Behavior and Sound Production by Longspine Squirrelfish *Holocentrus rufus* During Playback of Predator and Conspecific Sounds

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Abstract

Fishes and marine mammals make sounds and listen for predators and conspecifics, *i.e.*, they communicate underwater using sound. Longspine squirrelfish *Holocentrus rufus* are nocturnal reef fishes living in the Caribbean that commonly produce low-frequency sounds at dawn and dusk. In order to determine the reactions of longspine squirrelfish to sounds made by their conspecifics and by their potential predators, we performed experiments in which we played the grunting sounds of longspine squirrelfish and the echolocation and signature whistle sounds of bottlenose dolphin *Tursiops truncatus* through an underwater speaker on the reef at the Institute for Marine Studies, Calabash Caye, Turneffe Atoll, Belize. At the surface, a portable laptop computer was programmed to playback a series of sounds in the following sequence: pre-playback period with no sounds (8 min in experiment 1, 5 min in experiment 2), 700-Hz tone (10 min), longspine squirrelfish grunts (10 min), bottlenose dolphin echolocations (2 min), bottlenose dolphin signature whistle (2 min), and a post-playback without sounds (1 min in experiment 1, 2 min in experiment 2). Both the squirrelfish and dolphin sounds were recorded in the coral reef areas surrounding the study site. We also played a 700 Hz tone as a control. We monitored the sound production from free-ranging longspine squirrelfish in the area near the speakers where we played the sounds, and recorded behavioral responses of the fish using a digital video camera in an underwater housing with integrated hydrophone. To minimize acoustic disturbance, we used closed-circuit rebreathers (Inspiration) during the experimental playbacks. We compared the amount of time the squirrelfish stayed in view, how long the fish performed visual displays (fin erections), and how often they vocalized during each of the playback treatments. We found that the amount of time longspine squirrelfish remained in view did not significantly differ among treatments. Likewise, the duration of visual displays did not significantly differ among treatments. However, the fish appeared to perform fewer vocalizations during the playback of bottlenose dolphin sounds relative to vocalizations made during other playbacks. Longspine squirrelfish may be listening for the hunting sounds made by predators and responding to those sounds by performing fewer vocalizations.

Introduction

Fish use sound to communicate in a variety of ecological interactions such as courtship and mating, aggressive encounters, and to signal alarm (*e.g.*, Winn *et al.*, 1964; Myrberg, 1981; Crawford *et al.*, 1997; Mckibben and Bass, 1998). An example of such a sound-producing fish is the longspine squirrelfish *Holocentrus rufus,* which inhabits shallow coral reefs throughout the Caribbean Sea. They produce low frequency vocalizations at a dominant frequency of 75-600 Hz (Moulton, 1958; Fish and Mowbray, 1970; Carlson and Bass, 2000). Vocalizations are produced by the contraction of muscles that vibrate ribs surrounding the swim bladder (Winn and Marshall, 1963). These low-frequency sounds are produced during both day and night, but appear to increase after sunset

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(Luczkovich, 2002) with peaks at dawn and dusk related to territorial behavior; during the day the fish are inactive hiding in reef crevices and at night they venture away from their territories in search of food (Winn *et al.*, 1964). During the transition between day and night, they interact vocally at their territory boundaries.

Longspine squirrelfish behavior
The following summary of behavior and sound production of longspine squirrelfish is based on the description by Winn *et al.* (1964), except where noted. Longspine squirrelfish typically produce either single grunts or a series of grunts (staccatos). The fish are territorial and often maintain territories adjacent to other longspine squirrelfish territories. During the day, squirrelfish hover near reef crevices and at night, they are more active, but do not venture far from daytime territory (Collette and Talbot, 1972). Longspine squirrelfish display a variety of behaviors and vocalizations when their territorial boundaries are violated. Single grunts are often issued by resident fish when territories are violated by conspecifics. The resident fish may also erect its fins and dash at the encroaching fish. Should the resident and intruder meet, both individuals may shudder so that the whole body vibrates. Sometimes only the resident fish shudders. In the case of an abrupt meeting, the resident fish might infrequently initiate a staccato call. Longspine squirrelfish may also initate shudders, grunts, or staccatos when fishes of other species, such as the bluestriped grunt, violate territorial boundaries. Interestingly, Winn *et al.* (1964) found that longspine squirrelfish sounded longer staccatos when moray eels were introduced into their territories relative to other non-predatory fish such as mullets and grunts.

Marine Mammals and Predator-Prey interactions
Marine mammals also use sound in predator-prey interactions. For instance, bottlenose dolphins, change their direction of travel and orient toward playbacks of sound-producing (soniferous) fishes, suggesting that they listen passively for low-frequency prey sounds (Barros and Odell, 1990; Barros and Wells, 1998; Gannon and Waples, 2004; Gannon *et al.*, 2005). Bottlenose dolphins also produce high-frequency sound (active sonar) for echolocation (Au, 1993). Bottlenose dolphins may also employ low frequency 'pops' when locating prey (Remage-Heley *et al.*, 2006). Recent studies have suggested that some species of fish are capable of hearing and reacting to these sounds used by dolphins while hunting. Mann *et al.* (1998) conducted an experiment in which bottlenose dolphin echolocation clicks were played to captive shad *Alosa sapidissima*. The researchers used classical conditioning of heart rate and muscle activity to determine whether or not their experimental subjects could detect echolocation clicks. Their results suggest that shad can detect clicks with a peak frequency of 80 kHz. Mann *et al*. (1998) propose that shad evolved this ability to hear ultrasonic sounds in response to selective pressure brought about from predation by marine mammals. Remage-Healey *et al.* (2006) played bottlenose dolphin sounds to gulf toadfish *Opsanus beta* in their natural environment. The males of the species use vocalizations during courtship. The researchers found that these vocalizations were suppressed by as much as 50% when low frequency dolphin 'pops' were played within the hearing range of the toadfish. Additionally, the scientists took blood samples from the toadfish immediately following playback of the dolphin sounds. These samples were analyzed and found to have significantly elevated levels of the stress hormone cortisol. Finally, Luczkovich *et al.* (2000) recorded chorusing silver perch *Bairdiella chrysoura* in North Carolina waters, and found that, when bottlenose dolphin whistles also occurred, the intensity of perch chorusing dropped nearly eight fold. The perch resumed chorusing following the cessation of dolphin whistling. This significant drop in chorusing intensity was also observed when the researchers performed experiments involving the playback of recordings of dolphin whistles (3500-6000 Hz) to naturally occurring populations of chorusing silver perch (Luczkovich *et al.*, 2000). We believe that bottlenose dolphins are predators of the longspine squirrelfish, based on the fact that they normally consume fishes, and elsewhere it has been shown that up to 75% of the diet of this marine mammal is composed of soniferous fish species (Barros and Odell, 1990; Barros and Wells, 1998; Gannon and Waples, 2004).

Study Objectives

The purpose of our study was to determine the reactions of longspine squirrelfish to sounds of their conspecifics and their potential predators (bottlenose dolphin) on a coral reef in Belize. We observed the behavior of these fishes during the crepuscular period in a natural setting in order to determine what types of sounds they would respond to with naturally occurring background sound levels (sounds of other fishes, snapping shrimp, waves, etc.), but without any diver's bubbles and associated sounds. We examined the responses of free-ranging longspine squirrelfish to a variety of acoustic stimuli including vocalizations made by conspecifics and sounds produced by bottlenose dolphins recorded in nearby locations. We determined if these sounds would alter the amount of time longspine squirrelfish stayed out in the open, the duration of visual displays such as fin erection, and vocalization frequency of occurrence. We hypothesized that the amount of time in view, the display time, and the vocalization rate would be higher during the playback of the longspine squirrelfish vocalizations, and lower during the playback of the bottlenose dolphin sounds relative to the controls.

Methods

Playback Studies

Playback studies were conducted using an underwater speaker mounted on the bottom in 8 m of water on the Calabash Caye at dusk (1700-1900 local time in June 2005) (Figure 1). Sounds were played through a Clark AC339 underwater speaker. A portable car audio amplifier (Sony XPlode 200 watt) was used to drive the underwater speaker. Sounds were played back on a Panasonic Toughbook CF-29 using a pre-programmed Windows Media play list. Longspine squirrelfish were videotaped by divers using Inspiration rebreathers to allow the recording of fish sounds while minimizing the bubbles and sounds associated with SCUBA diving. Fish were videotaped and sound recorded using a Light and Motion Mako housing and Sony PC100 camera. The housing had an integrated hydrophone. Behavior was monitored during a series of playback treatments: 1) pre-playback period with no sounds (8 min in experiment 1, 5 min in experiment 2); 2) pure 700-Hz tone (10 min) as a positive control; 3) longspine squirrelfish grunts (10 minutes); 4) bottlenose dolphin echolocation (2 min); 5) signature whistle (2 min); and 6) a post-playback period with no sounds. Sounds were played back in sequence as noted above in both experimental trials; no attempt was made to randomize playback sequence because this was a preliminary study. Both the longspine squirrelfish and bottlenose dolphin sounds were previously recorded in the coral reef areas on Turneffe Atoll and stored in wav files (16 bit, 44.1 kHz).

Video Analysis

Tapes were reviewed and scored for the following responses. Dorsal fin erections (displays) were counted and timed during each of the various playback treatments. Also, the amount of time individual fish remained within view during each playback treatment was determined. Finally, we used Ulead Video Studio (8.0) software (www.ulead.com) to make wave files of the video soundtracks. These files were then made into spectrograms using Raven Lite 1.0 Bioacoustics Software (Cornell Lab of Ornithology, 159 Sapsucker Woods Rd, Ithaca, NY, 14850) so that sounds could be visualized and counted, and the frequency of occurrence of longspine squirrelfish vocalizations was determined for each playback treatment. It should be noted that low-light conditions during the dolphin playback made the analysis of longspine squirrelfish displays and time in view more difficult and potentially less accurate. Also, background noise in the dolphin whistle playback prevented analysis of longspine squirrelfish vocalizations.

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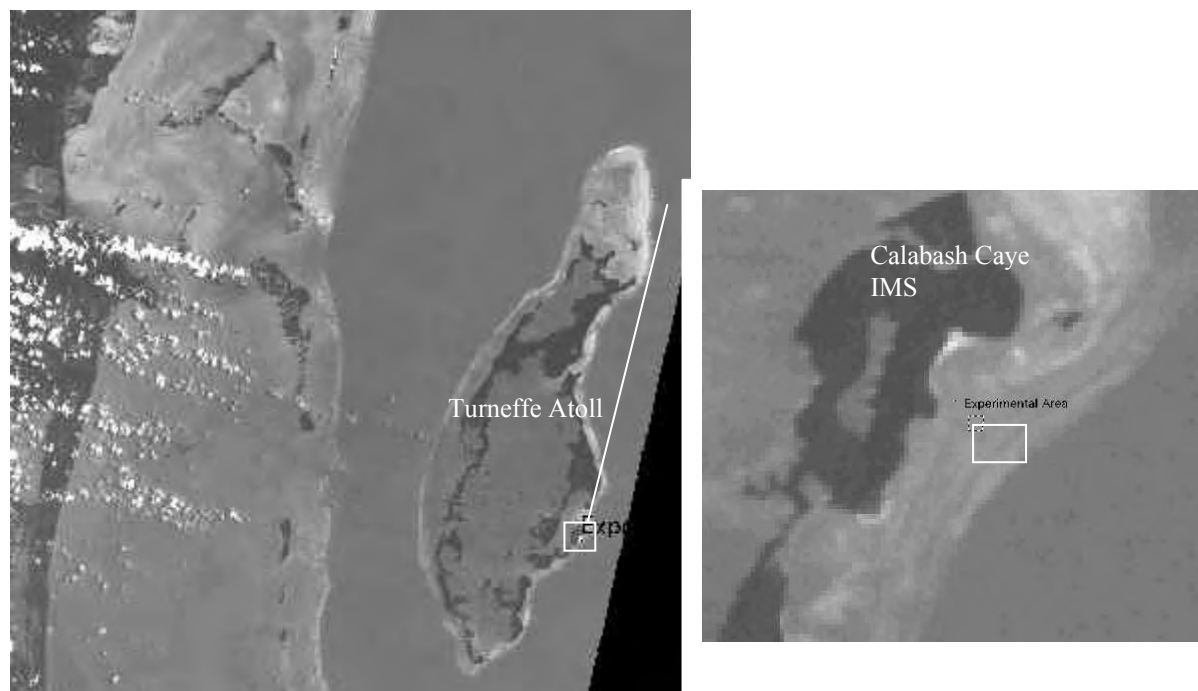

Figure 1. Landsat image of the coastal area of Belize, with inset of the experimental area for playback studies, near University of Belize Institute of Marine Studies (IMS), Calabash Caye, Turneffe Atoll, Belize.

Statistical Analysis

Although these were preliminary studies, we attempted a statistical test in spite of the low power to reject our hypotheses. The small sample size reported here (n=2) necessitated a non-parametric testing procedure and we report probability values for informational value, as it would be difficult in any circumstance to obtain a significant test at such low statistical power. We used a nonparametric Kruskal-Wallis (KW) test for comparison of median response variables (Systat Version 11.0, Systat Software, San Jose, CA, USA). We feel that plots of the response variables under variable treatments are very informative for our preliminary experiments. We plotted response variables with a box plot (also in Systat 11.0). Examination of the box plots allows the reader a reliable and quick assessment of the fish's responses for patterns that may be replicated under greater statistical power.

Results

Longspine squirrelfish did not appear to spend less time in view during the conspecific and dolphin sound playbacks, and they did not exhibit different durations of visual display. The amount of time individual fish remained in view during each playback treatment did not vary in a statistically significant way (KW test p=0.898, Figure 2). Likewise, no statistically significant differences were found among treatments in display duration (KW test p=0.610, Figure 3). No clear patterns were observable among median values shown in the box plots, although the range of observed values declined greatly during the longspine squirrelfish and dolphin sound playbacks and did not return during the post-playback period.
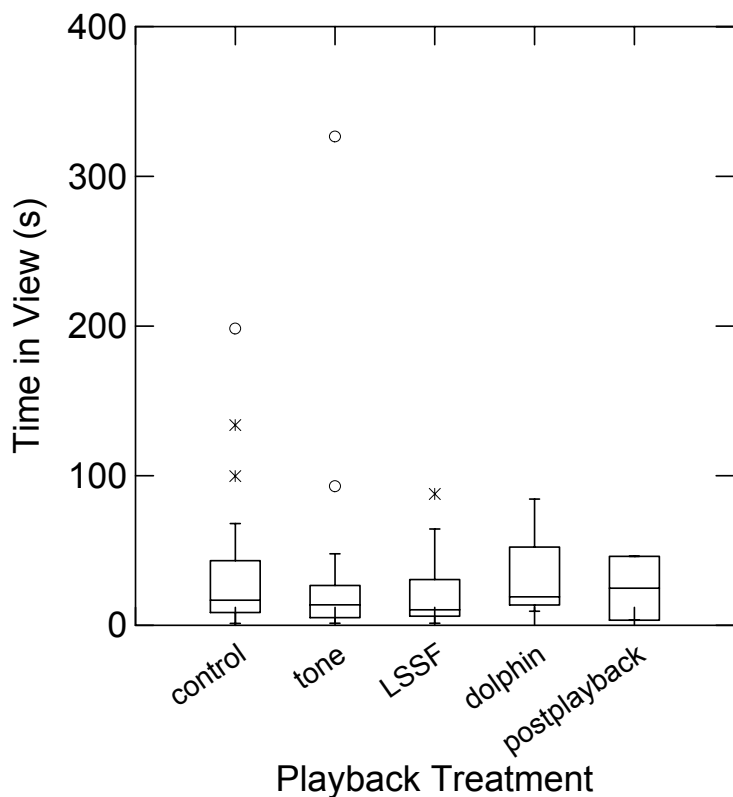
Figure 2. Box plot of the time in view (s) of individual longspine squirrelfish (LSSF) during various playback treatments. which shows the median as a horizontal line, a box showing the range in which 50% of the values fall (the mid-range, defined by the first and third quartile range boundaries), a vertical line above and below the box which shows the range of the observed values within a range equal to 1.5* the mid-range above and below the first and third quartile range boundaries. Values falling outside this range are indicated with asterisks (<3* the mid-range above or below the box limit) or circles (>3* mid-range above or below the box limit) and are considered far outside values.
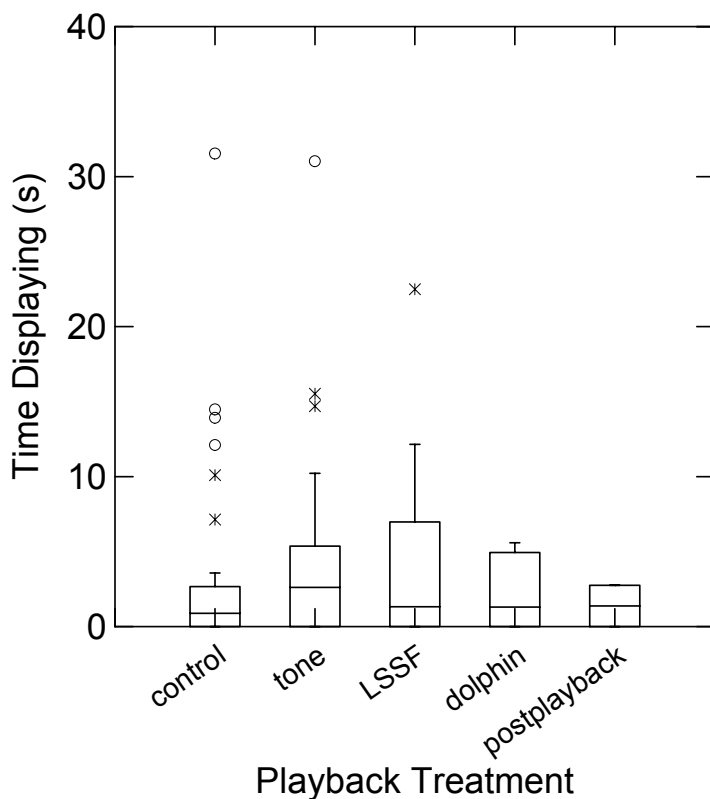
Figure 3. Box plot of the time spent displaying by longspine squirrelfish (LSSF) during various playback treatments. See Figure 2 for explanation of box plot symbols.

Vocalizations did not appear to vary under the pure tone and longspine squirrelfish playback treatments; however vocalizations became less frequent during dolphin echolocation playback (Figure 4). Experimental Trial 1 had high levels of natural grunting by longspine squirrelfish and there were several longspine squirrelfish in the experimental area. This resulted in a high variation in the rate of grunting between experimental trials. Nonetheless, vocalizations that had been recorded at median rate of 3.15 and 4.25 per minute during the control and 700-Hz tone periods dropped to 2.45 per minute during the longspine squirrelfish playbacks and 0.25 per minute during the dolphin echolocation playbacks. In addition, the range of values decreased greatly during these two playback treatments. Although no vocalizations occurred during the dolphin echolocation playback treatment in Experimental Trial 1, a single 'staccato' sound was recorded during that treatment in Experimental Trial 2. During the post-playback period, the vocalization rates increased again to a median of 3.15 per minute. This suggests that the vocalization rate was not declining as a simple function of time, as might be expected if it were declining as ambient light levels declined at dusk. Indeed, the opposite pattern would be expected to occur, as vocalizations should have increased as darkness increased. It appears that the dolphin echolocation sounds decreased the vocalization rate of longspine squirrelfish.
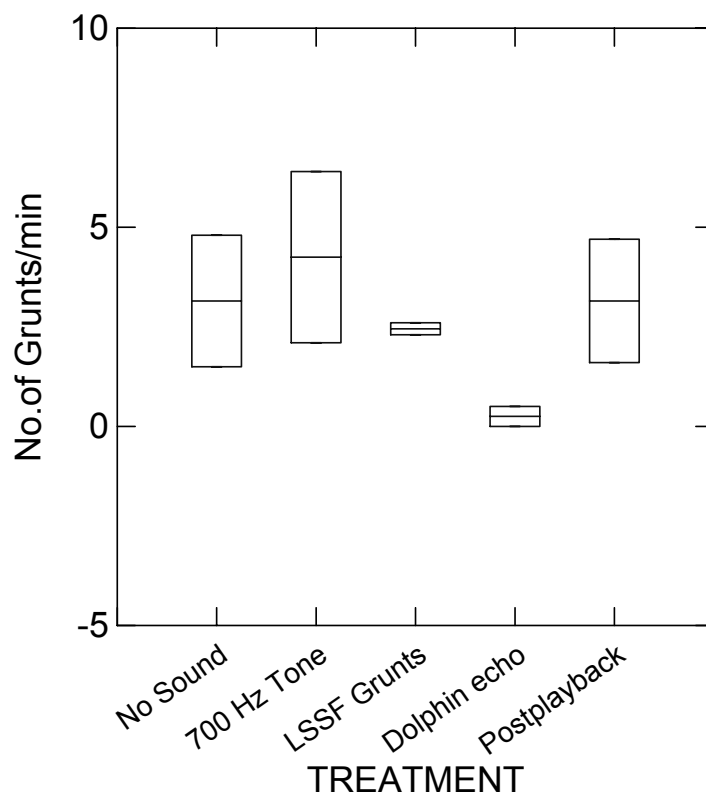

Figure 4. Box plot of the number of 'grunts' per minute by longspine squirrelfish (LSSF) recorded during each of the playback treatments. Although there was a non-significant statistical result, (KW test p=0.320), the statistical power was low (n=2) and there was a clear pattern of reduction in sound production during the dolphin playbacks. See Figure 2 for box plot explanation.

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Discussion

Our hypothesis that the amount of time in view, the display time, and the vocalization are higher during the playback of the longspine squirrelfish vocalizations was not supported by our preliminary observations. However, vocalization rate was lower during the playback of the bottlenose dolphin echolocation sounds relative to the controls.

The apparent suppression of longspine squirrelfish vocalizations during playback of bottlenose dolphin sounds is similar to the observed behavior of other species of fish such as Gulf toadfish (Gannon *et al.*, 2006) and silver perch (Luczkovich *et al.*, 2001). Both of these species suppressed vocalizations in apparent response to sounds made by bottlenose dolphin. Similarly, Mann *et al.* (1999) observed that shad seemed to be able to detect dolphin echolocation.

One squirrelfish staccato call occurred during one of the two dolphin echolocation playbacks. Winn *et al.* (1964) surmised that longspine squirrelfish use staccato calls as signals of alarm, and it is possible that our recorded stacatto was an alarm call to other nearby fishes. We noted another possible Holocentrid alarm call in an on-line video recording of cooperative hunting by grouper and moray eels in the Red Sea (Bshary *et al.*, 2006; Luczkovich and Keusenkothen, 2006).

The behavior we noted during playback differs somewhat from the results of Winn *et al.* (1964). Winn found that longspine squirrelfish, in an aquarium study, initially sought cover during playback of three types of fish sounds (longspine squirrelfish staccato, toadfish *Opsanus tau*, and big eye scad, *Selar crumenophthalmus*). Some fish emerged from cover after a minute or so. Our experimental subjects did not appear to seek cover during the various treatments. However, the Winn *et al.* (1964) experiments were performed in tanks, and it is possible that the behavior of the fish was influenced by their close proximity to the underwater sound source in an enclosed space.

Thus, it appears that fish inhabiting coral reefs are acoustically aware of their potential predators in the coral reef environment. We conclude that there is sufficient indication of 'acoustic avoidance' behavior in squirrelfish to dolphin echolocation calls that further verification in a controlled replicated study with randomized playback treatments is warranted. We hope to perform these experiments in the near future.

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