fig. 6) or net swimming ($r = 0.09$ to 0.32 , $p = 0.09$ to 0.89). Similarly, for the artificial sound, there was no directionality relative to the sound source for either swimming direction ($r = 0.21$, $p = 0.37$) or net swimming ($r = 0.10$, $p = 0.81$). Further, there was no relationship between starting distance from the sound source and the direction swum relative to the sound source.

The lack of evidence that larvae swam toward the source of the nocturnal reef sounds means we cannot reject the null hypothesis that *Chromis tripteralis* larvae cannot localize a sound source.

There was no indication that current direction or tide may have influenced swimming direction of the larvae. Larval swimming directions were uncorrelated with current directions at any location or under any sound conditions ($p > 0.20$, non-parametric angular-angular correlation test). Under ambient conditions, swimming directions at both windward and leeward locations were significantly directional (Rayleigh test, $p = 0.02$ to 0.00003) to the southeast (130 to 154°) when fish were observed under rising tides and when they were observed under falling tides. Fish observed when nocturnal reef sounds were being broadcast remained non-directional at both locations under rising and under falling tides (Rayleigh test, $p = 0.22$ to 0.83).

Table 3. Statistical comparisons of mean direction and dispersion between locations and acoustical conditions. Values are p for F test (mean direction) and for the Wallraff non-parametric test for angular dispersion. A 1-tailed Wallraff test was applied to dispersion data only if the results of the Rayleigh test for 2 treatments (Table 1) were different (i.e. one significantly directional and the other not). See Table 2 for abbreviations

Treatment	Mean direction	Angular dispersion
LNS vs LA	0.835 NS	0.045
LAS vs LA	0.002	–
LNS vs LAS	0.030	0.020
WNS vs WA	0.700 NS	0.205 (NS)
L+WNS vs L+WA	0.807 NS	0.048
LA vs WA	0.468 NS	–
LNS vs WNS	0.250 NS	–

Swimming speed

Relationships of swimming speed to the sonic environment differed among locations. At the leeward location, larvae swam about 5 cm s^{-1} faster when sound was broadcast than under ambient conditions (Table 3), regardless of the type of sound. At the windward location, there was no difference in swimming speed between nocturnal reef sound and ambient conditions (Table 4). The lack of a consistent pattern of swimming speed among conditions and locations means our tests of hypotheses involving swimming speed are inconclusive.

DISCUSSION

We defer a discussion of the southerly swimming of *Chromis tripteralis* larvae in ambient conditions to a separate paper (Leis & Carson-Ewart, unpubl.) that deals with swimming directions of several species around Lizard Island. Suffice it to say that this remarkably consistent behavior makes possible the experiments reported here.

One possibility that requires comment is that the larvae may swim to the south in the presence of anthropogenic sound (either the boat noise present in ambient conditions or the very different broadcast artificial sound), but not in their absence (i.e. when we were broadcasting nocturnal reef sounds, no boat noise was present — see ‘Materials and methods’). We doubt this

Table 4. Swimming speed (mean \pm SE) of *Chromis tripteralis* larvae. See Table 2 for abbreviations

	LA (n = 48)	LNS (n = 22)	LAS (n = 23)	WA (n = 39)	WNS (n = 18)
Speed (cm s^{-1})	24.79 ± 0.85	30.30 ± 0.79	30.66 ± 1.15	24.30 ± 1.34	25.60 ± 1.31

to be the case, but even if this were true, then our conclusions about hearing remain unaltered: we have still shown that the larvae can hear and that they can distinguish between sounds.

Chromis atripectoralis larvae reacted to nocturnal reef sounds played during the day by altering their normal swimming behavior from highly directional to non-directional. This clearly indicates that the larvae can hear and that they modify their behavior when they detect this sound of biological origin. They did not react in the same way to an artificial sound. If anything, the artificial sound resulted in the swimming becoming more directional and in a slightly different (more southerly) direction. This clearly indicates that the larvae can distinguish between a potentially biologically meaningful natural sound and an artificial sound that presumably conveys no biological information. We thus reject the first 2 hypotheses that *C. atripectoralis* larvae cannot detect underwater sound and that they cannot distinguish among sounds. These abilities certainly apply over a scale of 25 to 100 m (i.e. the distance from the sound source to where the larvae were released). Although the broadcast levels fell to near the background noise level beyond about 100 m, the loudspeaker is a much less powerful source than a reef. Also, the decrease in noise level with distance would be greater from a point source like a loudspeaker than from a distributed source like a reef. Nocturnal noise from reefs has been observed at levels above background noise for distances of more than 10 km (McCauley & Cato 2000). The possibility remains that the larvae may be able to detect such sounds from greater distances than the range of release distances we used.

We detected no indication that the larvae swam toward the source of the nocturnal reef sound. Some individuals did so, passing within visual distance of the speaker but no more than would be expected by chance alone. Nor did the larvae swim toward nearby (500 to 1000 m away) Lizard Island (or in any other particular direction) in response to the sound. Therefore, we cannot reject the third null hypothesis that larvae cannot locate a sound source that they can hear.

These behaviors did not vary on opposite sides of Lizard Island, indicating that the presence of the island and its reefs 1000 m away was apparently not a factor in determining which way the larvae of this species swam.

We can only speculate as to the reasons for the change in swimming behavior from highly directional to random. One possibility is that once they hear the nocturnal sounds from the reef, the larvae begin looking for the reef in the vicinity. This would not necessarily imply any ability to localize the sound source, simply a change from directed to non-directed swimming that would result in decreased net displacement from the area for the population of larvae as a whole. This

could enable larvae to remain near a reef, if not find it. It must be remembered, however, that although the population of larvae swam randomly, individual larvae still swam directionally—they did not each adopt a random swimming mode. Another possibility is that the broadcast of nocturnal reef sound confused the larvae, disrupting their response to whatever cues they use in ambient conditions to maintain a southerly heading. Nocturnal reef sounds differ from those during the day (Cato 1978, 1992, McCauley & Cato 2000), so it is possible that the larvae were simply confused by the conflicting signals (i.e. a sound occurring at the wrong time of day) and that this confusion resulted in a loss of a southerly heading. The main difference between day- and night-time sounds, however, is the greater occurrence and intensity at night, rather than major differences in acoustical characteristics. These speculations can provide starting places for further research on the matter.

Net swimming direction is what determines if a reef settlement site is located. Net directions are an interaction between the swimming direction and speed of the larvae and those of local currents. In all cases, the net direction observed was expected based on the swimming and current directions. Average speeds of currents in the area are somewhat lower than the average swimming speeds of the larvae (10 to 15 cm s⁻¹ vs 24 to 30 cm s⁻¹; Frith et al. 1986, Leis 1986). At the windward location, there was no directionality to net swimming directions when reef sounds were played. This is consistent with the expected interaction of the essentially bimodal current direction (N and SSE) encountered during this experiment with a random distribution of swimming directions. At the leeward location, the net swimming direction was significantly, although weakly, to the east. This is consistent with an interaction of the essentially bimodal (NNE and SSE), but slightly easterly, currents encountered during the experiment with a random distribution of swimming directions. However, the lack of any correlation between current direction and larval swimming direction indicates that swimming direction through the water column was not influenced by currents (similarly, distance from shore had little effect on swimming direction: it did not differ significantly between 3 distances from shore at the windward location, although at the lee location, the direction 500 m from shore was about 50° more easterly than were swimming directions either nearer or further from shore; authors' unpubl. data). This is not unexpected because it is difficult to conceive how larvae could even detect that they were in a current without an outside reference (Leis et al. 1996, Leis & McCormick 2002).

The results for swimming speed were ambiguous. At the leeward location, larvae swam about 20% faster in

the presence of broadcast sound than without it, yet at the windward location, no difference was found. Without replication of leeward and windward locations, it is impossible to assess the significance of this apparent difference in behavior between locations. However, even if the larvae did swim faster at the leeward location in response to the sounds we broadcast, they did not distinguish between the natural and the artificial sounds in relation to speed.

Given the temporal separation of the portions of this study, the possibility exists that larval behavior differed temporally, rather than in response to the sounds we broadcast. The ambient behavior was determined over an extended period of time (Table 1), so this seems unlikely. Under ambient conditions, data on at least 3 larvae were obtained on 7 d at each of the leeward and windward sites. On these 14 d, the average swimming directions varied from ESE to WSW (98 to 181° at the leeward and 109 to 259° at the windward location). In other words, the average swimming directions were relatively consistent from day to day and did not vary in a major way from the overall mean direction (135° leeward, 154° windward). Further, our sound experiments all took place over several days and in 1 case over several months. A very complex temporal alteration of behavior unrelated to sound would be required to duplicate the results we obtained. We conclude that this is extremely unlikely, and that the differences in swimming directionality we document here are due to sound and not to temporal aliasing.

Our original intent was to proceed beyond the observations reported here and to undertake additional study of swimming behavior by interspersing observations of larvae while the nocturnal reef sounds were broadcast with observations of larvae with the speaker switched off. We began making such observations, but these were quickly disrupted by 2 dangerous aggressive shark incidents. Previously, we had very few encounters with sharks—none of which had been aggressive—in several years of observing fish larvae in the pelagic environment, so it is possible that alternating broadcasting and non-broadcasting periods attracted or irritated the sharks, resulting in the incidents. Subsequent observations in the Sydney Aquarium's shark tank showed that carcharinid sharks did respond to switching the speaker on for 5 min and off for 5 min over 75 min. Two to 3 times more carcharinid sharks were present in the end of the tank near the speaker when it was turned off than when it was on ($p = 0.001$, Wilcoxon's rank-sum test). In any case, our *in situ* observations of this sort were discontinued for safety reasons and, because project funding ended, could not be resumed. In spite of our inability to conduct observations in a way that completely eliminated the possibility of temporal aliasing, the evidence pre-

sented here clearly indicates that larvae of 1 pomacentrid can hear.

This is the first demonstration that larvae of a coral-reef fish can detect sound and distinguish among sounds. In response to a biologically meaningful sound (but not an artificial sound), the larvae altered their swimming behavior from highly directional to random. In response to both biological and artificial sound, they may have increased their swimming speed, although this is equivocal. However, there was no clear indication that they attempted to locate the sound source.

Our observations were made only during the day. It is entirely possible that larvae would respond to sound differently at night. However, our experiment was not concerned with day-night differences in behavior; rather, we sought to determine if larvae of *Chromis atripectoralis* can hear.

The nocturnal reef sounds we broadcast contain a very complex mix of frequencies and intensities. Therefore, we cannot say which frequencies or intensities the larvae can hear and which of these induced the change in behavior. This is an area that requires much more work. Our dependence on larvae from light traps and the weather, both notoriously unpredictable, meant that we could not plan our work in advance. In particular, there were very few days over 3 summers when we had sufficient larvae of *Chromis atripectoralis* and suitable weather for the sound work. Future advances would be aided by a more predictable supply of larvae and less dependence on calm weather.

The only other demonstration of hearing abilities in a larval reef fish (a temperate tripterygiid) used light traps with and without a broadcast sound source (Tolimieri et al. 2000). In this case, the larvae responded to the sound by entering a light trap in increased numbers. Presumably, this involved locating the sound source next to the light trap from an unknown distance, but the possibility that the sound simply increased the tendency of the larvae already attracted to the light trap to enter it cannot be excluded. Light-trap catch efficiencies are apparently low (Meekan et al. 2000), so the near vicinity of a light trap presumably attracts many larvae that might be induced to enter the trap by an auditory cue.

We show here that hearing can operate over scales of 25 to 130 m. Other scales remain to be examined. True nocturnal reef sounds can be expected to produce signal-to-noise ratios comparable to those of our broadcast at much greater distances from the source, and thus should be audible at much greater distances. The light-trap study could not provide an estimate of the scale over which the tripterygiid larvae could hear and respond (Tolimieri et al. 2000). Further studies on larvae of other taxa are needed, but it is interesting that both the (unrelated) species studied thus far can

hear biologically meaningful sound. The ability may be widespread. Both studies utilized late (settlement) stage larvae. The hearing abilities of younger, less developed larvae remain unassessed.

If such hearing abilities are widespread, then the presumption that larvae can use reef sounds for navigation is strengthened. Larvae may be able to hear, but imaginative research on the ability of larvae to localize reef sound is needed before we can be sure that reef sound is an important cue used by fish larvae attempting to find reefs or to remain near them. In addition, research is needed on the minimum sound cue threshold above background that is detectable by larvae. This would enable estimates of the scale over which such navigation could operate. Sound propagates well under water, and the speed and endurance of reef-fish larvae make them very effective swimmers (Leis & Stobutzki 1999). So, if sound can serve as a cue in this way, the potential for long-range navigation by reef-fish larvae would be truly impressive. Even if larvae lack the ability to localize reef sounds, they may be able to minimize their displacement from the vicinity of a reef they can hear by adopting non-directional swimming. Our observations show that a population of larvae potentially can achieve this, even if individuals did not seem to.

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