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#### **GULF AND CARIBBEAN FISHERIES INSTITUTE PARTNERSHIP**

# THE PURR OF THE LIONFISH: SOUND AND BEHAVIORAL CONTEXT OF WILD LIONFISH IN THE GREATER CARIBBEAN<sup>§</sup>

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#### INTRODUCTION

Understanding the behavioral context of sound production is key to the application of passive acoustics to the study of marine organisms (Gannon 2008). Acoustic communication in fishes can be associated with different behavioral contexts, such as predator defense, distress, agonism, and reproduction (Ladich and Myberg 2006, Lobel et al. 2010). Acoustic signals associated with reproductive displays can function as advertisement to attract receptive females, usually at long distances (6–13 m) as produced by the Lusitania Toadfish (*Halobatrachus didactylus*; Alves et al. 2016), or they may function as courtship signals that are usually at close proximity (< 1 m) and commonly of low frequency, like those produced by gobies (Pedroso et al. 2012). Research on the types of sound signals produced during the behavioral displays of fishes is necessary to further the use of passive acoustic monitoring.

Here we present a low amplitude and potentially courtship– related sound produced by invasive lionfish (*Pterois* spp.), the first reported sound by lionfish in the wild. The behavior and associated sounds were recorded in the presence of multiple lionfish in both Puerto Rico and the Florida Keys during separate research projects. This short communication is intended to provide a brief characterization of this behavior and sound. Lionfish are known to be capable of producing sounds (Beattie et al. 2017), but the behavior associated with sound production in natural conditions has not been previously documented.

#### MATERIALS AND METHODS

#### Study sites

Video and audio recordings of lionfish behavior were collected *in situ* at 2 locations, one in Puerto Rico and one in the Florida Keys. Bajo de Sico (BDS) is an offshore seamount surrounded by deeper (300 m) waters located 27 km off the west coast of Puerto Rico. The recorder at BDS was placed on a tripod upon an isolated rocky outcrop with relief 1.5 m above the seafloor at a depth of 43 m to monitor reef fishes at a presumed multi–species spawning aggregation site. In the

Florida Keys, sounds were recorded 8.5 km southeast of Marathon, FL at a depth of 30 m in habitat consisting of patch reefs seaward of a reef shelf–edge. The hydrophone was placed near a small patch of reef about 5 m<sup>2</sup> with < 1 m of vertical relief and separated from other patch reefs by at least 5 m, specifically to record lionfish located on this patch reef.

#### Identification of behavior and associated sound

A synchronous audio/video camera (OpenCam; Loggerhead Instruments, Sarasota, FL) was used at BDS. It was composed of a low-frequency hydrophone (HTI, 96 min series) with a sensitivity of -170 dBV re: 1 µPa and frequency response between 2-30 kHz, rechargeable lithium battery pack and a Hack-HD video camera, all embedded in epoxy. This unit recorded video at 30 frames per second (fps) with 1920 X 1080 resolution in MPEG-4 format with an audio sampling rate of 48 kHz at 32 bits per sample. The video and audio were recorded simultaneously in single files with duration corresponding to the recording schedule on the same micro SD card (64 GB capacity). It was deployed at BDS from 1–5 May 2013 during the last quarter moon phase (2 May). The daily recording schedule was designed to last multiple days by reducing observations to crepuscular times when enough sunlight reached these depths, but with more recording time near sunset. In order to distinguish the files recorded in sequence, their duration was cycled sequentially at 10, 11 and 5 min and programmed to record at the following times: 0720-0730, 0740-0751, 0800-0805, 1500-1510, 1530-1541, 1545-1650, 1610-1620, 1630-1641, 1650-1655, 1700-1710, 1715-1726, 1730-1735, 1740-1750, 1800–1811, 1815–1820, and 1830–1835.

Underwater video and acoustic recordings from the Florida Keys were made with a digital acoustic recorder paired with a high definition video camera inside a PVC housing (LHC Cyclops; Loggerhead Instruments). This unit stores audio on one SD card and video on another with unique filenames that refer to the start date and time of the recording, which were used to

<sup>&</sup>lt;sup>§</sup> This article is based on a presentation given in November 2019 at the 72<sup>nd</sup> annual Gulf and Caribbean Fisheries Institute conference in Punta Cana, Dominican Republic

pair audio and video recordings. It has a low frequency hydrophone (HTI, 96 min series) with a sensitivity of -170 dBV re: 1 µPa and frequency response between 2–30 kHz, and is powered by 12 D–cell batteries located inside the housing. The Cyclops was mounted horizontally on a cement block 20 cm above the seafloor. Audio was recorded at a 44.1 kHz sampling rate and 16–bit resolution at duty cycles of 60 s every 10 min. Video was recorded at 30 fps with 1920 X 1080 resolution in MPEG–4 format. The unit's clock is reset at each deployment on both the audio and video recorders prior to deployment so any time drift between the deployment and recovery would be negligible. Recording duration ranged from 4–28 d between February and May 2019.

Video files with embedded audio from BDS were reviewed with noise cancelling headphones to detect sounds when lionfish were present in the video frame. Videos were trimmed to clips when the lionfish were present, and sounds were then extracted. Video and audio files that were recorded separately from Florida were synchronized in PowerDirector 14 (Cyber-Link) and reviewed with noise cancelling headphones.

#### Characterization of sound structure

Sounds heard during lionfish behaviors were initially classified as a lionfish call. These and similar sounds from BDS with high signal to noise ratio (S/N), above a threshold of 10 dB of background noise, and all similar sounds from Florida were pooled per site to describe them acoustically. Acoustic parameters measured included: total duration of call bouts, number of units or calls per bout, interval between calls, call duration, peak frequency, and sound pressure level (SPL). For purposes of characterization, we defined a call bout as a string of calls occurring within 5 s of each other. Intervals between successive calls and number of individual calls were measured within bouts. Call bout duration was measured as the time difference between the beginning of the first and end of the last call in the bout. The peak frequency within each call was calculated as the frequency at which maximum power occurred. Spectrograms and oscillograms of the sounds were created with Raven Pro 1.5 with a 1391-point (29 ms) Hann window (3 dB bandwidth = 49.6 Hz), with a 50% overlap and 2048-point DFT yielding time and frequency measurement precision of 14.5 ms and 23.4 Hz. To determine the SPLs of calls, only videos where lionfish were visible and estimated to be within a meter of the recorder were used. Sound pressure levels were first converted to linear pressure units (µPa) to calculate the mean then converted back again to SPL (dB re: 1 µPa), to provide a mean energy level for the sound. Power spectra of a lionfish call and of a similar clip of ambient noise were extracted from the spectrogram for comparison of sound strength.

At BDS lionfish were collected at similar depths as the recorders (45–60 m) by polespear with closed circuit rebreathers in order to evaluate their sexual dimorphism. All fish were measured as total length (TL) to the nearest 0.1 cm. A Student's t-test of independence was used to test differences in size of males vs. females. A temperature logger (Onset) was deployed at the seafloor near the recorder at BDS and a Vemco VR2Tx receiver with built in temperature sensor was deployed at 40 m on a wreck offshore of Marathon Key, Florida to measure seawater temperature (°C) at each site.

#### Results

#### Identification of behavior and associated sound

Recordings at BDS occurred during early morning and late afternoon hours completing a total recording time of 583 min in 58 separate recordings, of which 43 recordings were during hours of sufficient light to see lionfish behaviors. During one recording (3 May 2013 at 1830, 15 min before sunset) 2 lionfish swam directly in front of the camera, in close proximity to each other revealing a behavior accompanied by a stereotype sound described below (Supplementary Video 1). At the time of this sound, both lionfish were swimming slowly and in the same direction with the second fish closely behind the first, and both had all fins fully extended. The second fish appeared larger in size and darker in coloration. A bout of calls with intervals of silence occurred during the moment both fish pass directly in front of the camera, and was perceived strongest when the larger fish, the chaser, approached the camera and the sound waned as they moved away. These bouts of calls were present in 8 of the 58 video recordings from BDS, although without direct visual images of lionfish, and they were heard at least once daily and only between 1615 and 1840. Seawater temperature at depth on 3 May 2013 averaged 26.4°C (range 25.7 – 27.6 °C); sunset occurred at 1846.

In the Florida Keys, a sound of similar structure was recorded with lionfish visible in the video frame on 20 March 2019 at 1525 and 1535 (Supplementary Video 2). At that date and time there was a full moon, tide was falling, and the observation occurred 2 hr before sunset (1735) when seawater temperature measured at a similar depth 3.4 km away was 25.4°C. Both recordings showed 2 lionfish hovering above the sand underneath a coral overhang. Immediately at the start of the call bout the smaller lionfish turned toward the larger one which gently swept its tail twice and raised its dorsal fin. The smaller fish continued to face the larger for several seconds before returning to its original orientation, all of which occurred during the call bout. In the second video, the same 2 fish are observed resting on the substrate. At the start of the call bout, the fish that appeared to be smaller turned slightly towards the one that appeared larger, which then erected its dorsal fin. The angle of the lionfish in relation to the camera make it impossible to determine their coloration patterns. Video recordings 10 and 20 min after the two described above showed the lionfish in the same location and in close proximity without any display or sounds. Similar call bouts were recorded on 21 occasions in 119 hr of audio-only recordings. All sounds occurred between 1500 and 2030, with 81% (17) within 2 hr of sunset.

#### Characterization of sound structure

From the BDS deployment 18 bouts of high signal to noise ratio (S/N) sequential calls (Supplementary Audio 1) were selected from which 122 individual calls were used to measure acoustic parameters. From the Florida Keys data, 373 calls, from 21 bouts were measured. The sound resembles an inter-

Parameter	Florida Keys				Puerto Rico			
	N	Mean (sd)	Min.	Max.	N	Mean (sd)	Min.	Max.
Bout duration (s)	21	18 (11.4)	1.1	44.5	18	5.59 (4.95)	0.6	28.5
Calls per bout (number)	21	19.2 (12.7)	4	52	18	8.4 (7.4)	2	50
Call duration (s)	373	0.19 (0.05)	0.22	0.36	122	0.13 (0.02)	0.08	0.18
Inter-call interval (s)	352	0.68 (0.48)	0.1	4.7	260	0.62 (0.22)	0.05	1.50
Peak frequency (Hz)	373	246.7 (15)	172.3	279.9	122	256.3 (8.9)	234	281
Sound pressure level (dB re: 1 µPa)	373	67.3 (3.7)	76.8	56.6	16	65.8 (0.2)	72.1	60.2

**TABLE 1.** Values of acoustic parameters of lionfish calls. Sample size (N) refers to the total number of units used for each parameter. Spectrograms and oscillograms of the sounds were created with Raven Pro 1.5 with a 1391-point (29 ms) Hann window (3 dB bandwidth = 49.6 Hz), with a 50% overlap and 2048-point DFT yielding time and frequency measurement precision of 14.5 ms and 23.4 Hz. sd = standard deviation.

mittent purr of a constant tone ending in a slight down sweep. Acoustic parameters from each location are summarized in Table 1. Bouts consisted of multiple and variable numbers (2 – 52) of discrete tonal calls (Figure 1), but no calls were observed in isolation. In BDS and Florida, respectively, mean total duration of the call bouts was 5.6 and 18 s, each call lasted 0.13 and 0.19 s on average, with mean intervals between calls of 0.62 and 0.68 s. The peak frequency averaged 256.3 and 246.7 Hz, and mean SPLs were 72.1 and 67.3 dB re: 1 µPa, in BDS



**FIGURE 1.** Example of a call bout spectrogram, a call spectrogram, and a call oscillogram of a lionfish sound recorded from Bajo de Sico, Puerto Rico. A. Call bout spectrogram. B. Call spectrogram. C. Call oscillogram. Spectrograms with 50% overlap, DFT-2048.

and Florida, respectively. The relative amplitude was higher between 250 and 280 Hz in the power spectra with lionfish calls overlaid upon that of ambient noise from the Florida acoustic data (Figure 2).



**FIGURE 2.** Power spectra of a Pterois sp. call (black line) and of ambient noise (grey background), recorded at the Florida Keys. A 0-10 Hz band-stop filter was applied prior to plotting to remove electrical interference noise produced by the recorder (spectrogram with 50% overlap, DFT 2048).

#### DISCUSSION

Bioacoustic characteristics, i.e., the structure of the sound signal (number of pulses, time between pulses, pulse rate, etc.; Myrberg 1997), may provide information on the identity or condition of the sound producer, as well as information on the nature of the interaction (courtship, spawning, territorial defense, etc.). The bioacoustic parameters of the tonal calls of lionfish described here differ significantly from the 'hum' recorded in captivity by Beattie et al. (2017), which only occurred in the presence of multiple lionfish or when they were agitated. In captivity, pulses were much shorter in duration, had greater bandwidth, and reached 900 Hz in frequency (Beattie et al. 2017), suggesting that the hum may be associated with agonistic displays between conspecifics and is distinct from this study's purr. A narrow bandwidth, relatively longer duration call bout structure, and occurrence when 2 lionfish are interacting non-agonistically suggests the purr of the lionfish is associated with courtship. Sounds produced by *Pterois* spp. may be the result of drumming on the swim bladder, as both intrinsic and extrinsic sonic muscles have been found in this genus (Yabe 1985). However, more detailed investigations are needed since multiple sound production mechanisms are possible in Scorpaeniform fishes (Ladich and Fine 2006, Kasumyan 2008).

Only 2 lionfish behavioral displays have been recorded in the wild. One is an agonistic interaction described as a high intensity act of 2 presumed males in a head to head orientation (Fogg and Faletti 2018), although no sounds have been associated with that agonistic behavior. The other consists of lionfish courtship displays, which according to Fishelson (1975) include circling, side winding, following and leading in close proximity, with behaviors beginning shortly before dark and extending into the night. The reproductive behaviors of other pteroid fishes (Dendrochirus zebra) exhibited changes in coloration and considerable male activity and courtship display 30 min prior to sunset (Moyer and Zaiser 1981). Females initiate courtship by swimming away from the male, followed by a pursuit with the male circling the female and tactile stimulation with spines or pelvic fins (Moyer and Zaiser 1981). For P. miles in the Red Sea, spawning follows courtship shortly after sunset when males lead the female to the surface, culminating as the female releases a floating egg mass that is fertilized by the male (Fishelson 1975).

In our recordings, the diel timing of lionfish behaviors is similar to the timing of courtship behavior in the species' na-

tive range. Lionfish show sexual dimorphism during reproduction (Morris et al. 2009), and dimorphic growth where females do not reach the length of males in the Caribbean (Edwards et al. 2014), supporting our presumption that the fish observed in the video recordings represent male-female pairs. At BDS this could be interpreted as a female being pursued by a larger, presumably male fish, and in Florida it is possible a mating pair was observed, as males grow larger than females in the Gulf of Mexico (Fogg et al. 2019). This hypothesis is further supported by data from lionfish collected at BDS, where the mean length of females was 0.82 x that of males (males: mean TL = 321.4 ± 44.5 mm, n = 17 vs. females: mean TL = 265.4 ± 22.6 mm, n = 14; Student's  $t_{24,59}$  = 4.53, p < 0.001). Unfortunately, the video images that revealed the behavioral display were not of sufficient quality to measure the relative sizes of the fish.

Sound pressure levels recorded during call bouts in the wild were low (67.3 – 65.7 dB re: 1  $\mu$ Pa) compared to ambient noise, even with the fish observed near (0.5 – 1 m) the recorders. Mean background SPL in Florida during the time frame in which lionfish vocalizations were detected (1500 – 2100) was 66.57 (3.3) dB re: 1 $\mu$ Pa, rivaling the received pressure levels of calls themselves. Hence the lionfish call bouts may be classified as a specialized, quiet signal (Kasumyan 2008, Kaatz et al. 2017) generally elicited during courtship between individuals at very close range. The low amplitudes described here may require special hydrophones placed at proximity for detection.

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