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Vocal repertoire and sound characteristics in the variegated cardinalfish, *Fowleria variegata* (Pisces: Apogonidae)[a]

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JASA ARTICLE

Vocal repertoire and sound characteristics in the variegated cardinalfish, *Fowleria variegata* (Pisces: Apogonidae)[a)]

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ABSTRACT:

The variegated cardinalfish *Fowleria variegata* produces grunt and hoot calls during agonistic and courtship interactions. Both sounds are tonal and occur as single and multiunit calls. Grunts are of short duration with variable frequency spectra. Hoots are longer, have a higher fundamental frequency, and a more developed harmonic structure. Agonistic grunt calls and short hoot calls (1–2 hoots) are produced during chases and when striking an individual or a mirror. Grunts are produced primarily in male-female and mirror-image encounters, and short hoot calls are produced primarily in male-male interactions. During the reproductive period, long hoot calls (three and four hoots) are the main sound type in a mix-sexed tank and at Dongsha Atoll. These are likely produced by males because isolated females are silent, and isolated males emit long hoot calls. Courtship interactions are mostly silent, and males are silent after capturing eggs for oral brooding. Tank sounds peak at dusk to early evening with a smaller peak at noon, although there are dusk and dawn peaks at Dongsha Atoll. Tank sounds exhibit a semilunar rhythm with peaks at the new and full moon. Other cardinalfish species from the atoll produce grunts but not hoot calls.

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I. INTRODUCTION

About 167 of 488 families of actinopterygian fishes include 800–1000 (a minimal estimate) soniferous species (Fine and Parmentier, 2015; Parmentier *et al.*, 2021; Looby *et al.*, 2021; Rice *et al.*, 2022), who purposefully emit sound for defense, advertisement, or social cohesion (Myrberg, 1981; Myrberg *et al.*, 2006; Fine and Parmentier, 2015; van Oosterom *et al.*, 2016). Sonic families are distributed in a vast range of habitats, including coral reefs.

Purposeful fish sounds are typically produced by sonic muscle contractions and stridulation between hard structures. They tend to be of short duration, low frequency (<3 kHz), repetitive and usually consist of a series of wideband frequency pulses (Parmentier and Fine, 2016). Generally, swimbladder sounds are low-pitched, and stridulatory sounds are higher-pitched (Fine and Ladich, 2003; Ladich and Fine, 2006; Kasumyan, 2008; Ladich, 2014; 2019).

Cardinalfish (family Apogonidae) with about 380 species in 41 genera (Eschmeyer *et al.*, 2021) have not been reported to produce sound. They are small (most species are < 10 cm) nocturnal fish in tropical regions of the Atlantic, Indian, and Pacific Oceans (Hobson, 1991). Although primarily marine, some species live in brackish and fresh water (Allen, 1993). Most species feed on zooplankton and small benthic invertebrates (Barnett *et al.*, 2006), but little is known about their social behavior (Kuwamura, 1985; Gould *et al.*, 2014). Although a female cardinalfish, *Pterapogon kauderni* was considered to be defending her mate after reproducing (Kuwamura, 1985; Okuda, 1997), Kolm and Berglund (2004) reported new evidence that males, not females, are the main aggressors toward a conspecific intruder and attributed the key role in territory defense to males in established pairs. A male broods the fertilized eggs in his mouth, and reproduction repeats every 2–4 weeks (e.g., Roozbehfar *et al.*, 2012). Many cardinalfishes are aggressive, although their social behavior ranges from territorial to shoaling.[1] The Apogonidae contains several bioluminescent coastal species in the Indo-Pacific genera *Siphamia*, *Rhabdamia*, *Archamia*, and *Apogon* (Herring, 1992; Gon, 1996; Gon and Allen, 1998; Thacker and Roje, 2009; Gould *et al.*, 2016) who possibly use bioluminescence for camouflage, defense, predation, and/or communication (Davis *et al.*, 2016).

During a survey of the soundscape of a patch reef in seagrass-coral mosaic habitats (Lee *et al.*, 2019) at Dongsha Atoll (20°41056.5″N 116°43006.9″E), an unidentified harmonic fish sound was recorded repeatedly. Auditioning captive fish indicated that the variegated cardinalfish, *Fowleria variegata* (Fig. 1) is the sound source. This finding represents the first record of sound production in cardinalfishes.

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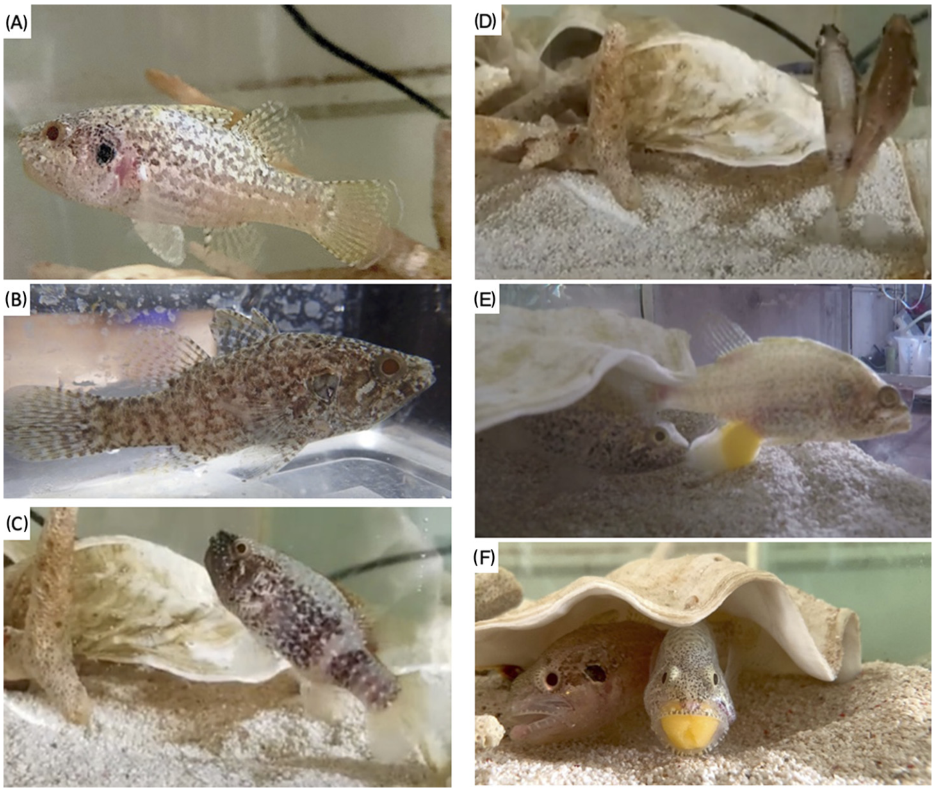

FIG. 1. (Color online) *F. variegata*, depicting the (A) male, (B) disturbed female, and (C) male in courtship. Notice the whitish pattern along the dorsal base extending to the stripe across the rear end of the body. (D) During courtship, the male (left) pushes sideward to the female (right). Notice the vertical pale strip anterior to the caudal peduncle was visible in both fish. (E) The male just prior to sucking the egg mass into his mouth cavity. (F) The male (right) had the egg mass in his mouth cavity and his mate was on the left side. Notice the female's opercle spot turned much darker after releasing the egg mass.

This paper (1) reports evidence for *F. variegata* as the source of the harmonic call, and (2) describes its vocal repertoire in males and females, (3) behaviors associated with sound types, and (4) circadian and lunar rhythms in sound production.

II. MATERIALS AND METHODS

Experimental and animal care protocols followed relevant international guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of National Sun Yat-sen University (IACUC No. 10735). Live fish collection at Dongsha Atoll was approved by the Marine National Park Headquarters.

A. Sample collection

Twelve *F. variegata* (seven males and five females; standard length, 55–79 mm) were collected in March 2019 (seven fish) and July (five fish) at a depth of 2–3 m from branching-coral patches of *Acropora* close to a seagrass bed in the lagoon at Dongsha Atoll. Fish were captured by scuba divers using 70% clove oil mixed with 30% ethanol and hand nets and transferred to acclimation tanks at National Sun Yat-sen University. The seven fish captured in March were kept in a community tank ($0.9 \times 0.45 \times 0.46$ m; water depth, 0.36 m) with filtered and recirculated seawater for prolonged sound recording (see below). Tank bottoms were covered with fine sand, small flower pots, dead coral branches, and shells of the giant clam *Tridacna gigas* for shelters. The aquaria were kept under natural photoperiod, and the temperature was $25 \pm 1\,°$C. The fish were fed with frozen shrimp every 2 days. Experiments started after the fish had acclimated to the environment and fed for one week.

B. Acoustic and visual recordings

Field recordings (sounds and videos) were made in the vicinity of a patch of *Acropora* using a calibrated automatic recording system (AUSOMS-mini AQM-002, Aqua Sound Co., Ltd., Kyoto, Japan[2]; middle mode; sensitivity, -187.94 dB re 1 V/μPa) and a GOPRO Hero 4 (24 frames/s-1, 44.1 kHz sample rate; San Mateo, CA). Recorders were tied to a coral patch close to the sea floor, and red light was used for video recording at night. Seven 24-h sound recording sessions and five 2 h video sessions were made: three sessions at dusk and two at night in April, June, and July 2018. Additional field recordings were made using a H2A hydrophone (Aquarian Audio Products, Anacortes, WA; frequency range, <10 Hz to >100 kHz) and Sony digital recorder (PCM-M10, Minato-ku, Tokyo, Japan) during low tide at a seagrass meadow (*Thalassia hemprichii*) to look for the harmonic calls.

Laboratory tests on the seven individuals to chart temporal changes (daily and lunar) in vocal activity included 43 days of sound recording (June 16–July 28th, 2020) in a community tank ($0.9 \times 0.45 \times 0.46$ m; water depth, 0.36 m). Six recordings (0500–0600, 0900–1000, 1200–1300, 1500–1600, 1800–1900, and 2200–2300 h) were made daily with the AUSOMS-mini AQM-002 sound recording system (Aqua Sound Co., Ltd., Kyoto, Japan).

The same fish were then used to reveal sound repertoire under various social and partner (solitary and different combinations of males and females) conditions. Fish were exposed to either mirror images (mirrors attached to all four walls) or conspecifics (no mirrors) without further acclimation: three male-male, nine female-male, and one female-female pairs. These treatments were conducted between 1800 and 1900 h in small glass tanks ($0.3 \times 0.15 \times 0.25$ m; water depth, 0.2 m) for 1 h after transfer from the community tank. Sound and visual interactions were recorded with a GOPRO Hero 4. Individuals were recognized by size, color pattern, or marks, and a code number was given to each individual. Due to the absence of external sexual secondary characters, sex determination was made after observations of reproductive behaviors (oral brooders were males and egg layers were females). When calm, coloration of males and females is similar, but both turn darker when disturbed.

C. Analysis of acoustic behaviors

GOPRO files were used to examine behaviors, and sounds were extracted to wav files using the AoA audio extractor setup freeware (version 2.3.7; Aoa Technology, Shenzhen, PRC). Sounds were digitized at 44.1 kHz (16-bit resolution) using AviSoft-SAS Lab Pro 5.2.13 software (Avisoft Bioacoustics, Glienicke, Germany).

Temporal parameters of sounds were measured manually from oscillograms, and frequency were measured from parameters from power spectra. The parameters for grunt calls included the number of pulses/grunt call, pulse duration (in ms), grunt call duration (in ms), number of cycles/pulse, and for hoot calls, the number of hoots/hoot call, hoot duration (in ms), hoot call duration (in ms), number of cycles/hoot, period (in ms) and rise time (in ms; amount of time taken for the waveform envelope to reach peak amplitude), number of harmonics, fundamental frequency (in Hz), and dominant frequency (frequency with the most energy). All of the parameters were expressed as mean ± standard deviation (SD). Definitions of these parameters follow Bolgan *et al.* (2019). Calling fish exhibited no external movements, which made it impossible to identify the caller. Therefore, further statistical analysis was not run.

D. Periodicity tests

Temporal changes in vocal activities (i.e., number of hoots per unit time) for periodicity were fit using the Cosinor analysis; the method approximates summation of least squares of time series data from a cosine function of assumed period (Nelson *et al.*, 1979; Mikulich *et al.*, 2003; Soong *et al.*, 2011). Computations were made using the software from the Circadian Rhythm Laboratory, University of South Carolina.[3]

III. RESULTS

A. Agonistic vocal repertoire

F. variegata produced grunt and hoot calls during conspecific chases and attacks and when exposed to mirrors. Grunts were composed of 1–15 pulses (Fig. 2). Single-pulse grunts varied from 10.5 to 22.5 ms (mean ± SD, 17.0 ± 3.3; $n = 34$) with 5–9 cycles (7.1 ± 1.1 cycles); fundamental frequency was 97.2 ± 11.8 ($n = 162$; Table I). The waveform of the grunt pulse was stereotypic with an amplitude peak at the second cycle (a rise time of ca. 4.9 ± 0.4 ms or ca. 28.8% of the pulse duration; $n = 34$) and a third cycle of decreased amplitude [Figs. 2(D) and 2(E)]. Sound energy spread unevenly to about 800 Hz but concentrated at frequency bands around 100 and 200 Hz [Fig. 2(C)]. Single-pulse grunts also appeared sporadically or in a series of 3–10 grunts with regular inter-grunt intervals (155.3 ± 46.4 ms; $n = 76$); the last grunt in the series might be a multi-pulse grunt.

Duration of multi-pulse grunt calls and number of pulses (range, 2–15) were linearly related ($Y = 16.17X - 4.95$; $R^2 = 0.91$; $n = 106$). Pulse duration was 16.8 ± 3.2 ms (range, 12–25 ms; $n = 34$) and number of cycles/pulse was

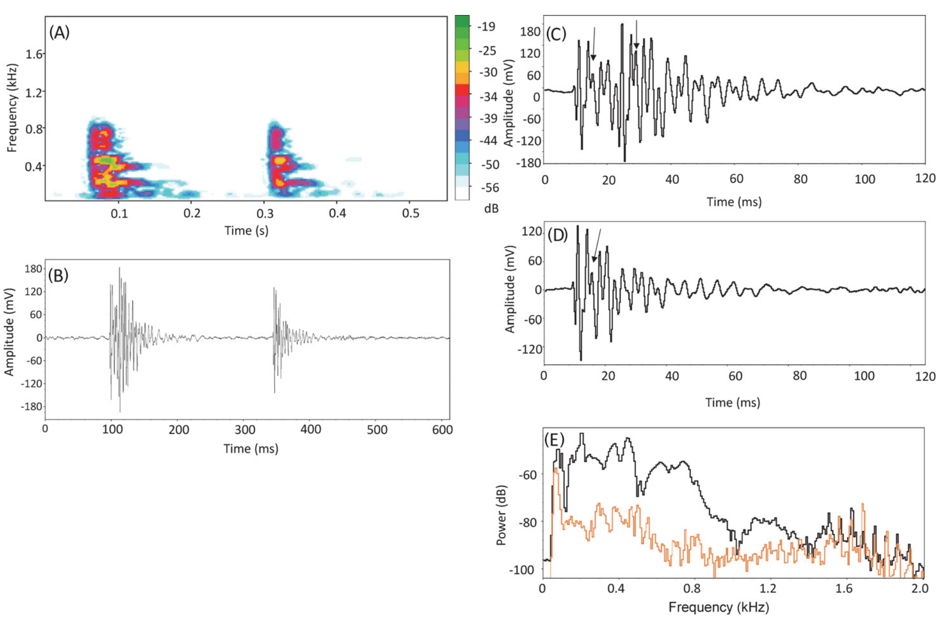

FIG. 2. (Color online) (A) Spectrograms and (B) oscillograms of a two-pulse (left) and a one-pulse grunt recorded during a male-female interaction. (C) Expanded oscillograms of the two-pulse and (D) one-pulse grunts. (E) Power spectra of the two-pulse grunt (black line) and background noise (brown line). Arrows in (C) and (D) point to the amplitude decrease in the third cycle. Fast Fourier transform (FFT), 256; overlap, 97%; Hamming window. Fundamental frequency = 207 and 199 Hz for the two-pulse and one-pulse grunts, respectively.

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TABLE I. Summary (mean ± SD) of acoustic features analyzed from sounds produced by *F. variegata* ($N = 12$). n, Number of data analyzed.

	Call or grunt duration (ms)		Number of pulses/grunt or hoots/call		Number of harmonics		Pulse or hoot duration (ms)		Inter-hoot interval (ms)		Number of cycles/pulses or range of pulses/hoot call		Fundamental frequency (Hz)	
Call type	Mean ± SD	n	Range	n	Range	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
Single-pulse grunt	17.0 ± 3.3 (10.5–22.5)	34	—	34	—	—	17.0 ± 3.3 (10.5–22.5)	34	—	—	7.1 ± 1.1 (5–9)	34	97.2 ± 11.8	162
Multi-pulse grunt	105.5 ± 62.3	34	2–15	34	6–16	156	16.8 ± 3.2 (12–25)	34	—	—	6.1 ± 1.1 (4–8)	34	87.2 ± 10.8	180
Agonistic hoot call	279.6 ± 153.4 (45–739)	160	1–5	160	1–6	32	66.0 ± 13.7	160	54.1 ± 17.9	110	10–15	32	191.0 ± 37.1	28
Community tank hoot call	456.5 ± 63.6	50	1–6	50	2–10	235	63.5 ± 6.4	241	41.5 ± 15.2	187	9–16	237	184.0 ± 6.4	50
Acropora coral hoot call	308.1 ± 52.1	49	1–6	49	2–11	189	56.1 ± 5.2	147	30.8 ± 9.1	98	9–16	147	229.2 ± 19.7	49

6.1 ± 1.1 (range, 4–8; $n = 34$). The fundamental frequency was 87.2 ± 10.8 (Table I). Multi-pulse grunt calls in series had irregular intervals between grunt calls. The waveform, power spectral density, duration, and number of cycles were similar to those of the single-pulse grunt [Figs. 2(D) and 2(E)].

Hoot calls were composed of 1–5 well-separated hoots (Figs. 3 and 4), and call duration (279.6 ± 153.4 ms; range, 45–739 ms) increased with the number of hoots in the call ($Y = 116.96X$ - 39.1 ms, $R^2 = 0.83$, $n = 160$). Hoot duration and inter-hoot interval were 66.0 ± 13.7 ms ($n = 160$) and 54.1 ± 17.9 ms ($n = 110$), respectively (Table I). Each hoot consisted of 9–13 cycles with a period of 5.3 ± 0.4 ms (range, 5.1–6.1 ms; $n = 36$; Fig. 3). Some hoots exhibited a slight decrease in frequency, resulting in a downward slope on a sonogram that is magnified in higher harmonics (Fig. 3). The waveform increased in amplitude with a peak at the 6–10th cycle (a rise time of 37.2 ± 6.4 ms; $n = 36$ hoots) before decreasing.

The first hoot within a call had a lower amplitude and maximum frequency than subsequent hoots (Figs. 3 and 5). Hoots had 1–6 harmonics with a mode of three; fundamental frequency varied from ca. 170–250 Hz, and dominant frequency was either at the first or second harmonic (Figs. 3–5; Table I). Thus, the fundamental frequency of hoots was approximately twice that of grunts.

B. Sound production in solitary individuals with no mirror

Solitary females were silent, but three of five solitary males produced calls with 1–6 hoots (Table II). A mouth-brooding

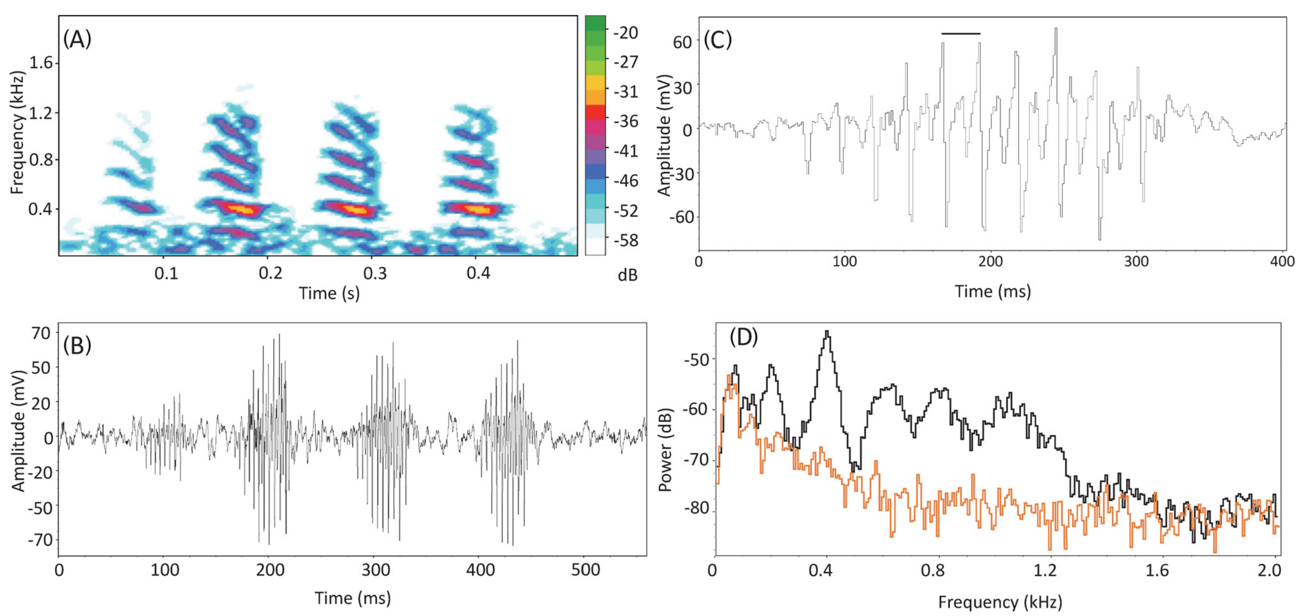

FIG. 3. (Color online) Spectrogram (A) and oscillogram (B) of a four-hoot call recorded in the *Acropora* patch. (C) Expanded waveform of the third hoot in (A) with 11 pulses, and (D) power spectra of the hoot call plus background noise (black line) and background noise (brown line). The bar in (C) shows the cycle period. FFT, 256; overlap, 97%; Hamming window, fundamental frequency = 206.2 Hz.

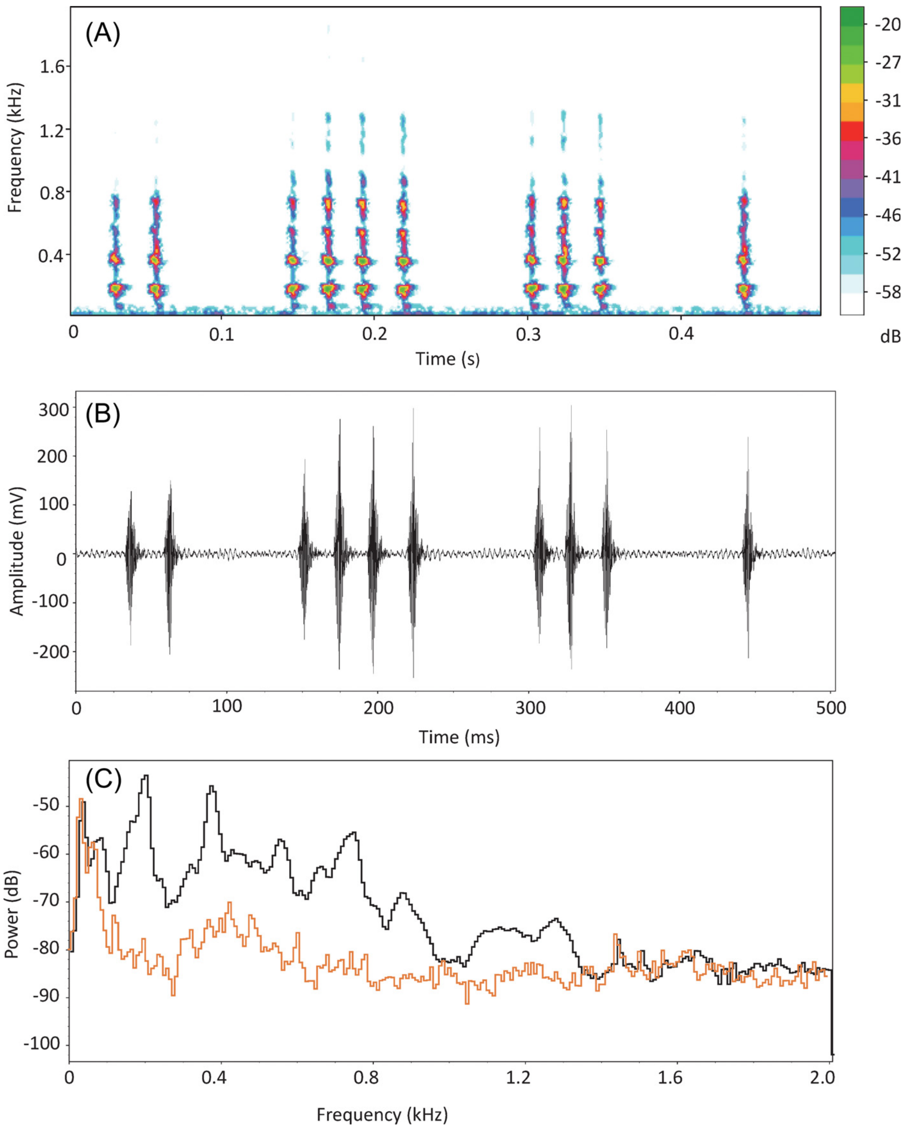

FIG. 4. (Color online) Four-hoot calls with one, two, three, and four hoots emitted by two captive males in an agonistic interaction, depicting the (A) spectrogram, (B) oscillogram, and (C) power spectra of the hoot calls plus background noise (black line) and background noise (brown line). FFT, 512; overlap, 94%; Hamming window.

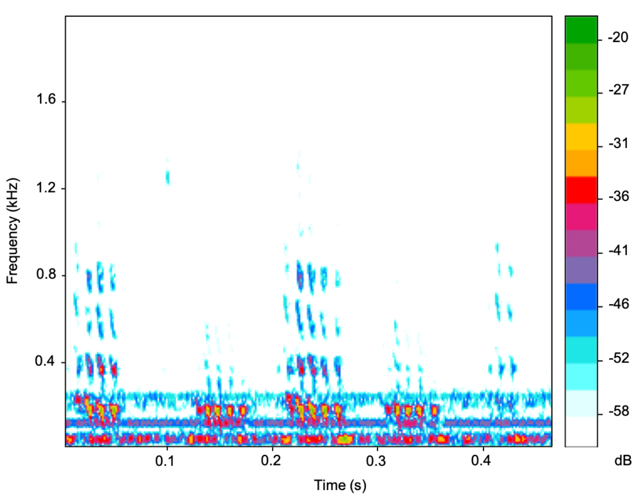

FIG. 5. (Color online) Spectrograms showing variation in frequency components of five (four-, five-, five-, five-, and three-) hoot calls recorded in the community tank, likely made by multiple fish. The fundamental frequency is ca. 179 Hz (arrow). FFT, 512; overlap, 88%; Hamming window.

individual and the smallest male did not call. Two males called almost continuously for up to 2 h, while the third vocalized for 9 min. Vocalization rates in these three varied from 17.9 ± 5.5 to 34.7 ± 1.7 calls/min, and they produced primarily 3- or 4-hoot calls. The less active male produced relatively more single-hoot calls (Table II).

C. Sound production in solitary individuals with mirror image (Table II)

Males and females might emit calls when facing or parallel to the mirror. Males ($n = 5$) were aggressive and launched biting attacks toward the mirror image. They emitted 1–5 hoots and 2–4 pulse grunt calls. The smallest individual emitted grunts, whereas the other four individuals emitted only hoot calls. Females ($n = 3$) produced single and multi-pulse grunts and hoot calls. The smallest individual emitted only hoot calls, but the two others emitted only grunts. More males responded to mirror images with hoot calls and more females responded with grunts, but this

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TABLE II. Occurrence of grunt and hoot calls varied in duration, measured in number of pulses and hoots per call, respectively, under different social contexts. The percentage value is in parentheses.

	1	2	3	4	5	6	7	8	9	10	11–15	Total
Mirror												
Hoot call (number of hoots/call)												
Male	265	81	44	12	1	—	—	—	—	—	—	403
	(65.8)	(20.1)	(10.9)	(3.0)	(0.3)							
Female	175	13	1	1	—	—	—	—	—	—	—	190
	(92.1)	(6.8)	(0.5)	(0.5)								
Grunt (number of pulses/grunt)												
Male	19	10	19	10	2	—	—	—	—	—	—	60
	(31.7)	(16.7)	(31.7)	(16.7)	(3.3)							
Female	256	64	119	30	15	13	8	5	2	2	—	514
	(49.8)	(12.5)	(23.2)	(5.8)	(2.9)	(2.5)	(1.6)	(1.0)	(0.39)	(0.39)		
Conspecific												
Hoot call (number of hoots/call)												
Male-male	70	16	6	6	2	—	—	—	—	—	—	102
	(69.6)	(16.7)	(5.9)	(5.9)	(2.0)							
Male-female	16	18	40	26	148	221	49	3	—	—	—	571
	(2.8)	(3.2)	(7.0)	(13.3)	(25.9)	(38.7)	(8.6)	(0.5)				
Grunt (number of pulses/grunt)												
Female-female	—	2	1	—	—	—	1	—	1	—	—	5
		(40.0)	(20.0)				(20.0)		(20.0)			
Male-female	58	14	18	21	12	13	13	3	4	4	8	168
	(34.5)	(8.3)	(10.7)	(12.5)	(7.1)	(7.7)	(7.7)	(1.7)	(2.4)	(2.4)	(4.8)	

should be treated as a hypothesis for future work rather than a statistically supported result.

D. Sound production and behavioral responses to conspecifics (Table II)

In male-female trials ($n = 7$), the fish sometimes stayed in close proximity and occasionally courted (see below). Larger females tended to drive away smaller males. The majority of sounds were grunts (1–15 pulses; mode 1-pulse) with a few short hoot calls that were likely emitted by the male (see below). Since the smallest male and smallest female emitted only grunts and hoot calls, respectively, when responding to mirror images, we expect that both call types might be present during intersexual social interactions. However, numerous hoot calls (1–8 hoots, mode of six) but no grunts were recorded, although it was not possible to determine which individual made the sounds.

In two male-male trials, fights occurred, and 1–5 hoot calls (mode 1 hoot) were emitted. In a female-female (one trial) interaction, only multi-pulse grunts (2–9-pulses) were emitted when one fish chased the other.

E. Sound production and periodicity in the community tank

Hoot calls but no grunts were recorded in the community tank during the 43-day period. They contained mainly 3–5 hoots (range 1–6 hoots, $n = 187$ calls; Fig. 5). Maximum vocalization peaked at 1800–2200 h and declined to a minimum at 0500–0600 h, and a smaller peak was also present at 1200 h [Fig. 6(A)]. A significant 24-h periodicity occurred [$p < 0.05$; Cosinor test; Fig. 6(B)], indicating that the temporal pattern appearing in Fig. 6(A) has a period of 24 h. Calls exhibited a 15-day rhythm, peaking around the new and full moon and gradually decreasing to silence after 7 days (Fig. 7). A mouth-brooding male was present when the number of hoot calls declined.

Hoot call duration, hoot duration, and inter-hoot interval were 456.5 ± 63.6 ms ($n = 50$ hoot calls), 63.5 ± 6.4 ms ($n = 241$ hoots), and 41.5 ± 15.2 ms ($n = 187$ inter-hoot intervals), respectively (Table I). Fundamental frequency was 184 ± 6.4 Hz ($n = 50$), and the percentages of hoot calls ($n = 104$) with the dominant frequency at the fundamental frequency and the second harmonic were 76% and 23%, respectively.

F. Hoot calls from the *Acropora* patch

Only hoot calls (3–5 hoots, $n = 49$) were recorded in the *Acropora* patch. Hoot call duration, hoot duration, and inter-hoot interval were 308.1 ± 52.1 ms ($n = 49$ hoot calls), 56.1 ± 5.2 ms ($n = 147$), and 30.8 ± 9.1 ms ($n = 98$ inter-unit intervals), respectively (Table I). Each hoot included 2–11 harmonics ($n = 189$). Mean fundamental frequency was 229.2 ± 19.7 Hz ($n = 49$; Fig. 3; Table I). Duration of hoot calls and number of hoots were linearly related ($Y = 82.33X + 4.41$, $R^2 = 0.73$). The percentages of hoot calls with the dominant frequency located at the fundamental frequency and the second harmonic were 55% and 46%, respectively ($n = 173$).

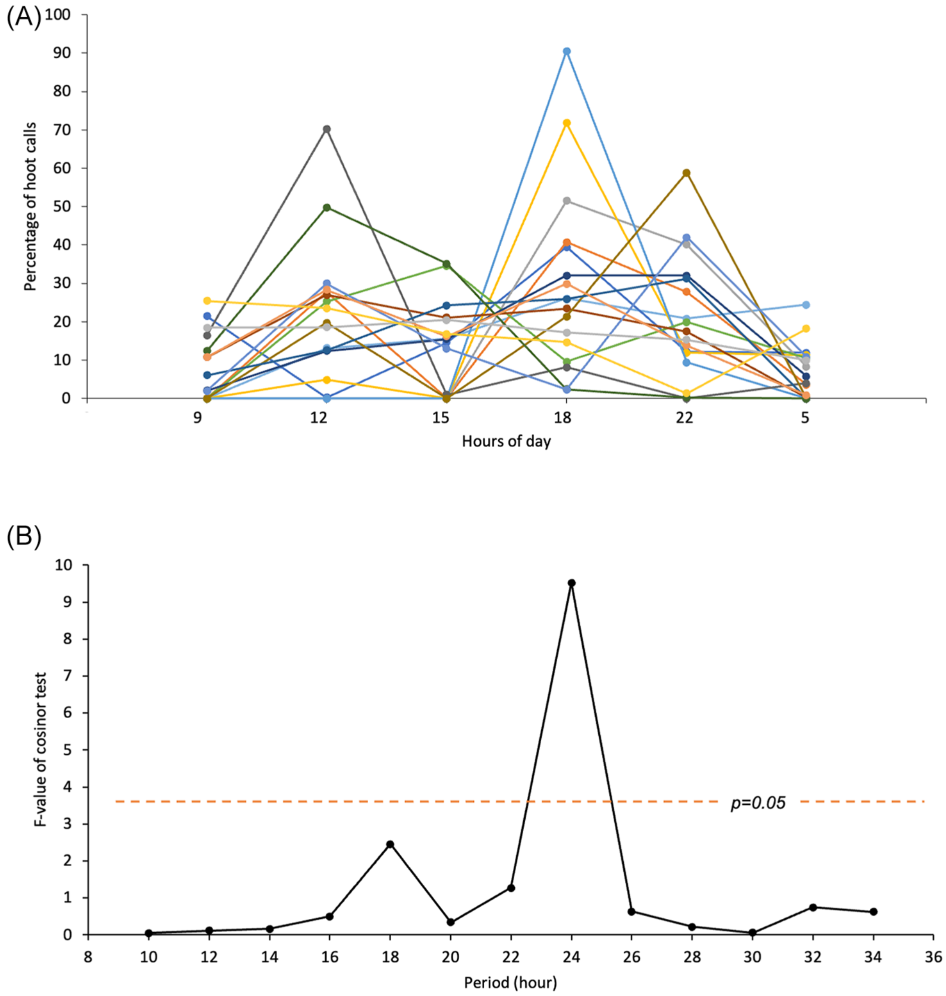

FIG. 6. (Color online) Temporal change in mean relative number of hoot calls in *F. variegata* in the community tank over multiple days. (A) Each line includes the six data points recorded in 24 h. The maximum and minimum number of hoots are at 1800 h and 0500 h, respectively. Percentage of hoot calls: number per call divided by total number recorded per day. (B) Test of diel rhythm in the number of hoot calls in the community tank. *F* values by Cosinor tests of period = 24 h is clearly above the threshold for significant rhythms supporting the diel rhythmic pattern shown in (A) has a period of 24 h.

In a three-hoot call with a duration of 300 ms, the hoot duration was ca. 53.2 ms and contained nine cycles (cycle period = 5.9 ms). A 5.9 ms cycle is equivalent to a fundamental frequency of 169.5 Hz, and the fundamental frequency from the power spectrum for this call was 170 Hz. Although the sonic mechanism has not been identified, it is likely that the contraction rate of a fast muscle determines the fundamental frequency as in toadfish (Fine *et al.*, 2001).

Hoot calls could be heard underwater in the field by divers at about 1 m from the coral patch where *F. variegata* were caught (also, see below). Hoots were also heard in the room outside of the community tank (Chang and Chen, 2020), suggesting a high sound pressure level.

Hoot calls occurred day and night. A 24-h recording session made on 21 April 2018 indicated that the number of hoot calls was lowest at ca. 12–13 h and peaked around 4–5 h and 17–19 h (Fig. 8). Preliminary analysis of field recordings on an 8-day continuous sound file made between 14 August 2021 (7 days before full moon) and 21 August 2021 supports vocalization peaks at dawn and dusk. Most hoot calls at dawn were weaker in amplitude, possibly representing sources further from the hydrophone. In summary, captive individuals emitted noon and dusk to early evening choruses, whereas there were dawn and dusk choruses in the field.

G. Courtship and spawning behavior in community tank

One incidence of courtship was witnessed: the male became darker and the female had a brownish color pattern and swollen belly through which the outline of the golden egg mass was visible. During courtship, the male and female hovered close together side by side. They swam slowly, rapidly oscillating their pectoral fins and tails, and their bodies quivered against each other. The male pushed the female by moving sideways into her. The dorsal part of the male's body turned whitish, the color extending across the front edge of the caudal peduncle as a vertical band pattern. The female remained light brown, although a faint vertical stripe might appear. On one occasion, when the pair was in the water column, a few transient knocks followed a clear thump sound ($n = 2$; Fig. 9). This was the only time these sounds were recorded, and they may be specific signals associated with courtship.

The pair entered the shelter, and the male with the throat and abdomen darker leaned toward the pale female in a parallel orientation. His tail beat, and he pushed the female sideways. As the female released the egg mass, the male moved closer to the female with his genital area close to the egg mass. Sperm release was not visible in the video.

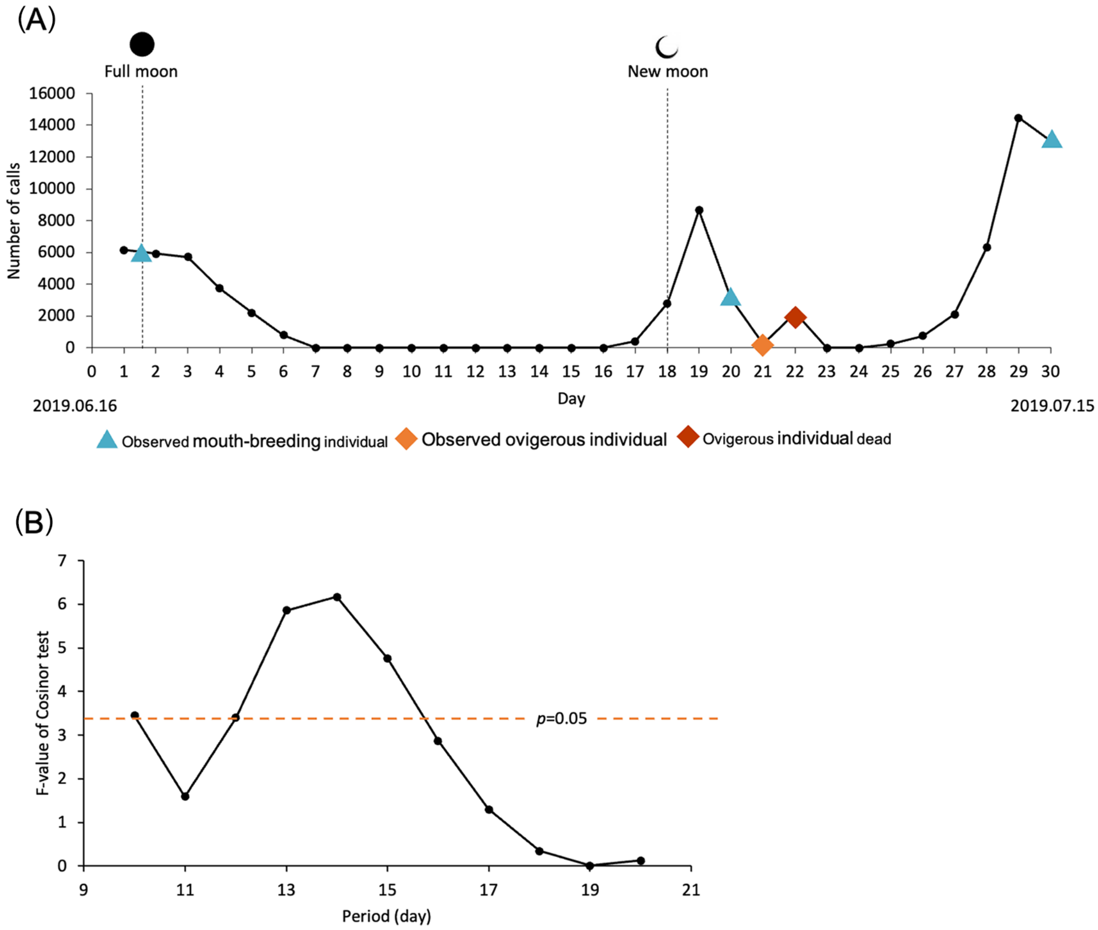

FIG. 7. (Color online) (A) Semi-lunar rhythm in emission of hoot calls in the community tank. The two distinctive peaks are associated with the new and full moon phases. (B) Test of semilunar rhythm in the number of hoot calls in a community tank. *F* values by Cosinor tests of 13, 14, and 15 days are clearly above the threshold for significant rhythms supporting a semilunar rhythm.

The female then swam slowly forward leaving the male. Before the egg clutch detached, the male bit and held the white structure at the rear end of the egg clutch, and then sucked the egg clutch into his mouth cavity [Figs. 1(E) and 1(F)].[4] We hypothesize that the white structure is a releaser that stimulates male egg capture. The female turned dark immediately [Fig. 1(F)]. No sound was recorded during spawning activity. Brood duration ranged from 9 to 16 days ($n = 14$).

IV. DISCUSSION

Harmonic sounds recorded in seagrass meadows and *Acropora* patches on Dongsha Island came from an unknown source. We auditioned various species in captivity and identified the emitter: the cardinalfish, *F. variegata*. It has a relatively diverse vocal repertoire, including harmonic hoot and grunt calls that vary in duration, number of pulses, and cycles per hoot and grunt (Figs. 2–4). The order Kurtiformes includes the Apogonoidei (Apogonidae) and Kurtoidei (Kurtidae; Betancur-R *et al.*, 2017). Sound production has not been reported in kurtids. Therefore, this is the first record of sound production for the order Kurtiformes.

Mirror and social interactions reveal that *F. variegata* is aggressive. Grunts and shorter hoot calls are emitted in agonistic contexts, and longer hoot calls are likely advertisement calls used to attract females. Males struck the mirror image while producing grunt or short hoot calls. Hoots of various lengths, including occasional longer ones, are emitted during male-male aggressive interactions. Hoot calls appear to serve dual functions: agonistic toward males (1–2-hoot calls) and advertising (4–6-hoot calls) to unseen females who might be attracted from longer distances. Grunts are emitted primarily during intersexual and female-female social interactions. No grunts were recorded in the community tank or field. As these sound types occurred during close intersexual encounters, they may also play a role in courtship. Because reproductive activity does not take place commonly, capturing these sounds in the field may be unlikely. Calling solitary males emit long hoot calls.

Grunts are somewhat tonal with energy concentrated at frequency bands around 100 and 200 Hz and energy to ca. 800 Hz [Fig. 2(C)]. Grunts consist of 1–15 adjacent pulses, each with a duration of ca. 17 ms. Although unrelated to toadfish, the cardinalfish sounds share similarities with grunts and boatwhistles of the oyster and gulf toadfish

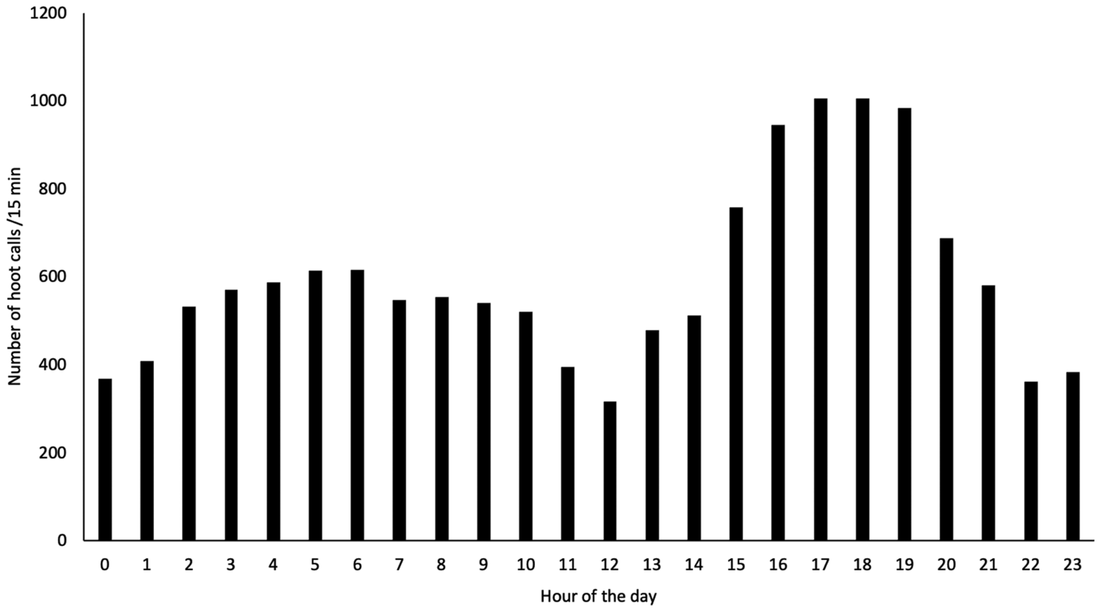

FIG. 8. Diel change in number of hoot calls from *Acropora* branching-coral patches. The sounds were recorded on 21 April 2018. Hour of the day in the *X* axis (0–23) represents the beginning and end of an hour (i.e., 0 = 00:00–00:59 a.m., 23 = 23:00–23:59 p.m.).

(Thorson and Fine, 2002; Fine and Thorson, 2008). Toadfish grunts are of shorter duration with a poorly developed harmonic spectrum compared to the tonal boatwhistle. Additionally, grunts sometimes form introductory notes preceding the boatwhistle, indicating an imperfect separation of the use of the two calls (agonistic vs courtship).

The rare appearance of transient knocks and thumps during courtship (Fig. 9) suggests that the fish's vocal repertoire may be more complex than described. Although most fish advertisement calls are emitted during courtship prior to spawning (Amorim, 2006), some fishes produce sounds during spawning as in the hamlet, *Hypoplectrus unicolor*, and the striped parrotfish, *Scarus iserti* (Lobel, 1992). However, more work is needed to establish the significance of these vocal signals to successful courtship or spawning.

Distortion of sound characteristics occurs within small tanks as resonant frequencies and reverberation influence propagation and spectro-temporal structure (Akamatsu *et al.*, 2002; Parmentier *et al.*, 2014; Jones *et al.*, 2019). Akamatsu *et al.* (2002) presented a practical procedure for sound recording and measurement in small tanks, suggesting that the sound should have frequencies lower than the tank resonant frequency, and the hydrophone should be placed within the attenuation distance of the sound source to minimize possible distortion. The dominant frequency of the hoots was ca. 200 or 400 Hz, which was below the minimum resonant frequency of the tank (calculated as 3053 Hz).

Shelter spaces were available in the community tank for each individual, and social interactions were infrequent in day time. Additionally, there was likely habituation to the presence of conspecifics that would not carry over to a novel situation in which fish were transplanted. Observations on the fish in the community tank at night were made using a Xiaomi Intelligent Camera (Xiaomi Co., Beijing, PRC) connected to a mobile phone, but detailed interactions were unclear due to the quality limitation of the camera. However, camera observations indicate that *F. variegata* is nocturnal and exhibits more movement at night, swimming further away from more limited daytime positions. Agonistic confrontations were rare in daytime. Actual courtship and spawning were recorded in the community tank, during which the gravid female might chase the male. No agonistic calls were noticed when chasing took place. It is likely that after social acclimation in the community tank with numerous shelters, intensive chases and fights were infrequent, and agonistic calls were not recorded. The small tank where behavior tests were conducted had limited space and no shelters, therefore, social-encounter chances increased. Under a high stress environment with no place for retreat, aggression intensity increased, leading to sound production.

The few data sets from the field and community tank reveal a difference in the diel pattern of vocalization—there are dawn and dusk peaks in the field (Fig. 8) vs a major peak at dusk to early evening and a minor peak at noon in the community tank [Fig. 6(A)]. As apogonids are nocturnal, they appear to leave their daytime shelters, likely, for nighttime foraging. The peak at dawn might be related to this diel movement.

An important question is about the distinction of *F. variegata* calls from those of other fishes on Dongsha Atoll. Several apogonid species occupy spaces in clumps of *Acropora*. However, only hoot calls were found in numerous sound files recorded at the site. Other cardinalfish on Dongsha Atoll, i.e., *Apogonichthyoides melas*, *Nectamia fusca*, *Ostorhinchus novemfasciatus*, and *Pristicon trimaculatus*, produced only transient single or multiple drum or knocks during agonistic interactions in tanks at the field research station at Dongsha Island (Chang, 2020). Additional recordings made on *Apogon timorensis*, *Archamia fucata*, *Ostorhinchus properuptus*, and *Sphaeramia nematoptera*

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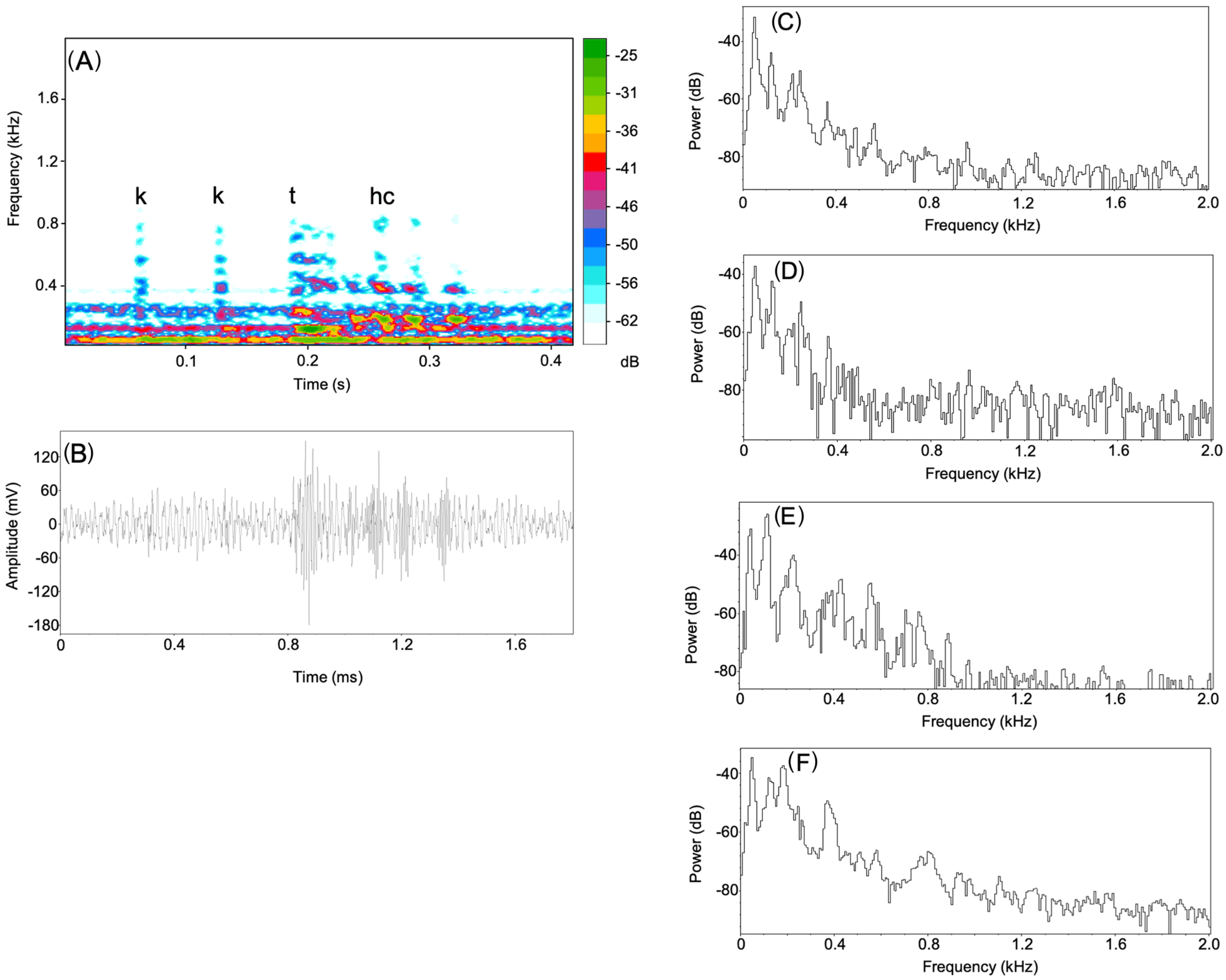

FIG. 9. (Color online) Spectrogram (A) and oscillogram (B) showing transient knocks (*k*), a loud thump noise (*t*), and a four-hoot call (hc) recorded in the community tank with two *F. variegata* engaging in courtship. The hoot call was likely from another fish and not from the courting pair. Power spectrum of the two knocks mixed with background noise, background noise alone, thump, and hoot calls are shown in (C), (D), (E), and (F), respectively. FFT, 512; overlap, 97%; Hamming window. Peak frequencies of the knock, thump, hoot call, and background noise are 203 Hz, 45 Hz, 117 Hz, and 179 Hz, respectively.

from Dongsha Atoll also revealed transient nonharmonic calls (Chang and Soong, 2022). Tonal sounds in the Ambon damselfish, *Pomacentrus amboinensis*, have also been reported sympatrically with *F. variegata* (Parmentier and Frederich, 2016). However, the calls of these two species are clearly different (Parmentier and Frederich, 2016) and unlikely to be confused. In addition to *Fowleria marmorata*, *Fowleria punctulata* has been reported from the Atoll (Chen *et al.*, 2011) although we have not collected them at our recording site. The present study supports the harmonic hoots in the coral patch as coming from the variegated cardinalfish, *F. variegata*, and further work is required to differentiate sounds and microhabitats of the three *Fowleria* species.

Harmonic sound types appear to be uncommon in the vocal repertoire among apogonid species recorded in this study, and *F. variegata* is exceptional in using this sound type (tonal and multiunit varied in call duration) for advertisement and courtship communication. In the noisy coral reef environment, competition for acoustic space is expected to be high. Therefore, harmonic sounds with a series of units will increase signal distinctiveness and likely support signal discrimination (Parmentier and Frederich, 2016); the characteristics of the hoot call in *F. variegata* fit this explanation. For Lusitanian toadfish males, higher calling rate, calling effort, and amplitude modulation in their advertisement calls could be more attractive to females (Amorim *et al.*, 2010); longer and more complex boatwhistles produced at a faster rate could be more attractive as well (Winn, 1967, 1972; Fish, 1972; Thorson and Fine, 2002). Similarly, longer hoots with more units could be more attractive to ripe females than the short sporadic agonistic grunts. A long 15-pulse grunt could last up to ca. 0.25 s, which approximates to the duration of a two-hoot call (Fig. 2).

Mouth-brooding males do not call. After we gently pulled the egg mass out of the mouth of an egg-brooding male, it emitted 151 hoot calls in 23 min (6.6 ± 7.0 call/min; range, 0–24). The egg mass may inhibit the male from calling or curtail his motivation. The silence of brooding males also suggests that male mates with a single female in each brood cycle. Finally, we suggest that the white structure on the egg mass functions as an "ethological" releaser that stimulates and enables the male to engulf the egg mass.

Calling males invest energy in prolonged oral brooding (i.e., up to 16 days without eating; Chang and Chen, 2020). These reproductive contributions indicate a possible prime role for the male in mate choice.

Social habits may determine channels most appropriate for communication. As apogonids show plasticity of aggregative behavior (i.e., from solitary to schooling), it is possible that such social flexibility can underlie plasticity in communicatory signals (Rastorgueff, 2020).

Meanwhile, in those bioluminescent cardinalfish species, acoustic signals might coevolve with bioluminescence. Alternately, sounds could be replaced by bioluminescence as in the inverse relationship of sound production and electric discharge in synodontid catfishes (Boyle *et al.*, 2014).

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[1]See https://www.lamar.edu/arts-sciences/biology/study-abroad-belize/marine-critters/marine-critters-3/cardinalfish.html (Last viewed December 3, 2022).
[2]See http://aqua-sound.com/ (Last viewed December 3, 2022).
[3]See www.circadian.org/main.html (Last viewed December 3, 2022).
[4]See supplementary material at https://www.scitation.org/doi/suppl/10.1121/10.0016441 for a video showing egg laying in the variegated cardinalfish, *F. variegata*.

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