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Testing the generality of acoustic cue use at settlement in larval coral reef fish

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Abstract. Some settlement-stage larval fish appear to be attracted to reef sound and may, therefore, use acoustic cues when orientating towards their settlement site. However, all work on the *in situ* response of coral reef fish larvae to sound in acoustic playback experiments has been carried out in the same location (Lizard Island, the Great Barrier Reef), and in some cases, using the same reef recording. It is therefore not clear how widespread acoustic cue use is. To test whether sound is a general and reliable indicator of reef settlement site, we conducted a similar experiment in a different coral reef region, where the coral reef habitat and therefore soundscape is less uniform in quality (Bohol, Philippines). Contrary to our predictions, in some cases we found that fish were not attracted to the broadcast reef sound. We suggest that this may be due to an artefact of the reef recording, possibly the location or the time of day the recording was made. Our results indicate that larval fish are more selective in their response to coral reef sound rather than just being innately attracted to generic reef sound. This highlights the need to assess anthropogenic impacts on the natural soundscape, as this could affect the ability of larval coral reef fish to acoustically detect a suitable settlement site.

Key words: coral reef fish, cue, sound, light traps, settlement

Introduction

Ten years ago, it was hypothesised that larval reef fish could use sound to locate a settlement site (Stobutski and Bellwood 1998). There are now data that show that as early as the embryonic stage, coral reef fish can detect sound and their sensitivity to sound increases with age (Egner and Mann 2005; Kenyon 1996; Simpson et al. 2005a). At the time of settlement, damselfish (*Pomacentrus nagasakiensis*) are as equally sensitive to sound frequencies as juvenile-stage fish, and therefore are physiologically able to receive acoustic information (Wright et al. 2005). Additionally, Pomacentridae larvae can determine the direction of a sound source and will swim towards reef recordings broadcast in a choice chamber (Leis and Lockett 2005; Tolimieri et al. 2004). This is not just a general phonotactic response but appears to be specific to reef sound as fish were attracted towards reef recordings, but not artificial pure tones (Leis et al. 2002).

Acoustic playback experiments have shown that reef fish are attracted to light traps broadcasting reef sound over the ambient soundscape (Leis and Carson-Ewart 2003; Simpson et al. 2004; Tolimieri et al. 2000), and higher natural settlement rates are seen on patch reefs that were associated with underwater speakers playing reef recordings, in comparison to silent control patches (Simpson et al. 2005b). Generally, settlement-stage fish are more attracted to the higher frequency components of reef sound (made predominantly by invertebrates), relative to the original recording and the filtered lower frequencies alone, so sound appears to be more than just a broad indicator of reef location and may provide specific information used in settlement site selection (Simpson et al. 2008).

The use of sound for orientation during settlement varies among families, however, with some families appearing not to respond to sound cues (Leis and Carson-Ewart 2003; Simpson et al. 2004). What is not yet understood is how widespread acoustic cue use is. With the exception of one study carried out on sub-tropical rocky reef fish (Tolimieri et al. 2000), the remaining seven *in situ* studies that have shown positive phonotactic responses of larval fish to coral reef sound were all carried out at Lizard Island. Four of these studies shared the same single reef recording as the test sound (Simpson et al. 2004; Simpson et al. 2005b; Simpson et al. 2008; Tolimieri et al. 2004), and the remaining three used another (Leis et al. 2002; Leis and Carson-Ewart 2003; Leis and Lockett, 2005). As a result, our knowledge of acoustic cue use in settlement-stage fish orientation is potentially very location specific and it has not been investigated in any other coral reef area, where the soundscape may be less consistent due to variability in reef quality. We questioned the generality of acoustic cue use by testing the response of larval coral reef fish to sound in a different location. Using the same techniques that have previously been used to assess the attraction of settlement-stage fish to broadcast reef sound at Lizard Island (i.e. coupling light traps with underwater speakers), we carried out a similar experiment on settlement-stage coral reef fish in the Philippines. Light traps collect phototactic larval reef fish at the end of their pelagic phase, and the comparison of catch rates in the sound treated vs. the silent traps can be used to assess the attraction of settlement-stage fish to the broadcast sound treatment (Leis et al. 2003; Simpson et al. 2004; Simpson et al. 2008; Tolimieri et al. 2000). We predicted that if sound is a general and reliable indicator of reef location, it will be used by settlement-stage fish in this different study area, therefore higher numbers of fish would be attracted to the sound, in comparison to the silent control treatment.

Methods

Traps (designed by Ecocean, St Clément de Rivère, France) were set at surface moorings located in a sea channel to the northeast of Pangapasan Island, Bohol, central Philippines (10°01.1'N, 123°56.2'E). The moorings were anchored on a sandy substrate in water of 10-12 m depth. There was no reef present within 50 m of each mooring and they were separated by c. 400 m to prevent acoustic overlap of the different traps broadcasting sound. The area at which the broadcast sound was detectable over the ambient reef sound was estimated to be 20-50 m (see Fig. 1).

Each night, two sound and two silent traps were each pseudo-randomly assigned to a mooring, so each treatment was tested multiple times at each position during the experiment. The sound systems consisted of an MP3 player, a 12V lead-acid battery, 18W Universal Amplifier Module (Kemo-Electronic GmbH, Lanhen, Germany), and an Electrovoice UW30 underwater speaker (Lubell Labs, Columbus, OH, USA). This played the sound treatment on continuous loop through the night, which was a recording taken at 8.40am on the 16th June 2007, at Black Forest Reef, a marine protected area located to the southwest of Bohol (09°31.228'N, 123°40.991'E). The recording was taken using an Edirol R1 recorder, and a HTI-96-MIN omni-directional hydrophone with a built in preamplifier (High Tech, Inc., Gulfport, MS, USA) and processed using Audacity 1.2.6. (a free digital audio editor available at http://audacity.sourceforge.net/) to delete artificial artefacts (e.g. the sound of distant boat engines) and produce a clean one-minute recording.

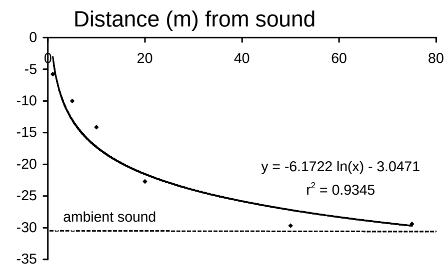

Figure 1. The distance over which the sound treatments were detectable over the ambient sound level. Sound intensity (root mean squared measured in relative dB) was measured at increasing distances from the sound system playing back a pure tone sound.

The experiment was conducted over 21 nights from the 4th-24th July. Traps were deployed at dusk, left overnight and collected at dawn, when the catches were transferred to separate polystyrene cool boxes and transported by boat to a nearby aquarium facility in Matabao, Bohol. Reef fish were separated from the rest of the catch (which consisted primarily of invertebrates and clupeids) and identified to family, or when possible, species level and counted. The fish were then given to Ecocean for rearing for a release scheme.

Analysis

There are no data available on the behaviour of larval fish upon entering light traps, therefore we do not know if it is a fair assumption to treat each captured fish as a statistically independent data point. For this reason, two approaches were taken for the analysis. A sign test, which makes no assumptions on the independence of fish caught, was used to test the null hypothesis that the number of nights with the largest catch would be the same for the silent and sound treated traps. As this test has a low power to detect a treatment difference when the number of testable nights per family is low (after excluding ties), the second approach estimated the effect of the sound treatment on the number of larval fish caught by fitting a generalised linear mixed effects model (GLMM). This method does assume larvae entered the trap independently, however it has the benefit of including the temporal and spatial variation that is characteristic of larval fish distribution and occurrence in light traps. Counts were grouped by family and families for which fewer than 10 individuals were captured over the experiment were excluded. Counts of fish per family were not normally distributed (Shapiro-Wilk test, $W = 0.1878$, $p < 0.001$). A logarithmic link function and Poisson error distribution was specified as the data set was bounded by zero and the variance in counts per family was not equal. As there was inter-family variation in abundance, the number of fish caught per mooring and the number of fish caught per day over the lunar cycle, these were fitted as random effects. An interaction between sound treatment and family was fitted as a main fixed effect. As a significant trap unit effect was not found it was dropped from the model. The models were fitted using maximum likelihood.

Deviance statistics (estimates of how well the model captures the data) were generated for each model with and without the explanatory variables. To obtain the significance levels of the explanatory variables, the deviance statistics were compared using Chi-square tests. All analyses were implemented in R (R Development Core Team 2007).

Results

Twenty nights of data were collected, with 78 trap deployments (39 sound and 39 silent) as bad weather (on the 8th night) caused all traps to be retrieved early. A sound system failed on one occasion and on another a mooring was stolen, preventing a silent trap from being set, leading to a total of 18 nights data with sound treated and silent control trap deployments.

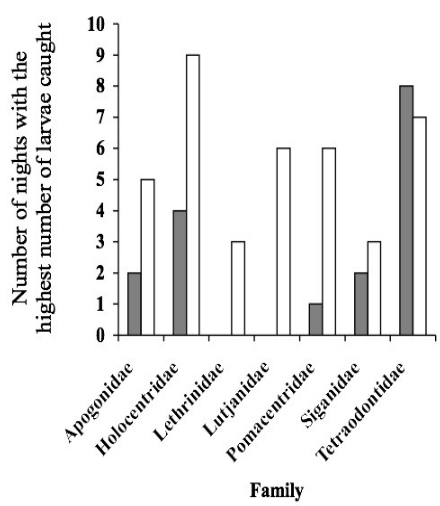

Figure 2. Number of nights with the greatest catch per treatment deployed with speakers (grey) and without (white) from the 4th-24th July 2007, Bohol, the Philippines.

A total of 326 larval coral reef fish from 14 families were caught (see Table 1). The four most common families (Apogonidae, Holocentridae, Siganidae and Tetraodontidae) comprised 75% of the total catch.

Table 1. Summary of catches of settlement-stage coral reef fish larvae caught in light traps with broadcast reef noise (sound) and without (silent). Low catch rates prevented analysis of some of the families. Results (significance levels) of the sign tests (per family) and the generalised linear mixed effects model (GLMM) (families grouped according to their direction of response to the sound treatment) are shown (see methods for details).

Family	Silent	Sound	Total	Sign test	GLMM
Apogonidae	31	12	43	0.226	
Holocentridae	22	13	35	0.133	
Lethrinidae	8	2	10	0.125	
Lutjanidae	14	4	18	0.015	< 0.001
Pomacentridae	13	1	14	0.062	
Siganidae	57	26	83	0.500	
Tetraodontidae	45	44	99	0.500	0.718
Blennidae	1	1	2		
Carangidae	2	2	4		
Chaetodontidae	0	1	1		
Mullidae	2	5	7		
Scaridae	0	2	1		
Sphyraenidae	1	4	5		
Syngnathidae	2	2	4		

Sign tests showed that one family (Lutjanidae) was caught in greater numbers on significantly more nights in the silent traps (see Table 1; Fig. 2).

In contrast, the less conservative GLMM that takes into account other spatially and temporally variable factors found that there was a variable response of fish families to the sound treatment (family: sound treatment interaction, χ^2: 14.41, p= 0.025). When the seven most abundant families were grouped according to their direction of response to the sound treatment, six (Apogonidae, Holocentridae, Lethrinidae, Lutjanidae, Pomacentridae and Siganidae) were caught in higher numbers in the silent traps in comparison to the sound (χ^2: 15.240, p<0.001). There was no difference between the sound and silent treatment in catch rates for the Tetraodontidae (*post hoc* Mann-Whitney, W: 118.5, p= 0.718).

Discussion

Settlement-stage larval fish were not attracted to the broadcast reef sound. We predicted that if fish could detect and were attracted to reef noise, there would be higher catch rates in the sound treated light traps. The opposite effect was found for the Lutjanidae, where significantly more fish were caught in the silent than in the sound treated traps. There was an overall trend, when the abundant families were grouped together, for higher catch rates in the silent treated traps. This result is in contrast to those from four previous acoustic playback studies in which fish (of the families caught in this study: Apogonidae, Holocentridae, Lethrinidae and Pomacentridae) were attracted to broadcast reef sound (Leis and Carson-Ewart 2003; Simpson et al. 2004; Simpson et al. 2005b; Simpson et al. 2008). Our results are consistent with those of Leis et al. (2003), who demonstrated that the attraction of settlement-stage apogonids and pomacentrids to sound varied with location. In that study larvae responded positively to the sound treament at inshore but not offshore sites. There are two possible explanations for the lack of congruence with the findings that settlement-stage fish are attracted to reef sound: 1) there was a negative effect between the design of the traps used and the sound treatment 2) there was an artefact of the recording we broadcast for the sound treatment that acted as a repellant to settlement-stage larval fish.

We used a light trap that has a more open entrance than did those used at Lizard Island. This could mean that fish entering the trap were more vulnerable to predation. So if for example, the sound treatment also attracted predators, this could reduce the number of fish caught. Without any data on the rate of predation on fish entering the trap, this explanation, as with any other on a potential trap type and sound treatment interaction, is speculative. However, this is unlikely to have contributed to our finding that settlement-stage fish were caught in higher numbers in the silent traps, as when the Ecocean traps were used at Lizard Island in 2007-8, the most commonly caught families were more abundant in the sound treated traps (Heenan, pers. obs). This asymmetry also is unlikely to be the result of the fish caught in our study being unable to detect the sound treatment, as if this were the case, one would expect an equal number to be caught in the silent and sound treated traps. Without further experimentation, we do not know if this represents a general avoidance of coral reef fish larvae to sound in this region of the Phillipines or if it was specific to the recording used for the sound treatment.

There are two aspects of the recording itself that may have been repellent to settlement-stage coral reef fish. The first concerns the variation in reef sounds: they vary with time (season, moon phase and time of day); and the biological chorus has cyclical patterns in intensity, peaking during summer evenings around the new moon (Cato 1978; Radford et al. 2008). This coincides with when larval fish arrive in highest density to recruit to the reef (Dufour and Galzin 1993; Irisson and Lecchini 2008). While settlement-stage fish are attracted at night to nocturnal reef recordings (Leis and Carson-Ewart 2003; Leis and Lockett 2005; Simpson et al. 2004; Simpson et al. 2005b; Simpson et al. 2008; Tolimieri et al. 2004), they do not respond to nocturnal reef noise during the day (Leis et al. 2002; Tolimieri et al. 2004). Due to logistical reasons, the test recording we used was taken in the morning (8am), however *in situ* observations of released larvae showed that they orientate away from the reef during the day in Australia (Leis and Carson-Ewart 2002). In this study larval fish were repelled by a daytime recording, therefore this result supports the diel dependent nature of larval attraction to sound, suggesting that 1) they can perceive the difference between the sound of a reef at night and during the day and 2) they use this information to time their approach to the reef.

The second aspect of the test recording relates to the difference between the area where it was taken and played back. Located 60 km away, the recording was chosen as it was a marine protected area, with high fish diversity and abundance, and we believed it to be a biologically rich in sound. Some settlement-stage larval fish appear to imprint to their natal reef site by olfaction (Arvedlund and Nielsen 1996; Arvedlund et al. 1999; Gerlach et al. 2007), and so as embryonic stage fish can hear sounds (Simpson et al. 2005a) it is plausible to suggest that imprinting may also occur to natal reef sounds. If this were the case in this study fish may have been affected by the non local aspect of the test sound. However, in four separate studies, larval fish at Lizard Island were attracted to a recording taken at Feather Reef, which is located over 300 km away, which shows that larval fish will respond to a non local recording. Instead, we suggest that the test recording sounded sufficiently different from the ambient acoustic conditions that were characteristic of the playback site (a channel flanked by two reefs that had degraded to urchin and algal dominated reefs), that it caused fish in this area to avoid the played back sound.

This is the first *in situ* acoustic playback experiment on settlement-stage coral reef fish performed outside of the Great Barrier Reef, and we found that in contrast to these previous studies, catches of larvae decreased due to the sound treatment. This suggests that larvae are more selective in their response to reef sound, rather than having a generic innate attraction. Given the potential for habitat degradation, overfishing and anthropogenic sources of sound to modify the natural soundscape, acoustic surveys are needed to compare the soundprints of different reefs. Furthermore, experiments are required to determine the selectivity of acoustic cue use in settlement-stage fish, as it seems possible that this could affect the ability of larvae to acoustically detect a suitable settlement site.

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