Behavioral plasticity in larval reef fish: orientation is influenced by recent acoustic experiences

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Animals use many different cues to orient in their environment, solve directional movement challenges, and select suitable habitat. Recent work has highlighted the importance of the ambient soundscape in providing orientation cues for larvae of coral reef fishes at the key life-history phase when they recruit from open ocean to coral reef environments. In this study, we combined acoustic conditioning with binary choice chambers and used 442 settlement-stage larvae from 4 Pomacentridae (damselfish) species (*Pomacentrus amboinensis, P. brachialis, P. moluccensis,* and *P. nagasakiensis*) to test whether responses to acoustic cues are fixed or whether behavior is influenced by recent acoustic experience. Over 8 trials, groups of wild-caught larvae that experienced noise (natural reef noise or artificial tone noise) during a 12-h conditioning period showed a positive directional response to reef noise in the chambers. Groups conditioned with reef noise responded adversely to the tone noise, whereas groups conditioned to the artificial tones were subsequently attracted by them. This plasticity in behavior suggests that settlement-stage larval reef fish (~20 days old) are influenced by, and can retain information from, recent acoustic experiences. Behavioral plasticity may enable greater control by larvae over their selection of settlement sites but could also mean that anthropogenic sounds have more than masking effects on the orientation behavior of fishes. *Key words:* acoustic cues, choice chambers, coral reef fishes, orientation, Pomacentridae. *[Behav Ecol 21:1098–1105 (2010)]*

The ability of animals to sense their surroundings, make directional decisions, and select appropriate habitat is key to their survival. Cues used for solving orientation and navigation challenges are diverse in nature and may include visual, auditory, olfactory, magnetic, and kinetic information. The value of a cue depends on the medium in which it is propagated, the relevance of the information it carries, and the ability of the receiver to detect and interpret it. As a result, hierarchies of cues may be used to resolve spatial challenges with multiple cues operating over different distances and with varying degrees of accuracy (Quinn and Dittman 1990; Kingsford et al. 2002). For coral reef fishes, a key life-history process is settlement, the transition period when larvae return from an open ocean larval stage to recruit to benthic habitat where they metamorphose into their juvenile stage and will live out their adult lives (Leis 1991; Leis and McCormick 2002; Leis 2006). This process greatly influences population dynamics and so determines patterns in connectivity between populations and controls the replenishment of harvested species. Settlement behavior of coral reef fish larvae is modified by visual and olfactory cues (Lecchini et al. 2005), and recent work has found that the larvae of reef fishes and some crustaceans are also attracted to the noises emanating from potential settlement sites (Montgomery et al. 2006).

Initial work using recordings of biological reef noise (consisting of invertebrate "crackle" and fish vocalizations) identified an attraction of crab zoea and megalopae (Jeffs et al. 2003), temperate water tripterygiid blennies (Tolimieri et al. 2000), and settlement-stage coral reef fishes (Leis et al. 2003; Simpson et al. 2004) to reef noise when broadcast around light traps. This attraction persists in coral reef fishes when light traps are replaced with patch reefs constructed from coral rubble (Simpson, Meekan, et al. 2005). Recent work has found that different components of the acoustic signature of a reef are important to fish at different life stages, suggesting that the response to reef noise may change with ontogeny. Adult and juvenile reef fishes are attracted by low-frequency fish vocalizations (Simpson, Jeffs, et al. 2008), whereas settlement-stage reef fishes are attracted by higher frequency invertebrate crackle (Simpson, Meekan, et al. 2008). Whether this is due to changes in auditory preference or changes in hearing abilities (e.g., thresholds of response at different frequencies) is not known, and would benefit from further work, although an in situ study of the swimming behavior of settlement-stage *Chromis atripectoralis* found that the quality (not just quantity) of the acoustic signal influences the response of fish if natural reef noise is compared with artificial pure tones (Leis et al. 2002).

Reef noise is a complex signal that is produced by, and varies according to, the local fauna (Cato 1980; McCauley and Cato 2000; Radford et al. 2008a, 2008b). These sounds propagate hundreds to thousands of meters from reefs within the hearing range of larval fishes (Wright et al. 2005, 2008, 2010; Mann et al. 2007) and can be detected using hydrophones from 10 s of km from the source (McCauley and Cato 2000).

If settlement-stage fishes (days to weeks in age) were to use reef noise to select among habitats rather than swim toward any reef, they would need to retain and discriminate between several acoustic experiences. Adult fish can learn to respond to acoustic stimuli in the context of classical conditioning studies, with negative reinforcement (e.g., electric shock: Fay 1969), positive reinforcement (e.g., food reward: Yan and Popper 1991), and instrument shock avoidance (Popper 1971) all yielding repeatable results. With regard to fish discriminating between sounds, goldfish (*Carassius auratus*) are able to perceive both pitch and timbre (Fay 1995), whereas adult koi carp (*Cyprinus carpio*) are able to distinguish between John Lee Hooker (blues) and Johann Sebastian Bach (classical), and even use this information to characterize novel music by genre (Chase 2001). However, the effect of the acoustic experiences of larval fishes in altering and guiding their orientation is as yet untested.

In this study, we used settlement-stage damselfish collected by light traps to test for the first time whether directional responses to auditory cues are fixed or can be modified by recent acoustic experiences. Until now, the degree of behavioral plasticity in settlement behavior, and the influence of local conditions on behavioral preferences, was not known. After a period of potential acoustic conditioning, where fishes were held for 12–14 h in a safe environment with various acoustic signals, we tested for directional responses in binary choice chambers. By this approach, we addressed the following questions: 1) Can fish orient with respect to acoustic cues?; 2) Do fish have an inherent preference for natural reef noise?; 3) Do fish show a natural aversion to an artificially generated noise?; and 4) Is the behavior of fish affected by recent experiences?

MATERIALS AND METHODS

This study was carried out over 5 nights during a summer new moon in November at Lizard Island Research Station (lat 14°41′S long 145°27′E), Great Barrier Reef, Australia. All work was carried out under permits held by the Australian Institute of Marine Science and issued by the Great Barrier Reef Marine Park Authority, and animal handling and testing techniques were designed using guidance from the Association for the Study of Animal Behavior and the Animal Behavior Society (ASAB/ABS 2006). According to the composition of light trap catches from the previous night, settlement-stage fishes from the genus *Pomacentrus* (*P. amboinensis, P. brachialis, P. moluccensis,* and *P. nagasakiensis*; 10–14 mm standard length) were used on each day. These 4 species were all phonotactic in an earlier study (Simpson et al. 2004), and are easily identified, so the stress to the fish of sorting and handling was minimized. By using congenerics, the experiment could run on consecutive nights despite variation in the catches at species level.

Conditioning environment

We collected fish each morning at dawn from (silent) light traps set in 12 m of water over a sandy bottom 700 m in front of the research station and brought them in aerated containers back to the station where they were divided into 6 tanks (60 × 30 × 30 cm) in similar size and species composition groups (12–30 per group, depending on availability; Figure 1A). The tanks remained shaded throughout the day (to slow metamorphosis of larvae into postsettlement fish) and were randomly allocated to one of 3 treatments (2 tanks per treatment): 1) Reef: natural reef noise recorded at new moon at dusk at Feather Reef (lat 17°32.28′S long 146°21.36′E, Great Barrier Reef, Australia); 2) Tone: an artificial pure tone mix of equal amplitude 50, 100, 200, 400, 800,
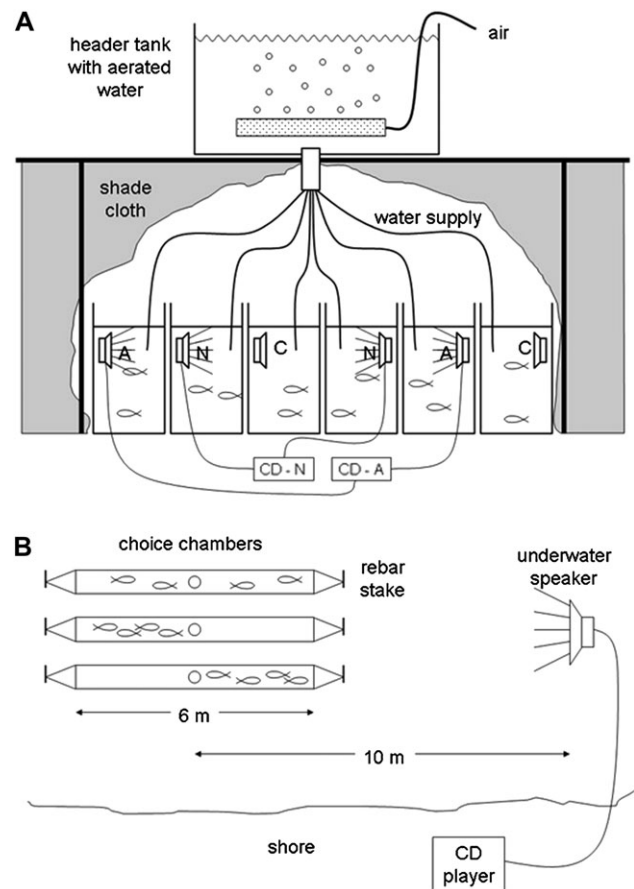

Figure 1
(A) Acoustic conditioning environment and (B) choice chamber testing environment. (A) Prior to testing, larvae collected from light traps were kept in 6 tanks under shaded conditions with a supply of aerated water. Two tanks were randomly allocated each day to natural reef noise (N), which was played from a portable CD player through speakers housed in plastic bags (CD – N), 2 received artificial tone noise (A, from CD – A), and 2 were controls with no noise (C). NB: Tanks were 20 cm apart and isolated from each other using polystyrene tiles for acoustic insulation. (B) Choice chamber testing environment. Choice chambers were constructed of clear polythene tubing (50 cm Ø), with a central opening for introducing fish and were moored in 1–2 m water. Here, the speaker is located to the north of the chambers, and as an example, fish in the top chamber show no response, in the middle chamber show an aversion to the broadcast sound, and in the bottom chamber show an attraction to the sound. Half of the trials had the speaker to the south of the chambers.

1600, 3200, 6400, and 12800 Hz sounds; and 3) Silent: a control with no broadcast noise.

The reef noise recording we used in this study consisted of invertebrate "clicks" and fish vocalizations and had been found to elicit positive directional swimming responses in larval reef fishes in earlier studies (Simpson et al. 2004; Tolimieri et al. 2004; Simpson, Meekan, et al. 2005; Simpson, Meekan, et al. 2008). The Tone treatment was generated to produce a different quality of sound while controlling for the quantity of noise (e.g., still covering the same spectral range at similar intensities as the reef noise). It should be noted that previous studies where single recordings have been used to study the response of animals to broader phenomena have recently been challenged (Slabbekoorn and Bouton 2008), and in current work, we now use multiple recordings to investigate general responses to classes of sounds. However, in this study, we

were investigating whether the behavior of larvae is influenced by specific sounds they have previously experienced.

The sound treatments were played on continuous loops throughout the day, using portable CD players and computer speakers housed in plastic bags in each tank. The Silent control treatment had similar speakers in the tanks that remained silent. The acoustic conditioning environment in each of the 6 tanks during playback was measured using a Sonatech 8178 hydrophone lowered to the center of the tank and recorded using a Sony DAT TCD8. As intended, the sound levels in the reef and pure tone tanks were substantially greater (root mean square [rms] broadband power = 133 dB re 1 μPa) compared with the silent tank (115 dB re 1 μPa). To avoid the noise of bubbles from aeration, we set up the tanks without direct air supplies and instead supplied each with highly aerated water at a rate of 10 ml s^{-1} from a header tank (Figure 1A). The acoustic treatments were presented to the fish in the conditioning tanks throughout the day for a total of 12–14 h. After this period (soon after dusk), we subdivided fish from each of the 6 tanks into 3 perforated plastic pots, and the resulting 18 pots were carried in a dark box filled with aerated water to the shore.

Test environment

We used cylindrical choice chambers, which were similar in concept to those used in earlier studies (Stobutzki and Bellwood 1998; Tolimieri et al. 2004; Leis and Lockett 2005). Three chambers were moored in 1–2 m of water over a sandy bottom using rebar (steel rod) stakes at each end. Chambers were arranged parallel to each other, to the shore, and to the nearest reef (250 m away), in a N-S direction in 1–2 m of water (Figure 1B). Each chamber, 6 m long × 50 cm diameter, was made with clear polyethylene "layflat" tubing and filled with seawater so was acoustically and visually transparent. The chambers had central sealable openings through which fish were introduced and openings at each end so we could release the fish alive after each trial.

On each night, we carried out 1 or 2 sets of 3 trials, using an underwater speaker (Lubell Labs Inc., Columbus, OH; LL964, frequency response 0.2–20 kHz) placed 10 m to the north or south of the chambers (randomly allocated) for the first set and moved to the opposite end if there was a second set of trials. In total, we conducted 8 sets of trials, with the speaker at each end 4 times (to account for wave direction, current, or moon direction effects). In each trial, one pot of fish (containing 3–13 fish) from each of the 3 conditioning treatments was selected at random. The fish were released into the 3 chambers (one chamber per conditioning treatment, randomly allocated) and given 5 min to acclimatize. Throughout this study we used groups of fish rather than individuals in the chambers because Tolimieri et al. (2004) had found that individuals responded to reef noise randomly in choice chambers (perhaps due to being highly stressed by isolation) but groups elicited a positive response (perhaps through quorum sensing of preferences with imperfect individual responses, see Codling et al. 2007). After this, one of the 3 conditioning sound treatments (Reef, Tone, and Silent) was replayed using the speaker. Test sounds were replayed at a broadband source level 156 dB re 1 μPa, which when measured at the center of chambers were ~145 dB re 1 μPa (rms broadband power). In contrast, without playback the ambient noise at the study sites was measured during the experiment at 117 dB re 1 μPa. Assuming a cylindrical model of propagation of sound in the chamber (Vermeij et al. 2010), broadcast sounds would have a 3 dB range within the chamber depending on position of the fish, and throughout the chamber, the test treatments would be clearly audible by larval pomacentrids at this stage in

their development (Egner and Mann 2005; Wright et al. 2005, 2010).

In each trial, the test treatment was presented for 10 min, after which time we counted the number of fish in each half of each chamber while snorkeling with torches; playback of the treatment sound continued during census and torch light was not directed at fish until they were being counted. For each chamber, 2 snorkelers started from the center and moved apart toward each end, thus preventing free-swimming fish being counted more than once. This census was repeated for the other 2 chambers within a set of trials, after which we released the fish alive and the speaker was moved to the other end of the chambers for a second set of trials. To avoid potential observer bias, the snorkelers did not know which conditioning treatment had been allocated to each chamber until after counting the responses of the fish.

Statistical analysis

We analyzed the test results in 2 ways (following: Tolimieri et al. 2000; Tolimieri et al. 2004; Leis and Lockett 2005). First, making the assumption that larvae in each trial were moving independently of each other, we used Wilcoxon's matched-pairs tests with a H$_0$ that within the 8 replicates per treatment, there would be no trend in the number of larvae at each end. Second, allowing for the fact that fish behavior could be interactive, perhaps by schooling in the chamber and pooling of imperfect senses leading to a group response (Codling et al. 2007), we used Sign tests to test the number of group responses (dominant direction of the individuals in the chamber) toward and away from the speaker. We also tested the influence of the geographic orientation of the tubes and the position of the speaker using Wilcoxon's matched-pairs tests on the outcomes of the 8 Silent replicates where a sound treatment was not presented.

RESULTS

There were marked differences in the acoustic signals used in the 3 treatments for this study (Figure 2). The acoustic signatures of the natural reef noise and artificial tone mix were both broad spectra in profile, and during playback, these sounds were matched for intensity in the conditioning tanks (rms broadband power = 133 dB re 1 μPa) and in the choice chambers (145 dB re 1 μPa, Figure 2). In the tanks, the tone mix produced dominant pure tone signals, which remained dominant during playbacks in the sea except for the lowest frequency spikes. In contrast, the reef noise did not have any dominant frequencies in the tank or in the sea, but in both scenarios peaked in intensity at around 1.1 kHz. The silent tanks had lower sound levels (115 dB re 1 μPa), and this decreased magnitude was also seen in the sea (117 dB re 1 μPa), where only ambient background noise prevailed (Figure 2).

In total, we tested 442 fish in 8 sets of trials during this study (Conditioning treatment: R-159, T-140, S-143; Test treatment: R-160, T-137, S-145, where R = natural reef noise, T = artificial tone noise, and S = silent control; Table 1). The numbers between treatments varied slightly because ensuring exact replicate sizes would have demanded far greater sorting and handling, and thus stress, to the fish.

The larvae that had been exposed to natural reef noise during the day responded positively to natural reef noise in the chambers (overall 70% moved toward the sound, Figure 3A). This was true whether fish within trials were treated as individuals (Wilcoxon's matched-pairs test, P value = 0.031) or as groups (Sign test, P value = 0.016; Table 1, Figure 3B).
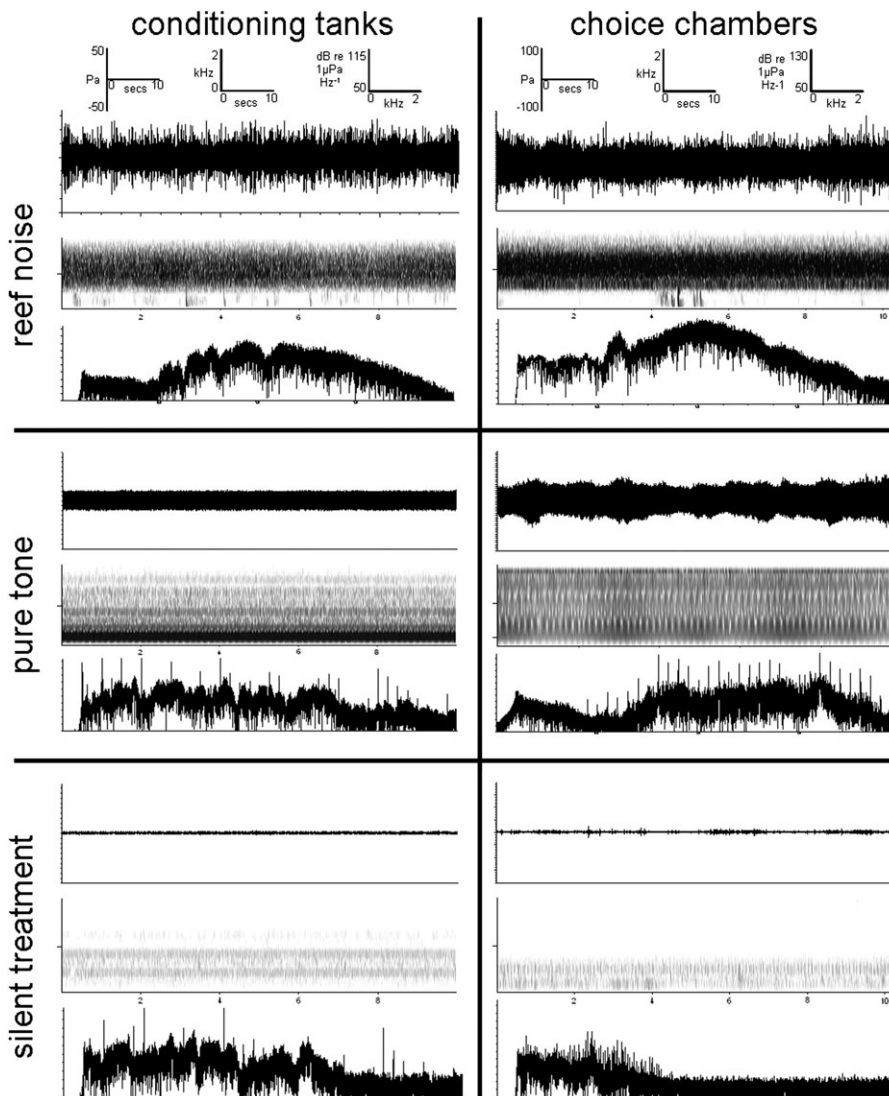
Figure 2
Analysis of the acoustic environments in the conditioning tanks (left tiles) and open water choice chamber trials (right tiles) for 3 treatment groups. Reef noise (top) was playback of a recording recorded at new moon at dusk at Feather Reef; Tone noise (middle) was an artificial pure tone mix of 50, 100, 200, 400, 800, 1600, 3200, 6400, and 12800 Hz sounds; and Silent (bottom) was a control treatment with no broadcast noise. Each tile consists of 3 components taken from a 10 s sample: top—amplitude; middle—spectrogram; bottom—spectral level analysis. Figure scales and units are given at the top of each column, and all analyses were limited to the spectral range 0–2 kHz because this is the range of hearing in Pomacentridae fishes (Egner and Mann 2005; Wright et al. 2005, 2010; Maruska et al. 2007).

In contrast, larvae that had been exposed to natural reef noise responded negatively to tone noise in the chambers (overall 67% moved away). The larvae exposed to the artificial tone noise during the day also showed a positive response toward the natural reef noise (73% moved toward the sound). In contrast to the fish conditioned with natural reef noise, fish that had experienced tone noise during the day moved toward this noise when it was presented in the choice chambers (70%), and this response was significant when behavior was considered within trials at both the individual and group level. In contrast to the significant positive and negative responses of fish that had experienced either reef or tone sound treatments during the day, fish presented with noise (reef or tone) for the first time in the choice chambers following a silent day showed no emergent directional response at the end of the 10-min test period.

Finally, there were no consistent directional responses in the 3 control scenarios where fish were left for 10 min in the choice chambers with the speaker remaining silent so only ambient noise was present. This was true irrespective of the conditioning experiences during the day and whether fish were treated as individuals or as groups within trials. Further analyses of these trials found there was no significant effect of the geographic orientation of the tubes during the 8 silent

trials (69 fish moved northwards, 76 fish moved southwards; Wilcoxon's, $n_{s/r} = 6$, $P = 0.563$). Similarly, there was no significant effect of the position of the speaker during the 8 silent trials (65 fish moved toward and 80 fish moved away from the speaker; Wilcoxon's, $n_{s/r} = 6$, $P = 0.219$).

DISCUSSION

This study is the first to identify plasticity in the response of settlement-stage reef fishes to their acoustic environment. Until now, studies of response to sounds have been equivocal as to whether attraction to reef noise is fixed or can be modified by experience. Although a difference between daytime and nighttime responses to noise has been inferred (Leis et al. 1996; Stobutzki and Bellwood 1998) and later tested (Tolimieri et al. 2004), the general consensus of studies is limited to the fact that free-swimming larvae are attracted to reef noise at night (Tolimieri et al. 2000; Leis and Carson-Ewart 2003; Simpson et al. 2004; Simpson, Meekan, et al. 2005; Simpson, Meekan, et al. 2008). This study identified clear differences in the responses of larvae according to recent acoustic experiences.

The larvae used in this study were wild caught so experienced natural soundscapes until the 12 h of conditioning

Table 1

Outcomes of directional choice chamber trials using 3 test treatments for Pomacentridae larvae previously exposed to the treatments in acoustic conditioning tanks

			Test Treatment					
			Reef		Tone		Silent	
			Toward	Away	Toward	Away	Toward	Away
Conditioning Treatment	Reef	Individual larvae	40	17	16	32	24	30
		Number of trials	8		8		8	
		Group response	6 (2) 0		1 (1) 6		1 (2) 5	
		Wilcoxon Prob.	**0.031**		**0.047**		0.312	
		Sign test	**0.016**		0.063		0.109	
	Tone	Individual larvae	33	15	32	14	22	24
		Number of trials	8		8		8	
		Group response	6 (1) 1		6 (2) 0		3 (1) 4	
		Wilcoxon Prob.	**0.047**	**0.031**	0.813		0.188	
		Sign test	0.063	**0.016**	0.5			
	Silent	Individual larvae	28	27	24	19	19	26
		Number of trials	8		8		7	
		Group response	3 (2) 3		5 (1) 2		1 (2) 4	
		Wilcoxon Prob.	1		0.578		0.188	
		Sign test	0.656	0.227		0.188		

Seven or 8 replicate trials were conducted for each combination, and the predominant group responses are given (numbers in parentheses indicate ties) with the Wilcoxon's matched-pairs and Sign test probabilities. Significant responses are indicated in bold ($\alpha = 0.05$). "Toward" indicates a positive directional response to the direction of the speaker.

preceding the trials. This experience consists of a few days of embryonic development on the reef, during which time they experience both the near-field particle motion and far-field pressure components of reef noise (Simpson, Yan, et al. 2005; Mann et al. 2007), followed by a pelagic phase of a few weeks when they will have experienced a temporally and spatially heterogeneous soundscape according to lunar phase, time of day, their proximity to reefs, and the local noise-producing reef fauna (McCauley and Cato 2000; Lammers et al. 2008; Radford et al. 2008a), with received sounds predominantly in the far field. Each larva may have a unique acoustic history for its early life depending on its dispersal trajectory (or perhaps not if it schooled during larval life: Planes et al. 2002; Codling et al. 2007), but randomized sorting of larvae into conditioning and then test treatment groups should have eliminated any biases due to this natural variation. On capture, larvae were treated in conditioning tanks for 12 h when they will have experienced both the particle motion and pressure components of the broadcast sounds. Once in the experimental choice chambers the relative importance of the near-field particle motion element of the broadcast treatments would be reduced because they are further from the speaker, although particle motion would still be well within in the range of direction for pomacentrids larvae (Mann et al. 2007). The difference between conditioning and test conditions highlights the difficulty in testing directional acoustic responses, which must be conducted in open water to ensure a gradient within the chamber. To keep the ratio of particle motion to pressure constant between conditioning and chamber trials, it would have been necessary to carry out the conditioning in open water (with multiple underwater sound systems and each treatment in a different bay to avoid cross-contamination) or use lateral line ablation (e.g., using $CoCl_2$ or aminoglycoside antibiotics) to control the detection of near-field particle motion. Without these expensive or intrusive modifications, this study is not able to disentangle the relative importance of near-field and far-field sounds.

Where larvae had experienced noise during conditioning (whether it was reef or tone noise), when presented with reef noise in the choice chambers they responded positively by swimming toward this natural sound, suggesting that there is a general attraction to natural reef noise even if it has not been experienced immediately prior to the trial. This result corroborates those of earlier choice chamber and playback experiments but in a new experimental arrangement that, without traps, provides greater freedom of movement for larvae than in previous studies (Tolimieri et al. 2004; Leis and Lockett 2005). Whether larvae were able to localize the source of the sound (resolving the 180° ambiguity problem, Montgomery et al. 2006) or sampled gradients within the chamber (acousticotaxis) is unclear without in situ observations, but within the 6 m length of the chamber, larvae successfully oriented toward the sound. The main contrast in our results was for larvae that had experienced noise during the day (reef or tone) and were then presented with tone noise in the chambers. Larvae that had experienced reef noise avoided the tone mix, a response also observed by Leis et al. (2002), but if conditioned to this tone mix, their response was reversed and they swam toward this artificial noise.

No directional response to noise (reef or tone) was observed within the 10-min test period for larvae from the silent conditioning group. This is a result which contrasts with earlier choice chamber studies where unconditioned light-trap-caught fish subsequently responded positively to broadcast reef noise (Tolimieri et al. 2004; Leis and Lockett 2005) and negatively to tone noise (Leis et al. 2002) over the period of a whole night. This study measured immediate (10 min) responses to sound, and so the lack of a consistent response from the silent treatment group to either of the test sounds suggests that eithera) directional responses to sound (positive to reef noise and negative to tone noise) are not immediate, but if experienced for the first time may be preceded by a period of sampling and/or processing of information, or b) the sudden onset of noise elicits a startle response whereby initially the fish freeze and show no movement. Further work using this methodology could explore the time taken to elicit a response in the silent group (perhaps through continual monitoring using infrared illumination and IR sensitive video
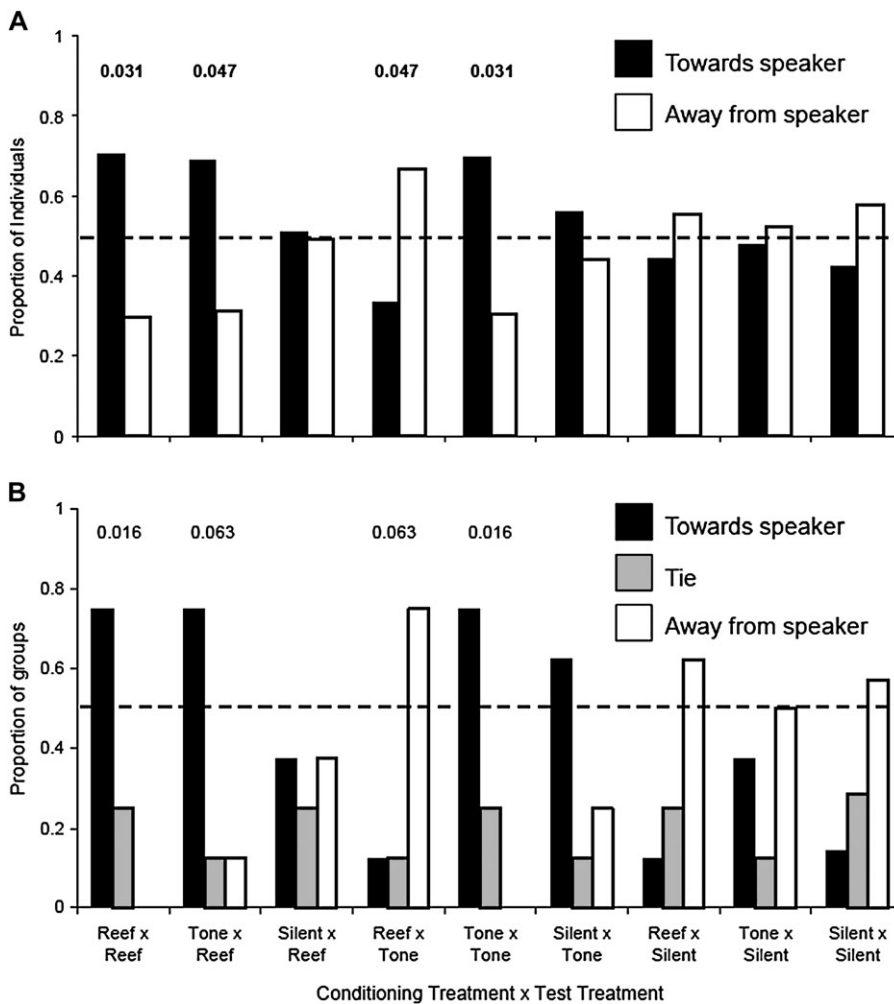
Figure 3
Directional response of (A) individuals and (B) groups of Pomacentridae larvae to 3 test treatments following a day in acoustic conditioning tanks. Dark bars represent larvae and groups that were in the half of the choice chamber toward the speaker after 10 min, and white bars represent larvae and groups that were in the half of the chamber away from the speaker (gray bars in B represent ties). Three treatments were used for conditioning and tests (Reef, Tone, and Silent) resulting in 9 combinations which were tested over 8 replicate trials with 43–57 larvae per combination (group size varied from 3–13, mode = 4). The dashed line indicates the prediction of the H_0 that there would be no individual or group preference, and P values <0.1 for Wilcoxons (A) and Sign (B) tests are given with significant results in bold.

cameras) and also measure the duration over which acoustic conditioning influences behavior.

This study identifies a behavioral plasticity in the response of settlement-stage fish to noise, which may facilitate in habitat selection. This process could be deliberate, whereby larval fish acoustically sample several potential settlement sites over a period of hours from a safe distance away from the "wall-of-mouths" (Hamner et al. 1988) and make comparisons prior to the risky act of settlement. Alternatively, larval fish could have preferences for certain habitat noises and move around, at a safe from reefs, until a certain threshold of attractiveness is met inducing an "urge to settle." By either mechanism, acoustic sampling would be an extremely useful behavior given that settlement mortality is severe (56%: Almany and Webster 2006; 61%: Doherty et al. 2004; 66%: Simpson 1999) and so multiple attempted settlement events are highly risky, and it would make use of the fact that coastal habitats have distinct sounds (Radford et al. 2010). The duration over which acoustic experiences can influence behavior is not tested in this study, but if sounds experienced during early development (including during embryonic development, Simpson, Yan, et al. 2005) fix the selection criteria which determine later behavior, this could facilitate self-recruitment back to natal sites (Jones et al. 1999; Jones et al. 2005). The ecological importance of chemical imprinting on natal cues is well documented in migratory salmon (Dittman and Quinn 1996) and in clownfish (Arvedlund and Nielsen 1996),

whereas several forms of acoustic imprinting have been described in birds (Lorenz 1937), although elements of imprinting, including the existence (or not) of a "critical period," whether it is irreversible, and the direct and indirect relationship of the cue and the downstream responses remain highly variable and sometimes contentious (see reviews by Bateson (1966) and Bolhuis (1991)). Because the fish used in this study were wild caught, using light traps moored near to coral reefs, they were not naive to reef noise. As with all Pomacentridae, they would have experienced audible ambient reef noise during embryonic development on the reef (Simpson, Yan, et al. 2005) and also experienced audible ambient reef noise around the time of capture (Mann et al. 2007). This study is not able to test the effects of the 12-h conditioning period in isolation from this earlier experience, but it did find significant differences in the behavior of the 3 treatment groups suggesting recent experience during the experiment had a marked influence on behavior. The potential for acoustic imprinting in fishes would be a worthwhile subject for further investigation, perhaps by exposing developing larvae to a suite of natural sounds at natural levels over several days in captivity and then testing for their responses. By this approach, it would be possible to test whether early experiences can determine responses to specific or broad classes of cues and whether experiences can determine responses to novel stimuli later in development.

Considering that vocalization, hearing and acoustic communication have been studied across the animal kingdom for many decades, it is surprising that the study of the passive use of soundscapes by animals for gleaning information about potential habitats is relatively unstudied. Studies of pigeon movements have suggested that background infrasound may provide a cue for navigation, and this cue may be disrupted by anthropogenic sources of infrasound including Concorde's sonic boom (Hagstrum 2001). Recent work has found that Eurasian reed warblers (*Acrocephalus scirpaceus*) and sedge warblers (*Acrocephalus schoenobaenus*), 2 migrating passerines, respond selectively to the acoustic signatures of potential landfall sites (Mukhin et al. 2008). Furthermore, adult birds show stronger preferences than juveniles, suggesting that previous acoustic experience may influence habitat recognition. For adult coral reef fishes, that will have experienced their local soundscape because larval settlement and are particularly attracted to the lower frequency sounds of other fishes (Simpson, Jeffs, et al. 2008), this could provide a mechanism for the homing behavior reported in displacement studies (e.g., Marnane 2000). This current study suggests that the response of animals to the surrounding soundscape may have a degree of plasticity that is altered by recent experience, even where the model is 3-week old larval damselfish. Understanding the importance of the soundscape for orientation and navigation, and the potential disruption of natural behavior by anthropogenic sources of sound, clearly merits further study in the field of bioacoustics.

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