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Acoustic communication in the Bocon toadfish (*Amphichthys cryptocentrus*)

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Abstract The soundscapes of many coastal habitats include vocalizations produced by species of the family Batrachoididae (toadfish and midshipman). We describe the calling and grunting behavior of male *Amphichthys cryptocentrus*, a tropical toadfish, and predict how these vocalizations are influenced by conspecifics. We recorded individual males, which produced broadband grunts and multi-note, harmonic "boatwhistle" calls. Grunts were either in combination with calls or stand-alone. We used a null model to test if these latter grunts were produced at random or in response to calls from conspecifics. The model supports the hypothesis that grunts were in response to calls from neighboring males, suggesting acoustic competition. Using the most conservative estimate of hearing abilities we predict that males responded to the second harmonic of neighbor's calls (230 Hz) at amplitudes of approximately 100–125 dB re 1μPa^2/Hz. We also observed that call and grunt rates increased when males were exposed to higher rates of acoustic activity from neighboring fish. Fish used grunts to respond to background calls that occurred at different amplitudes, suggesting they responded to the calls of multiple neighboring fish and not just the highest amplitude neighbor. This communication with multiple fish within hearing range suggests a communication network in which the spatial distribution of individual toadfish relative to one another will impact their vocal behavior. Thus, the density and distribution, and not just abundance, of these toadfish at a given site will influence the characteristics of the chorus and the role of this species in the local soundscape.

Keywords Toadfish · Batrachoididae · *Amphichthys cryptocentrus* · Acoustic communication · Bioacoustics · Soundscapes

Introduction

Marine soundscapes can provide a source of information to monitor biodiversity (Lammers et al. 2008; Lammers and Munger 2016) and may influence the settlement behavior of marine larvae (Montgomery et al. 2006). The biological component of habitat-associated soundscapes is the collection of sounds produced by soniferous species and at its fundamental level is a product of the acoustic behavior of individual organisms. Soniferous animals produce sound either intentionally as they engage in acoustic communication (Patek 2001; Amorim 2006; Colleye and Parmentier 2012; Tricas and Boyle 2014) or unintentionally as they go about routine activities such as feeding and

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defending (Versluis et al. 2000; Radford et al. 2008; Tricas and Boyle 2014). Therefore, the habitat-level trait of the soundscape, with its associated temporal and spatial characteristics, is generated by patterns at the species and individual level. Fishes are a major contributor to biotic marine soundscapes, and many species engage in acoustic communication that results in species-specific temporal and spatial patterns of acoustic activity (Locascio and Mann 2008; Montie et al. 2015; Mooney et al. 2016) and the sharing of acoustic niche space (Ruppé et al. 2015). Species of toadfish and midshipman (Batrachoididae) provide well-studied examples of acoustic communication in fishes (Bass and McKibben 2003) and are found worldwide in many near-shore habitats (Greenfield et al. 2008). Understanding how individual behavior may scale up to influence a chorus composed of many individuals may help inform the spatial and temporal variation in the fish-generated component of coastal soundscapes.

Toadfish males construct and occupy burrows from which they emit calls, typically described as boatwhistles, to attract females. After spawning, the eggs and larvae are demersal and the male remains in the burrow, defending the young (Gray and Winn 1961). The vocal repertoire and call parameters vary across the eight of 79 species whose acoustic behavior has been described (Mosharo and Lobel 2012). In general, sound types in this family consist of growls and hums (Bass and McKibben 2003), croaks (dos Santos et al. 2000), short, broadband grunts (Thorson and Fine 2002a; Fine and Waybright 2015), and the harmonic “boatwhistle” call used primarily for mate attraction (Bass and McKibben 2003). Calls have also been observed to be produced in the defense of the nest by males of *Halobatrachus didactylus* (Vasconcelos et al. 2010; Conti et al. 2015). Grunts are produced right before calls (Talvoga 1958) or as stand-alone utterances, singly or in combination, in agonistic and distress situations by both males and females (Gray and Winn 1961; dos Santos et al. 2000; Amorim et al. 2006; Maruska and Mensinger 2009; Fine and Waybright 2015). Males of some species produce grunts that are inserted non-randomly in the calls of neighboring males, i.e. they appear to purposefully overlap a neighbor’s call with a grunt (Thorson and Fine 2002a; Mensinger 2014). These grunts, called tags in this context (Thorson and Fine 2002a), are predicted to be a form of stealth acoustic competition (Mensinger 2014). This tagging behavior is hypothesized to decrease a call’s attractiveness by altering the fundamental frequency as perceived by females when they hear the call and grunt occurring together (Mensinger 2014). Several factors influence the acoustic behavior of toadfish species. These include individual traits such as age and physical condition (Mitchell et al. 2008; Amorim et al. 2010; Vasconcelos et al. 2015), environmental conditions such as season (Breder 1968; Fine 1978; Wall et al. 2013), lunar phase (Rice and Bass 2009; Maruska and Mensinger 2009) and the presence of predators (Remage-Healey et al. 2006), and social conditions such as the call rate of neighboring males (Fish and Offutt 1972; Remage-Healey and Bass 2005) and avoidance of call overlap (Winn 1972; Fish 1972; Thorson and Fine 2002b). Social factors are of particular importance given that hearing the calls of neighboring fish may be necessary to induce calling in an individual (Remage-Healey and Bass 2005), and reproductive success is highly dependent on calling behavior (Vasconcelos et al. 2012). Indeed, the radiation pattern of sound emanating from the swim bladder may have evolved to reduce the sound level received at the ears to allow a fish to both call and maintain the ability to listen to the calls of surrounding males (Barimo and Fine 1998).

Long-term simultaneous recordings (45 days) conducted at four tropical reefs in the Bocas del Toro Archipelago in northwestern Panama (Salas et al., in preparation) revealed the toadfish *Amphichthys cryptocentrus* (Valenciennes, 1837) to be a dominant contributor to the soundscapes across the region. This species is the sole member of its genus and inhabits coastal waters from Panama to Brazil (Greenfield et al. 2008). It was also observed that the diel and daily patterns of calling varied between the four reef sites, both in the duration of the nightly chorus, the rate at which the amplitude of the chorus diminished from its peak, and the rate of sporadic calls produced throughout the non-chorusing period. We predict if these differences were driven solely by abundance the same patterns would be observed across the sites, with only differences in chorus amplitude. However, since the temporal pattern of the chorusing differed between sites, we predict that the spatiotemporal variation is due to the social factors that influence calling behavior. Achieving a better understanding of how social factors mediate acoustic behavior in individuals of this species will inform the importance of their density and spatial distribution in influencing the characteristics of the chorus.

This study investigates the acoustic behavior of individual males of *A. cryptocentrus* in response to conspecifics. Our goals were two-fold. Our first objective was to describe the boatwhistle calls and grunts of *A. cryptocentrus*. We also used a null model to test the hypothesis that the grunts that occurred independent of calls were strategically placed to overlap the calls of neighboring males. If supported, this would suggest these grunts serve a competitive function as observed in *Opsanus tau* (Mensinger 2014). The second objective was to predict if the acoustic behavior of an individual male is driven by the nearest (and thus perceived loudest) neighbor or if a male instead responds to multiple neighbors in its vicinity. Evidence of the latter would suggest that the social factors that mediate calling could have an amplifying effect: the more calls an individual male hears, the more that fish will call and grunt, which will increase the vocalizations of additional fish nearby. We predict this propagation of behavioral influence would affect the characteristics of the chorus and its role in the soundscape.

Methods

We took advantage of a group of toadfish that was using the structure provided by the water pipes for the wet lab at the Smithsonian Tropical Research Institute's Bocas del Toro field station. The pipes are supported at intervals with concrete bricks, and toadfish males used this structural support to excavate their burrows, as they would use coral and rock (Hoffman and Robertson 1983). After confirming the presence of a fish inside a burrow, we used rope to measure a path one meter from the entrance of the burrow where toadfish would be observed to be sitting (the presumed calling position) to the placement of the hydrophone/recorder package (nRUDAR, Cetacean Instruments, range: 1 Hz to 22,050 Hz, sensitivity: −159.52 dB re 1 V/μPa). The hydrophone was approximately 30 cm above the substrate and water depths ranged from approximately 1.5 to 3.5 m. The hydrophone signal was recorded continuously at a sample rate of 44.1 kHz for one full night and was retrieved the following morning for each individual fish we sampled. We recorded thirteen fish on separate nights in July 2015. Of the 13 fish, two fish either did not vocalize or they moved to another borrow location before calling began. The position of a third, relative to another individual, made it difficult to distinguish with confidence the identity of the calls and was not used. Therefore, our analysis focuses on ten individual males. Since the fish are resident in their burrows with only the heads visible, we confirmed species identification by video recording, during the day, three fish emerging from their burrow to capture an urchin dropped outside the entrance (see Online Resource 1).

We used amplitude to differentiate the calls and grunts from the focal males from the calls and grunts of neighboring males in Raven (Version 1.4; Cornell Lab of Ornithology). Focal males are defined as those we specifically targeted to record on a given night, and neighboring males are the fish that produced the vocalizations not attributed to the focal fish. Both waveforms and spectrograms were used to identify vocal events produced by the focal fish and the times at which they occurred so that they could be selected from the recordings for analysis in MATLAB (Version R2012a; Mathworks). The highest amplitude calls and tags were assumed to be produced by the focal male, and the remaining calls and tags were considered to be produced by neighboring males. We reference the justification in Thorson and Fine (2002b) for this approach of using amplitude differences to assign vocalizations to specific fish.

Analysis of call characteristics

A. cryptocentrus produced boatwhistles composed of one or more distinct notes, and we focus the analysis on the single and double note calls since these were the predominant types. One or more broadband grunts directly preceded the first note (Fig. 1; see Online Resource 2 for audio), termed "pre-call grunts" here. They also produced grunts independent of calls, termed "isolated grunts". Call parameters, chosen to reflect those used in Mosharo and Lobel (2012), were measured using a custom MATLAB script and temporal measurements were made within the waveform of the vocal events. Note length is defined as the time elapsed between the start and end of a note. Call length was defined as the time elapsed between the start of the first note and the end of the last note, thus excluding pre-call grunts but including internote intervals, which is the elapsed time between the notes of a call. Pre-call grunts were included in call length when the grunt merged into the first note (as in the background calls in Fig. 2g). Similarly, we also observed a call variant where two or more notes appear merged together (as in background

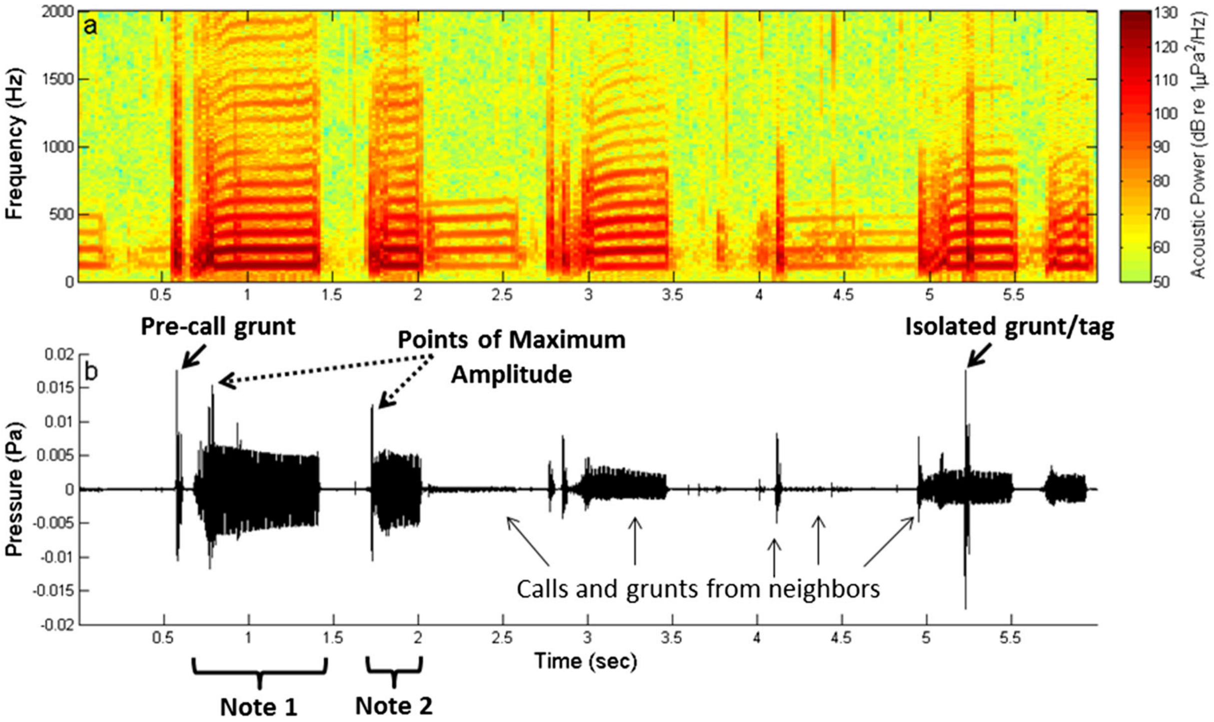

Fig. 1 Example recording and explanation of terms. A spectrogram (Online Resource 3) of six seconds from a recording of Fish 6 is shown in (**a**) and the corresponding waveform in (**b**). In this sample, Fish 6 produced a double note call with a single pre-call grunt. The points of maximum amplitude in the notes are indicated in the waveform. Fish 6 also produced an isolated grunt that overlapped a background call, and given the placement of this grunt it likely served as a tag. Calls and grunts that occurred at lower amplitudes than that observed by Fish 6 reflect vocalizations produced by fish inhabiting nearby burrows. Audio of this recording can be found in Online Resource 2

call in Fig. 2g), which we counted as all part of a single note. Call events (grunts and notes) were considered distinct only when the amplitude returned to ambient levels between events (as in Fig. 2b, e).

Point of maximum amplitude within a note is a potential diagnostic character for toadfish (Rice and Bass 2009). We calculated the point of maximum amplitude for single note calls and for the first and second notes in double note calls. In the waveform of a note, we found the maximum absolute amplitude and divided the time at which it had occurred in the note by the length of that note.

We determined overall call rate and the call rate of single and double note calls for the peak period of calling. We observed that at the beginning of a nighty calling bout, calls and/or grunts for some fish were sporadic until building into the chorusing period. We removed these initial vocalizations and time periods to calculate call and grunt rates that would better represent the rate at which these sounds are produced. For eight of the fish we extracted from the analysis a range of 1 to 19 calls over a period of 8 to 95 min, respectively. For five of the fish we extracted a range of 2 to 21 tags over 10 to 54 min, respectively.

To consider the effort made by fish to produce calls, we calculated both muscle contraction time and the number of muscle contractions per minute (as in Thorson and Fine 2002b). Multiplying the mean number of seconds per minute during which the swim bladder muscles are contracting (muscle contraction time) by the fundamental frequency provides the average number of muscle contractions per minute. To calculate muscle contraction time T_{mc}, we used Eq. 1, where R_s is the rate (calls per minute) at which single calls are produced, R_d is the rate (calls per minute) of double calls, D_f is the mean duration (in seconds) of the first note, and D_s is the mean duration (in seconds) of the second note:

$$T_{mc} = \left(R_s\, D_f\right) + \left(R_d D_f\right) + \left(R_d D_s\right) \qquad (1)$$

We selected fifty calls for each fish to determine the fundamental frequency and the sound level at the

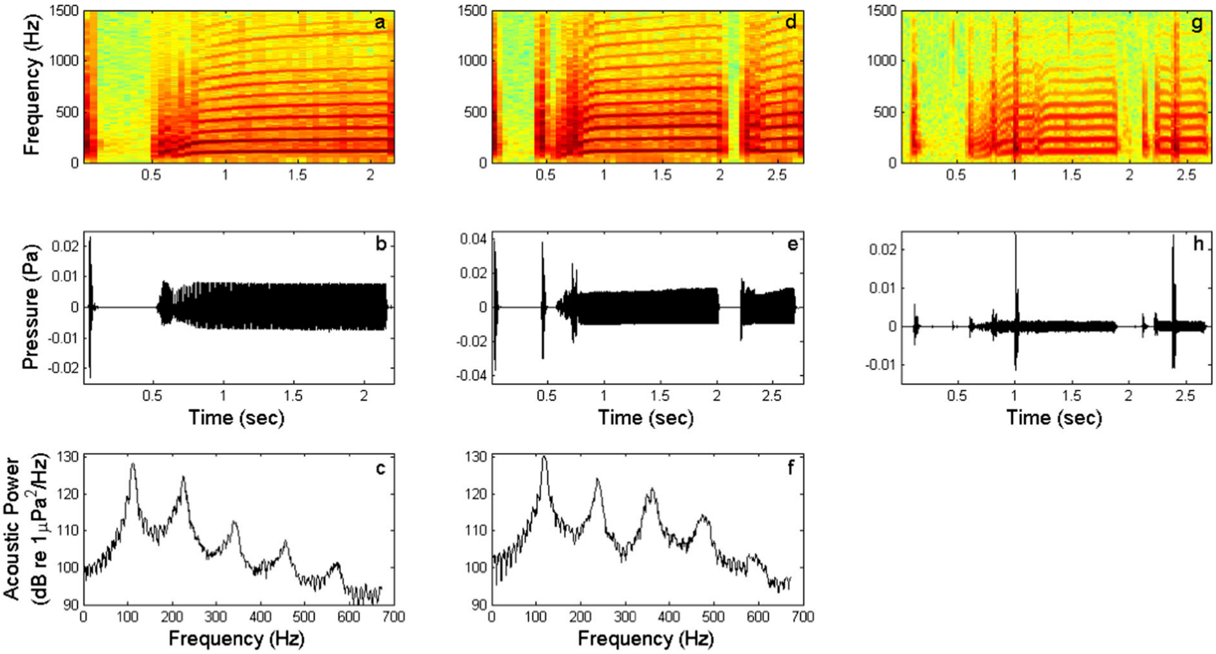

Fig. 2 Examples of vocalizations from *Ampicthyses cryptocentrus* males. A call composed of a pre-call grunt and a single note from Fish 1 is represented in three forms: (**a**) spectrogram, (**b**) waveform, and (**c**) power spectrum of the note (the grunt and the interval between the grunt and note is excluded). A call composed of a pre-call grunt and two notes from Fish 2 is also represented by a (**d**) spectrogram, (**e**) waveform, and (**f**) power spectrum of the first note. The initial broadband, grunt-like portions of the notes are visible in the spectrograms and correspond to the peak pressures in the waveforms. The fundamental frequencies of these calls are the lowest frequency peaks of the power spectra, and the dominant frequencies are the peaks with the highest power. In these two calls, the fundamental and dominant frequencies are the same. Two grunts that function as tags by Fish 3 are represented by a (**g**) spectrogram and (**h**) waveform. The background call tagged by Fish 3 provides an example of two less typical call variations: 1) a grunt that appears to merge into the first note, and 2) a note composed of seemingly two or more notes merged together. The parameters used to make the spectrograms and power spectra are found in Online Resource 3

fundamental frequency and second harmonic. The harmonic that has the greatest energy is the dominant frequency. The peak period of calling for each fish was divided into five time periods and ten calls were randomly selected from each period. If the randomly selected note was atypical (e.g. pre-call grunt merged into a call or notes merged together), or had been tagged by a high amplitude neighboring fish, the call was not used. If the call was a double note call, we used only the first note. Call evaluation was conducted in Raven and the time of the beginning and end of the notes was selected using the waveform view. These time markers were imported into a MATLAB script and spectrograms were produced for each call as described in Online Resource 3. We averaged over the time axis to produce a mean power spectrum for each call. In addition, the grunts preceding these calls were used to analyze grunt duration, peak pressure (0 to greatest peak; $\mathrm{dB}_{\mathrm{peak}}$ re 1 μPa), and dominant frequency. If the call had more than one pre-call grunt, we used only the first grunt. Dominant frequency was determined by computing a spectrogram (Online Resource 3) for the grunt, including portions of the recording before and after the grunt (composed of ambient sound) to reach the necessary window size for the spectrogram analysis. We averaged across the time axis to create a mean spectrum; the peak amplitude in this spectrum was the dominant frequency. This amplitude, however, is not the grunt amplitude we report, which was calculated by using the maximum absolute amplitude in the waveform of the grunt.

There are limitations to conducting sound level estimates of a long wavelength sound in shallow-water. However, we suggest that measuring outside the near-field provides confidence that these measurements reflect the sound level of these vocalizations (as argued in Barimo and Fine 1998). The near-field boundary can be approximated using the Rayleigh distance, which is equal to $\pi a^2/\lambda$ (Kinsler et al. 1999). For wavelength (λ) we use 12.8 m, which is the wavelength of a 117 Hz sound (fundamental frequency of these calls) in water with an approximate sound speed of 1500 m/s. We use for *a*, the longest dimension of the swim bladder, the value of 5 cm as used by Barimo and Fine (1998). This gives a Rayleigh distance of approximately 0.06 cm. The most uncertain term is *a*, and even if we double this value the distance (0.2 cm) remains well below the recording distance of 1 m.

Testing the placement of the isolated grunts with a null model

We tested if fish produced grunts at random or in response to specific acoustic stimuli. We used a null model (Fig. 3) to test the hypothesis that grunts not associated with calls (isolated grunts) are strategically placed to overlap the calls of neighboring males. We used amplitude of the acoustic environment that directly preceded the occurrence of a grunt to test this, and not observations of grunts overlapping calls. During the chorusing period the calling behavior of multiple individuals produces a high rate of calls from the group. Therefore, given the prevalence of these calls in the background, an isolated grunt from a focal fish that overlapped a call produced by a neighboring fish does not necessarily indicate that the grunt was purposefully inserted into that call. We predicted that if the calls of nearby males served as acoustic stimuli for grunting behavior then the distribution of amplitudes preceding these grunts would be different than if the grunts were produced at random. We therefore compared the amplitudes of the acoustic stimuli preceding the isolated grunts to the amplitudes preceding randomly placed “null grunts”. A null grunt is defined as a randomly selected time point in the recording.

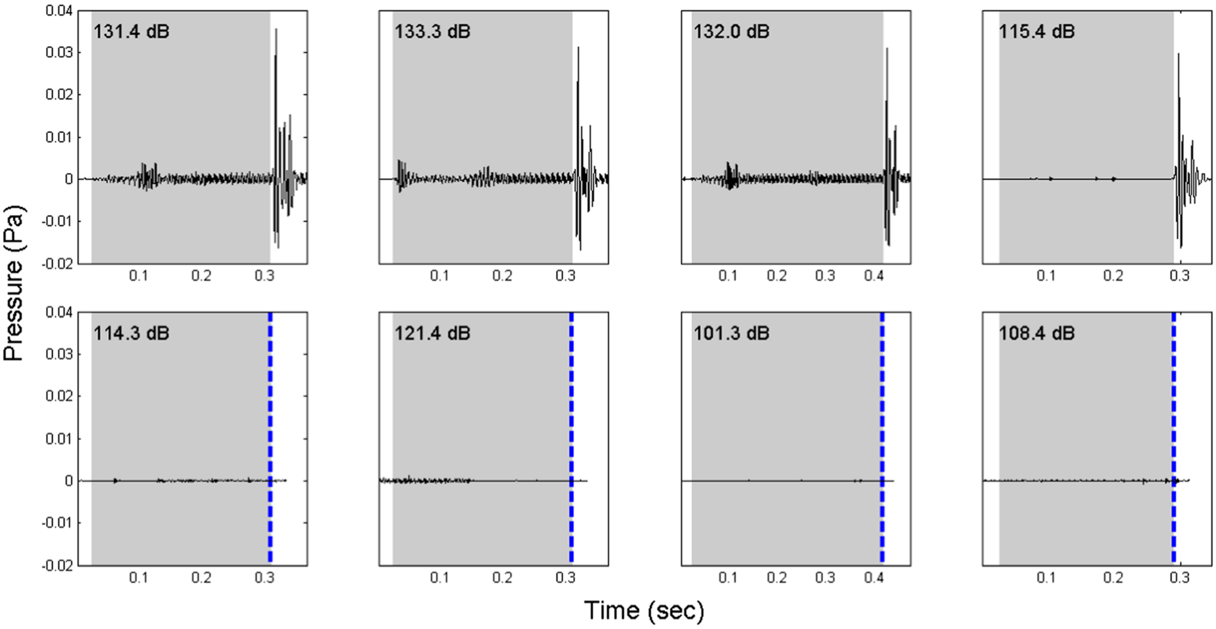

Fig. 3 Explanation of null model used to test the placement of the isolated grunts. The top row shows waveforms for four isolated grunts chosen from Fish 10, and for the sake of clarity in this model description we imagine that these are the only grunts in the analysis. To investigate the potential acoustic stimuli eliciting grunts, we found the peak pressures occurring before the grunts in sections of the recording illustrated by the shaded regions of the waveform. These segments included the beginning of the background call that the grunt overlapped up until the beginning of the grunt. The top right graph is an example of an isolated grunt that occurred without any discernible acoustic stimulus. In such cases, the peak pressure was found in a segment of the recording that was of a duration equal to the mean of the durations used for the first three grunts (e.g., the length of the shaded region in the top right is equal to the mean of the lengths of the shaded regions in the first three graphs in that row). The peak pressure ($\mathrm{dB_{peak}}$ re 1 μPa) of the waveform in each shaded section is in the upper left corner of each graph. These amplitudes are compared to the pressures found preceding the null tags (dashed vertical lines in the bottom row), which are randomly selected time points in the recording. As done for the observed isolated grunts, we found the peak pressure occurring in the waveform before the null grunt’s placement. The segments of the recording in which to find these peak pressures were equal to the durations used for the isolated grunts (e.g., the length of each shaded region in the bottom row is equal to the length of the shaded region in the graph directly above it). The peak pressures found preceding the null tags were compared to those preceding the isolated grunts using a Wilcoxon rank sum test

We first found the maximum amplitudes of the acoustic stimuli that preceded the isolated grunts made by the focal fish. We randomly selected 10% of the isolated grunts of each fish (but ensured a minimum of 50 and capped the analysis at 150). Fish 2 was an exception as this fish only produced seven isolated grunts in total, so all seven grunts were used. Using the spectrogram and waveform views in Raven, we extracted selections from the waveforms that preceded the grunts but did not include the grunt (Fig. 3). We included in the selection the background "event" that the grunt overlapped or followed, which was typically another toadfish call or less often a grunt. There were instances where this event also included a snap from a snapping shrimp; for example, a small number of randomly selected grunts followed the beginning of a neighbor's call and a shrimp snap that occurred at the same time as the call. These snaps were produced by species of *Synalpheus* and occurred at high rates in these recordings. In such cases (<10%) we resampled the grunt so that there was presumably only one likely acoustic stimulus (Thorson and Fine 2002a), even if the snap did not include the lower frequencies likely to be audible to the fish (Vasconcelos and Ladich 2008). We imported these selections into MATLAB and used a custom script that calculated the peak absolute sound pressure level (dB_{peak} re 1 μPa) in order to make the most conservative estimate of hearing sensitivity. A small subset of isolated grunts either 1) occurred during a period of ambient noise (no fish calls present), or 2) overlapped a series of low amplitude background calls making it not possible to discern a specific call the fish may have been targeting, if any. Since there was no preceding acoustic event to inform the time duration in which to identify the peak pressure, we found the peak pressure in a duration of the recording equal to the mean of the selections used for the other grunts (see Fig. 3).

We next found the peak absolute sound pressure level found in the recordings preceding the placement of the null grunts. For each fish we randomly selected a number of null grunts equal to the number of isolated grunts analyzed. We selected null grunts only from the peak period, as used for call and grunt rates, to avoid biasing the results with inclusion of a time period with few toadfish vocalizations. We identified the peak sound pressure that occurred before the random placement of the null grunts. The time periods of the recording in which to find these peak pressures was equal to the time durations used for the isolated grunts (Fig. 3). We resampled a null grunt if the preceding selection would include any portion of a grunt or call from the focal fish. We ran this null model 100 times for each fish, and compared the peak pressure preceding the isolated grunts to the peak pressure preceding the null grunts using a Wilcoxon rank sum test.

We also measured the latency time of the isolated grunts, defined as the time passed between the peak pressure and the beginning of the isolated grunt. This reflects the time it took for the fish to respond to the acoustic stimulus of the neighbor's call. For this measurement we excluded the grunts that did not appear to be in response to a specific toadfish vocalization.

Analysis of the background acoustic environment

We used a subset of the isolated grunts selected for comparison to the null model to further analyze the acoustic stimulus that may elicit grunting behavior. This subset included the grunts for which we measured latency time, but we excluded from this subset the grunts that had a selection of pre-grunt background content less than 93 μs in length. This is equal to the size of the spectrogram window used, and we did not want to sacrifice frequency resolution of the harmonics by decreasing window size. The number of grunts excluded for this reason ranged across the fish from one to 10. We created spectrograms (Online Resource 3) of the selections of the recordings that directly preceded these grunts (as represented by the gray boxes in Fig. 3). We used these spectrograms in two ways. First, we averaged across the time axis to produce a power spectrum, and then we averaged all spectra to create an average power spectrum for each fish. This spectrum represents the mean distribution of energy across the frequencies best detected by toadfish (Vasconcelos and Ladich 2008) that each fish presumably heard prior to emitting grunts. We used the maximum amplitude found in each fish's mean spectrum to test if call and/or grunt rate was correlated to the amplitude of the neighboring fish calls.

We wanted to investigate if the focal fish were responding to one or multiple neighbors; we make the prediction that if they are responding to more than one neighboring fish, the variation in maximum amplitude in the background calls the focal fish presumably responded to would be greater than the variation observed in the calls of the focal fish. The second use of the spectrograms created of the pre-grunt content was to compare the maximum amplitude found in each of these spectrograms to the maximum amplitude found in the spectrograms produced for the 50 calls selected for each fish.

To test if a focal fish increased its call rate in response to the rate of calling of neighboring fish, we used isolated grunt rate as a proxy for neighbor call rate. We make the assumption that if the null model supports isolated grunts are produced non-randomly to overlap the calls of nearby toadfish, then these grunts can serve as a reliable indicator of the rate of background vocalizations the fish could detect and were motivated to respond to. However, as described for the null model, there were grunts produced by the focal fish that did not overlap a distinct background toadfish call or at times any acoustic stimulus. As such, we removed the percentage of grunts for each fish that fell into this category. This is conservative, as grunts that do not overlap a distinct call could still be responding to toadfish-generated acoustic stimuli.

We tested the relationship (Pearson correlation) between call rate and the following parameters to investigate the trade-offs between call parameters and potential energy constraints: call length, the percentage of single note calls, and muscle contraction time. We also tested the relationship between call rate and percent moon illumination for the nights the individual fish were recorded given evidence of a lunar pattern to the calling behavior of this species (Salas et al., in preparation).

Results

Species level description of vocalization parameters

Individual level parameters are found in Table 1 and mean parameters representing the species level are found in Table 2. Calls were composed of one to four notes (mean = 1.32, SD = 0.47; Table 2), with one and two note calls the predominant call type (Table 1). We observed only two triple note calls (Fish 2) and a single quadruple note call (Fish 5). Calls were preceded by zero to four grunts, with one grunt the most typical (mean = 0.93, SD = 0.3). Calls were on average approximately 1 s in length (mean = 0.98, SD = 0.41), with the first note of the call longer (mean = 0.80) and more variable in length (SD = 0.23) than the second note (mean = 0.36, SD = 0.08). Isolated grunts were a mean length of 0.049 s (SD = 0.009). Single note calls were produced more frequently than double note calls; single note call rate was 2.78 calls/min (SD = 1.47) compared to the mean rate of double note calls of 1.31 (SD = 0.81). The mean call rate was 4.13 calls/min (SD = 1.84), with individual variation in rate (Table 1). Of the fish that produced advertisement calls, the maximum mean call rate was 5.79 call/min (Fish 3) and the minimum mean call rate was 0.56 calls/min (Fish 2). Fish 10 did not call but produced grunts. The mean rate of isolated grunt production over the recording period was 7.31 grunts/min (SD = 4.98), with a minimum rate of 0.069 grunts/min (Fish 2) and a maximum rate of 17.1 grunts/min (Fish 3). Calls and grunts were produced at a low rate per minute initially (Fig. 4), as discussed in the Methods for the calculation of call and grunt rates, but rates quickly rose and were seen to reach as high as 34 grunts/min (Fish 3) and 14 calls/min (Fish 4) during the peak period. In general, grunt rates were greater than call rates. The mean muscle contraction time was 3.68 s/min (SD = 1.18). The mean points of maximum amplitude was 11.6% (SD = 7.5%) into the first note and 6.1% (SD = 12.7%) into the second note. The coefficient of variation for call parameters was relatively low both within and between fish (Table 1 and Table 2). The greatest variation was observed between individuals in the rates of overall calls, single and double note calls, and isolated grunts.

Calls had a mean fundamental frequency of 116.6 Hz (SD = 2.52) at a mean amplitude of 128.3 dB re 1μPa^2/Hz (Table 2). The fundamental frequency was most typically also the dominant frequency except in the case of Fish 4 and Fish 5, which had greater mean energy in the second harmonic (Table 1). However, the difference in amplitude between the fundamental frequency and the second harmonic was for some fish minimal, such as by only 0.08 dB as seen in Fish 6. The dominant frequency of the broadband grunts occurred typically between 100 Hz and 300 Hz with a mean dominant frequency across the fish of 180 Hz. The mean peak pressure was 146 dB re 1μPa and the highest amplitude we observed was 154 dB re 1μPa.

Null model test of grunt placement

The median peak pressure preceding isolated grunts was significantly greater than the peak pressure that preceded the null grunts (Fig. 5). For all fish, the results of the Wilcoxon rank-sum test were significant ($p < 0.05$) for all 100 runs of the null model. Nine of the ten fish produced a high number of isolated grunts (range of

Table 1 Parameters (mean ± SD) for the boatwhistle calls and grunts recorded from 10 *A. crytocentrus* males

	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6
# Pre-call Grunts	0.94 ± 0.23	1.24 ± 0.43	0.93 ± 0.26	0.92 ± 0.32	0.85 ± 0.45	0.88 ± 0.33
# Notes	1.2 ± 0.4	1.97 ± 0.26	1.24 ± 0.43	1.42 ± 0.49	1.5 ± 0.5	1.29 ± 0.46
Call Length (sec)	1.03 ± 0.34	2.1 ± 0.25	0.97 ± 0.34	0.88 ± 0.37	1.25 ± 0.41	0.93 ± 0.39
Note 1 Length (sec)	0.90 ± 0.16	1.15 ± 0.14	0.81 ± 0.16	0.67 ± 0.197	0.93 ± 0.23	0.74 ± 0.156
Note 2 Length (sec)	0.40 ± 0.048	0.49 ± 0.04	0.38 ± 0.053	0.31 ± 0.047	0.39 ± 0.057	0.38 ± 0.057
Internote Interval (sec)	0.23 ± 0.04	0.17 ± 0.048	0.24 ± 0.031	0.18 ± 0.034	0.27 ± 0.036	0.26 ± 0.04
Call Rate (calls/min)	3.99	0.56	5.79	5.9	2.8	4.97
Single Note Call Rate (calls/min)	3.19	0.019	4.04	3.37	1.42	3.49
Double Note Call Rate (calls/min)	0.8	0.53	1.41	2.53	1.39	1.49
Point Max Amp Note 1 (%)	0.10 ± 0.0077	0.067 ± 0.02	0.091 ± 0.017	0.12 ± 0.0284	0.074 ± 0.0205	0.14 ± 0.026
Point Max Amp Note 2 (%)	0.018 ± 0.0015	0.013 ± 0.0029	0.037 ± 0.033	0.039 ± 0.0144	0.009 ± 0.0085	0.018 ± 0.0043
Mean Muscle Contraction Time (sec/min)	3.93	1.06	4.97	4.73	3.13	4.26
Mean Muscle Contractions/Minute	459.0	126.8	580.5	548.2	367.1	505.7
Tag Rate (tags/min)[a]	5.78	0.0687	17.11	11.26	5.44	11.17
Pre-call Grunt Lenth (sec)	0.044 ± 0.005	0.051 ± 0.005	0.052 ± 0.013	0.054 ± 0.006	0.053 ± 0.013	0.042 ± 0.004
Latency Time	0.13 ± 0.066	0.162 ± 0.18	0.11 ± 0.076	0.124 ± 0.061	0.11 ± 0.085	0.15 ± 0.072
# Calls Observed	538	91	769	555	359	595
# Tags Observed[a]	877	7	2838	1306	787	1519
Call Fundamental Frequency (Hz)	116.8 ± 1.75	119.6 ± 1.21	116.9 ± 1.36	115.9 ± 1.82	117.3 ± 1.72	118.7 ± 1.80
Call Amplitude at Fundamental Freq (dB re 1 $\mu Pa^2/Hz$)	132.2 ± 0.88	131.6 ± 0.93	130.2 ± 0.71	124.2 ± 1.11	119.7 ± 0.99	127.7 ± 0.91
Call Second Harmonic Frequency (Hz)	234.2 ± 2.87	239.8 ± 2.17	234.4 ± 3.0	232.3 ± 2.88	234.5 ± 3.04	238.8 ± 3.37
Call Amplitude at Second Harmonic (dB re 1 $\mu Pa^2/Hz$)	129.0 ± 0.67	126.7 ± 2.11	128.6 ± 0.82	125.3 ± 1.27	122.1 ± 1.12	127.6 ± 1.27
Pre-call Grunt Peak Pressure (dB_{peak} re 1 μPa)	147.0 ± 1.14	152.6 ± 0.76	147.9 ± 0.65	143.6 ± 1.84	140.7 ± 1.47	145.1 ± 0.53
Pre-call Grunt Dominant Frequency	190.6 ± 40.7	189.7 ± 29.7	184.2 ± 30.1	177.0 ± 32.4	176.2 ± 27.8	189.1 ± 26.4

	Fish 7	Fish 8	Fish 9	Fish 10	CoV Within (Range %)
# Pre-call Grunts	1.03 ± 0.19	0.93 ± 0.28	0.93 ± 0.27	NA	18.4–52.9
# Notes	1.34 ± 0.48	1.44 ± 0.50	1.05 ± 0.22	NA	13.2–35.8
Call Length (sec)	1.28 ± 0.39	0.81 ± 0.31	0.83 ± 0.27	NA	11.9–42
Note 1 Length (sec)	1.03 ± 0.18	0.58 ± 0.085	0.76 ± 0.2	NA	12.2–29.3
Note 2 Length (sec)	0.45 ± 0.044	0.28 ± 0.036	0.47 ± 0.056	NA	8.2–15.4
Internote Interval (sec)	0.28 ± 0.043	0.25 ± 0.04	0.21 ± 0.042	NA	12.9–27.7
Call Rate (calls/min)	2.48	5.75	4.96	0	–
Single Note Call Rate (calls/min)	1.6	3.2	4.71	NA	–
Double Note Call Rate (calls/min)	0.87	2.54	0.25	NA	–
Point Max Amp Note 1 (%)	0.11 ± 0.0273	0.16 ± 0.0178	0.11 ± 0.015	NA	7.7–30.1

Table 1 (continued)

	Fish 7	Fish 8	Fish 9	Fish 10	CoV Within (Range %)
Point Max Amp Note 2 (%)	0.019 ± 0.0048	0.038 ± 0.0064	0.021 ± 0.0047	NA	8.5–94.4
Mean Muscle Contraction Time (sec/min)	2.94	4.04	4.07	NA	–
Mean Muscle Contractions/Minute	341.3	465.8	460.7	NA	–
Tag Rate (tags/min)[a]	5.13	6.59	8.8	1.73	–
Pre-call Grunt Lenth (sec)	0.045 ± 0.004	0.049 ± 0.007	0.052 ± 0.004	NA	8.3–24.9
Latency Time	0.13 ± 0.055	0.129 ± 0.052	0.14 ± 0.061	0.16 ± 0.073	40.3–111
# Calls Observed	282	682	574	0	–
# Tags Observed[a]	553	862	1216	147	–
Call Fundamental Frequency (Hz)	116.1 ± 1.73	115.3 ± 3.14	113.2 ± 1.1	NA	0.97–2.7
Call Amplitude at Fundamental Freq (dB re 1 $\mu Pa^2/Hz$)	131.2 ± 1.18	128.7 ± 1.57	128.9 ± 0.71	NA	0.54–1.2
Call Second Harmonic Frequency (Hz)	232.8 ± 3.15	231.8 ± 5.58	226.9 ± 2.41	NA	0.91–2.4
Call Amplitude at Second Harmonic (dB re 1 $\mu Pa^2/Hz$)	127.6 ± 0.95	125.7 ± 1.76	126.4 ± 0.96	NA	0.52–1.7
Pre-call Grunt Peak Pressure (dB_{peak} re 1 μPa)	148.1 ± 1.06	145.5 ± 1.25	144.7 ± 0.74	NA	0.44–1.3
Pre-call Grunt Dominant Frequency	162.3 ± 35.0	172.5 ± 32.7	180.8 ± 29.9	NA	15.7–21.6

The point of maximum amplitude for the first and second note was calculated by dividing the time of the maximum absolute amplitude in the waveform by the length of the note. Mean muscle contraction time (number of seconds per minute the swim bladder muscles were contracting) was calculated using Eq. 1 (Methods). The mean number of muscle contractions per minute was calculated by multiplying the mean muscle contraction time by the fundamental frequency. Latency time is the time elapsed between the peak pressure occurring before a tag and the tag. The fundamental frequency and second harmonic and the amplitudes at these frequencies were determined from the first two peaks in power spectra of the first note of 50 calls selected for each fish. The peak pressure of the pre-call grunts was determined using the maximum absolute amplitude in the waveform. The dominant frequency of the pre-call grunts was determined as the peak frequency in power spectra of the grunts. The minimum and maximum Coefficient of Variation values across the individuals are reported for select parameters for each fish (CoV Within)

[a] The term tag also refers to "isolated grunts". We use here the terminology proposed in Thorson and Fine (2002a) given support that these grunts were intentionally used to overlap the calls of neighobring fish

NA indicates this parameter could not be measured since Fish 10 did not produce calls

Table 2 Species mean parameters for the boatwhistle calls and grunts recorded from *A. crytocentrus* males

	Species Mean ± SD (Range)	N	CoV Between (%)
# Pre-call Grunts	0.93 ± 0.3 (0–4)	4445	12
# Notes	1.32 ± 0.47 (1–4)	4445	18.8
Call Length (sec)	0.98 ± 0.41 (0.29–3.22)	4445	36.1
Note 1 Length (sec)	0.80 ± 0.23 (0.29–2.17)	4445	21.2
Note 2 Length (sec)	0.36 ± 0.08 (0.17–0.63)	1401	17.9
Internote Interval (sec)	0.24 ± 0.05 (0.076–0.67)	1403	16.3
Call Rate (calls/min)[a]	4.13 ± 1.84	9	44.5
Single Note Call Rate (calls/min)[a]	2.78 ± 1.47	9	57.9
Double Note Call Rate (calls/min)[a]	1.31 ± 0.81	9	61.7
Point Max Amp Note 1 (%)	0.116 ± 0.075	4445	26.7
Point Max Amp Note 2 (%)	0.0605 ± 0.127	1401	38
Mean Muscle Contraction Time (sec/min)	3.68 ± 1.18	9	32.1
Mean Muscle Contractions/Minute	428.4 ± 136.5	9	31.9
Tag Rate (tags/min)[b]	7.31 ± 4.98	10	68.2
Pre-call Grunt Lenth (sec)	0.049 ± 0.0087	450	17.7
Latency Time	0.13 ± 0.071	10	54.6
Call Fundamental Frequency (Hz)	116.6 ± 2.52 (107.2–122.6)	450	1.6
Call Amplitude at Fundamental Freq (dB re 1 μPa^2/Hz)	128.3 ± 3.95 (117.4–133.3)	450	3.1
Call Second Harmonic Frequency (Hz)	233.9 ± 4.87 (214.1–244.2)	450	1.6
Call Amplitude at Second Harmonic (dB re 1 μPa^2/Hz)	126.5 ± 2.35 (119.6–130.1)	450	1.7
Pre-call Grunt Peak Pressure (dB$_{peak}$ re 1 μPa)	146.1 ± 3.34 (136.3–153.9)	450	2.3
Pre-call Grunt Dominant Frequency	180.3 ± 32.8 (108.5–303.3)	450	18.2

The species mean (± SD) and range were generated by either 1) pooling the vocalizations observed from all individuals, 2) pooling the subset of calls and grunts selected for sound level analysis, or 3) taking the mean of the values calculated for the individuals. Coefficient of Variation (CoV) was calculated using the mean values for each individual in Table 1

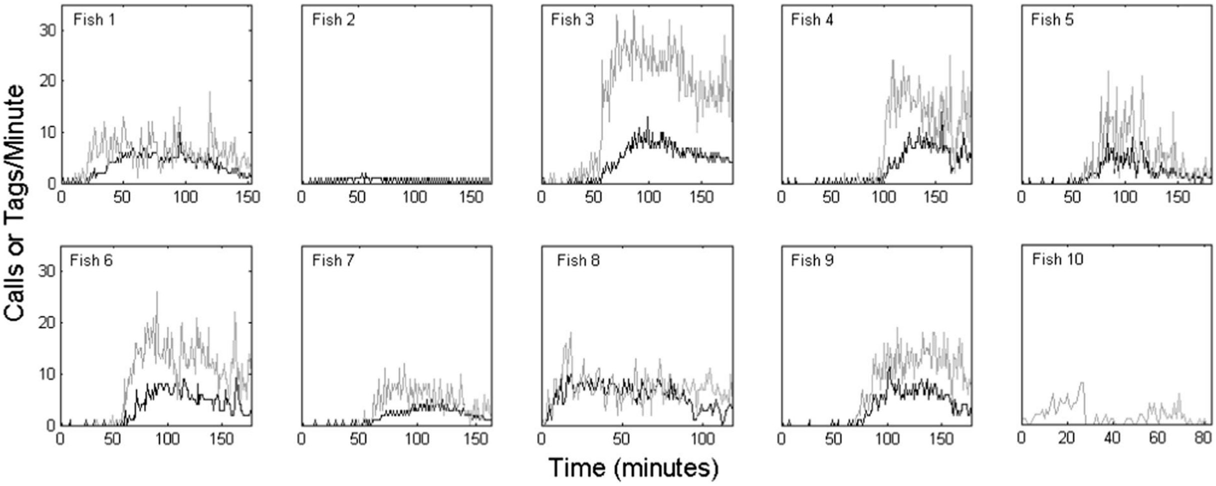

Fig. 4 Temporal variation in the rates of calls and isolated grunts. The number of grunts (also called tags) and calls per minute from the first event to the end of the analysis is shown in black (calls) and gray (grunts) for each fish. Fish 2 only produced seven isolated grunts, and Fish 10 did not produce any calls. In general, fish produced more grunts than calls and the rate of both varied throughout the chorusing period

147 to 2838), while Fish 2 produced only seven grunts (Table 1). The majority of isolated grunts overlapped background calls from neighboring toadfish. However, some grunts did not appear to be in response to a specific acoustic stimulus such as a discrete call or, less often, a grunt. The percentage of such grunts ranged from 2.0% to 19.7% for the nine fish that produced grunts at a relatively high rate. For Fish 2, three out of the seven grunts produced fit this category.

The mean time elapsed between the peak amplitude found in the recording and the beginning of the grunt (latency time) was 0.13 s (SD = 0.071).

Analysis of background calling environment

The mean frequency spectra of the acoustic environment preceding the isolated grunts (Fig. 6) peaks at the second harmonic, between 221 and 228 Hz for all fish but Fish 2. We used the maximum mean amplitude at the second harmonic to test the correlation between call and grunt rates and the amplitude of the calls of neighboring males. These correlations were not found to be significant (call rate: $r = 0.32$, $p = 0.37$, $df = 8$; grunt rate: $r = 0.031$, $p = 0.93$, $df = 8$).

The maximum amplitudes found in the spectrograms of the acoustic environment preceding the isolated grunts (Fig. 7a) were more variable than the maximum amplitudes found in the spectrograms of the focal fish (Fig. 7c). The frequency of the maximum amplitude in the content preceding the grunts occurred most typically at the second harmonic (Fig. 7b). The frequency of the maximum amplitude in the focal fish calls occurred most typically for three fish at the fundamental frequency, for three fish at the second harmonic, and for three fish at frequencies ranging between the first and second harmonics (Fig. 7d).

The significance of the null model supports the use of isolated grunt rate as a proxy for the call rate of neighboring fish. However, we did observe that some grunts did not overlap specific background calls, so we subtracted the percentage of grunts of this type from the total observed. The percentage of grunts removed from the nine fish that produced a high number of grunts ranged from 2 to 19.7%. For Fish 2, which only

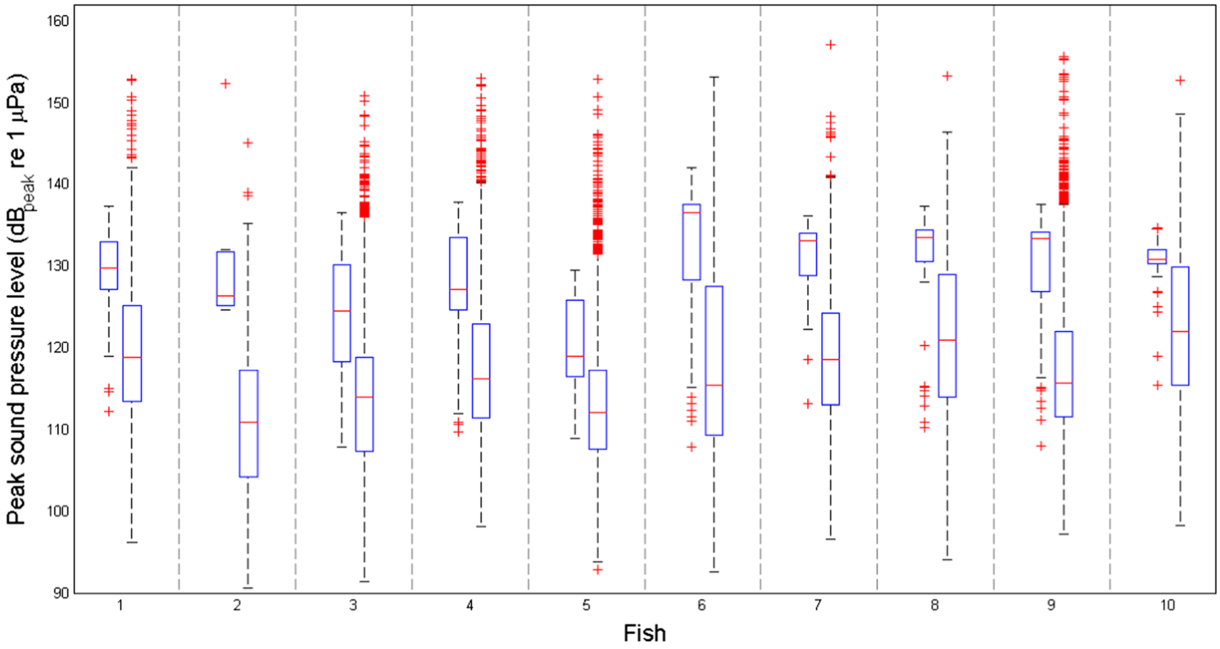

Fig. 5 Null model results: Comparing peak pressures that occurred before the isolated grunts and null grunts. Each set of boxplots (as demarcated with the vertical dashed lines) represents the results for one fish. Within each set, the boxplot to the left represents the peak pressures occurring before the isolated grunts, and the boxplot on the right represents the peak pressures occurring before the null grunts**.** The 100 runs of the null model are pooled for each fish. The isolated grunts produced by these toadfish males generally followed higher amplitude and less variable acoustic stimuli than would have occurred if the fish had been producing grunts at random. This suggests these grunts were in response to the vocalizations of neighboring males. Line in box = median; box = 25–75 percentiles; whiskers = most extreme data points not considered as outliers; red plus signs = outliers

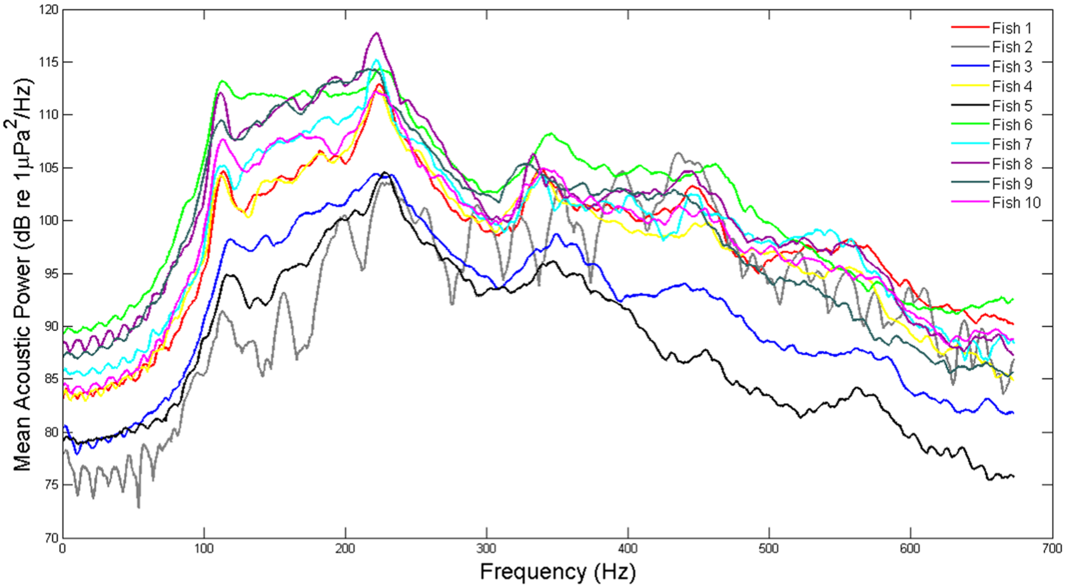

Fig. 6 Power spectra of the acoustic stimuli preceding the occurrence of isolated grunts (tags). These power spectra represent the acoustic environments to which the focal fish was exposed, on average, prior to emitting isolated grunts. The highest amplitude frequencies occurred around 220 Hz (except for Fish 2), which is approximately the second harmonic of the background calls tagged by the focal fish

produced seven grunts, 42.9% of grunts were removed. The correlation between call rate and this modified grunt rate was 0.84 ($p = 0.0024$, $df = 8$). This essentially matched the correlation between call rate and the unmodified grunt rate ($r = 0.86$, $p = 0.0015$, $df = 8$).

As call rate increased, there was a significant decline in mean call length ($r = -0.92$, $p << 0.05$, $df = 7$). The percentage of single note calls out of the total number of calls produced increased with call rate ($r = 0.67$, $p = 0.048$, $df = 7$). Mean muscle contraction time increased with call rate ($r = 0.96$, $p << 0.05$, $df = 7$). There was not a significant correlation between call rate and percent moon illumination ($r = -0.37$, $p = 0.28$, $df = 8$).

Discussion

We recorded ten *A. cryptocentrus* males in Caribbean Panama to describe the acoustic behavior of this species. The sedentary behavior of these fish within their burrows (Hoffman and Robertson 1983) and the low variation in amplitude and fundamental frequency within individuals (as in Barimo and Fine 1998; Thorson and Fine 2002b) allowed us to differentiate the calls of the focal fish and their neighbors. The placement of the isolated grunts suggests these males were intentionally using this behavior to overlap the calls of nearby males. Given this, we can predict the acoustic stimuli most likely to have elicited grunting behavior. Further, we suggest that males were responding to multiple individuals who occupied burrows around them. This result highlights the potential importance of the spatial distribution of these fish; we predict that the density of fish, and not just abundance, will impact the role of toadfish vocalizations in a habitat's soundscape.

Comparison of vocal behavior to other toadfish species

We compare the main features of the calls of *A. cryptocentrus* to the other species in this family whose acoustic behavior has been described (as summarized in Mosharo and Lobel 2012). The calls of *A. cryptocentrus* are preceded by a broadband grunt, similar to *Opsanus tau* and *O. beta* (Talvoga 1958). Five of the eight species compared in Mosharo and Lobel (2012) produce calls with only a single note, and while a single note is the most common call type for *A. cryptocentrus*, they also often produce double note calls. This makes them the fourth batrachoidid species observed to produce multiple note calls, and *A. cryptocentrus* is more similar to these species in call rate and call length than species that produce calls composed of only a single note (Mosharo and Lobel 2012). Like *O. beta*, the second note is shorter than the

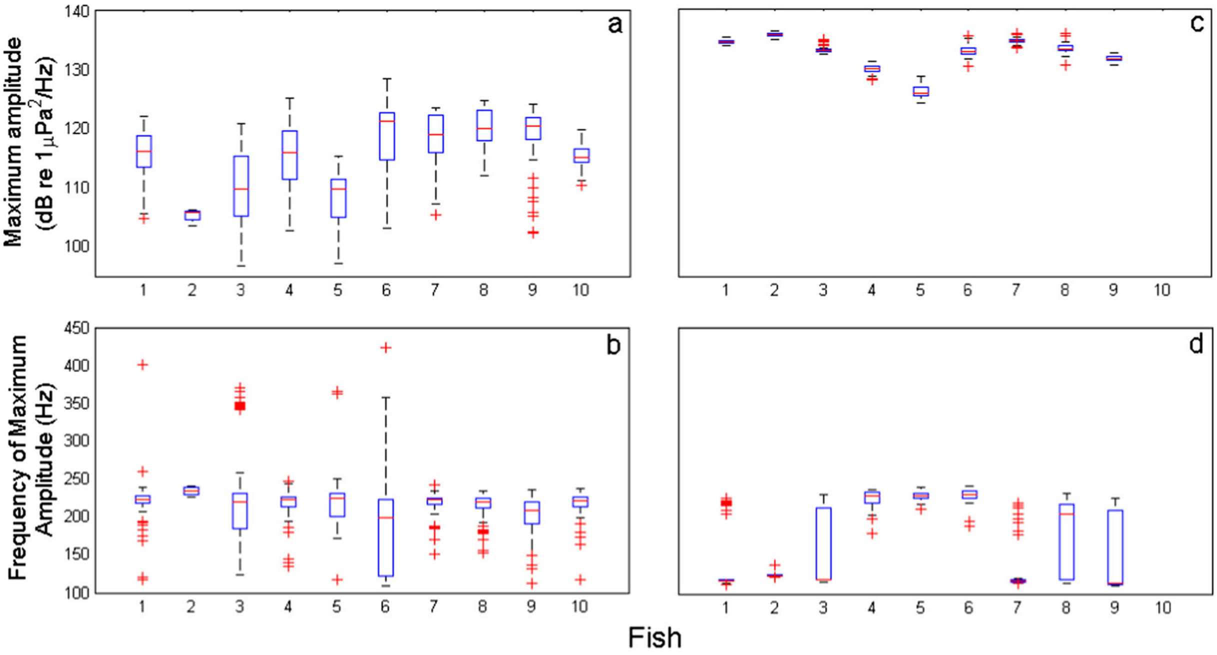

Fig. 7 Predicting the acoustic stimuli eliciting grunts and the number of neighbors tagged. We used the variation in the amplitudes of the calls that the focal fish tagged to predict if these grunts were in response to multiple neighbors or a single, high amplitude neighbor. If the focal fish produced grunts in response to multiple neighbors we predicted that the amplitudes of the calls they responded to with tags would be more variable than the amplitudes observed in the calls recorded from the focal fish. The maximum spectral amplitudes found in the portions of the background calls occurring before the tags are represented in (**a**), and the frequencies at which these amplitudes occurred is in (**b**). The maximum spectral amplitudes found in the calls of the focal fish are represented in (**c**), and the frequencies at which these amplitudes occurred is in (**d**). There are only nine boxplots for (**c**) and (**d**) because Fish 10 did not call. The greater variation observed in the calls of the neighboring fish compared to the calls of the focal fish supports males responded to multiple fish versus a single high amplitude neighbor. These results also suggest that fish were responding with grunts to acoustic stimuli at the second harmonic at amplitudes shown in (**a**), which is not always the highest amplitude frequency in the calls when recorded at a distance of one meter (**d**). Line in box = median; box = 25–75 percentiles; whiskers = most extreme data points not considered as outliers; red plus signs = outliers

first note (Thorson and Fine 2002b). The significant correlation between the percent of single note calls and call rate suggests that the removal of the second note may reduce the chances of a fish overlapping its own calls with those produced by neighboring males, as suggested for *O. beta* (Thorson and Fine 2002b). Shortening calls to include only a single note could also serve to minimize energy expenditure as call rate increases.

The fundamental frequency of a toadfish call is determined by the number of contractions per second of the muscles that surround the swim bladder (Fine et al. 2001). Thorson and Fine (2002b) suggest that the speed required of these muscles to produce sound underwater imposes a physiological constraint on the number of calls a toadfish can produce in a calling bout. Our results support this hypothesis, as despite *A. cryptocentrus* occurring in tropical waters, this species shows a comparable and even lower number of muscle contractions per minute than temperate species. Six of the nine fish had mean calling effort ranging between about 460 and 581 contractions per minute. This calling effort is similar to that observed for other toadfish species. Individuals of *O. tau* and *O. beta* produced 660 and 663 contractions per min, respectively (Thorson and Fine 2002b) and the calling effort of *Sanopus astrifer*, a tropical species, was 469 contractions per min (calculated from Mosharo and Lobel 2012). We can also consider the calling effort made during the peak of calling. Five of the nine fish had brief periods during which their call rates were greater than 10 calls per min (Fig. 4). Making the conservative assumption that these were single note calls and using the species mean for fundamental frequency and length of the first note (Table 1), the approximate number of contractions per minute during these peak periods was 933 contractions per minute. Fish 4 briefly had a peak rate of 14 calls per minute, thus producing 1088 contractions per minute (using the fundamental frequency and first note length of Fish 4). These values are comparable to the highest call efforts observed for *O. tau* and *O. beta* of 1033 and 1550 contractions per minute, respectively (Thorson and Fine 2002b). In total, these results support that *A. cryptocentrus* may have similar physiological constraints that bound their calling behavior and these fish exert a similar amount of effort as observed in other species to acoustic communication.

This species produces high intensity calls with respect to the ambient noise, with some fish producing calls with intensity greater than 130 dB re 1μPa^2/Hz at the fundamental frequency of the call (Table 1). The point of maximum amplitude occurred at a species mean of 11.6% into the first note and 6.0% into the second note. This pattern can be observed in the location of the peak of the waveforms of the notes in Fig. 1. This peak occurs during the grunt-like broadband portion that begins the notes. This initial broadband portion is also observed in the calls of several other toadfish species (Mosharo and Lobel 2012). After this peak, there is relatively little amplitude and frequency modulation. Rice and Bass (2009) suggest that the point at which the peak amplitude occurs in a note is a key parameter that distinguishes the calls produced by different batrachoidid species. More derived species appear to have a point of maximum amplitude sooner in the call than more basal species (Rice and Bass 2009; Mosharo and Lobel 2012). *A. cryptocentrus*, being more derived (Greenfield et al. 2008) reflects this possible phylogenetic pattern and is the first species observed to have the point of maximum amplitude depend on if the note is the first or second in the call.

Evidence of acoustic competition through tagging behavior

The null model supports the hypothesis that the isolated grunts are intentionally placed over the calls of neighboring male fish, as observed in *O. beta* and *O. tau* (Thorson and Fine 2002a; Mensinger 2014). We predicted that if fish were using grunts to respond to specific calls from nearby fish, the distribution of amplitudes that directly precedes these grunts would be different than if the grunts were produced at random. We observed that the grunts followed background content that was of greater and less variable amplitude than predicted by the null model. However, for all fish we did observe the upper whisker and outliers of the null model results to overlap and exceed the amplitudes observed to have occurred before the isolated grunts. This is explained in that these highest values in the null model reflect snaps from snapping shrimp that were close to the hydrophone (outliers) and high amplitude calls of neighboring fish. Thus, the placement of some fraction of null grunts followed the same amplitude of calls to which the focal fish responded; however, the overall results of the null model are significantly different than the observations, as predicted if the isolated grunts were non-randomly placed. The model results also support the observations we made in the spectrograms as we identified the vocalizations of the focal fish. The isolated grunts of the focal fish appeared in most cases to overlay the calls of neighboring males and typically occurred within the first, approximately third, of the call. Given these results, we henceforth use the terminology of “tags” to refer to these isolated grunts, sensu Thorson and Fine (2002a). We cannot know from this study the function of the tags, but we predict they are used as a form of acoustic competition as proposed for *O. tau* (Mensinger 2014) and/or as dominance displays as suggested for *O. beta* (Thorson and Fine 2002a). Grunts can also serve other functions, such as burrow defense (Gray and Winn 1961). This may account for the isolated grunts (2 to 19.7% of the subset selected for analysis) that do not appear to have functioned as tags given they did not overlap a discrete call from a neighboring male. These values are similar to the 12% of grunts that did not occur within a detectable boatwhistle call in *O. tau* (Mensinger 2014).

For a fish to tag a call produced by a neighboring fish, the call must be both audible and of sufficient amplitude to motivate a response. We next consider what specifically in the background calls might serve as the stimulus for a grunt. It is most conservative to predict that tags are in response to the highest amplitude content in the background calls. Thus, we hypothesize that the acoustic stimuli eliciting tags were the grunt-like, broadband portions that occurred at the beginning of a note and contained the point of maximum amplitude. The second harmonic (Fig. 6) was the highest amplitude frequency in the acoustic stimuli tagged by the fish in our study, likely a product of the fundamental frequencies attenuating most rapidly over distance (Fine and Lenhardt 1983). Maximum sound levels in the pre-grunt content occurred at the second harmonic as well (Fig. 7b), with most values ranging between 110 dB to 120 dB re 1uPa^2/Hz and the lowest at 97 dB re 1uPa^2/Hz (Fig. 7a). Audiograms do not exist for *A. cryptocentrus*, but we use the measured hearing sensitivity for other toadfish species to consider the likelihood that these sound levels were audible. Individuals of *Halobatrachus didactylus* (Vasconcelos and Ladich 2008) were sensitive to a 230 Hz sound (approximately the second harmonic of the calls in this study) at about 98 dB re 1μPa, suggesting that it is possible that *A. cryptocentrus* is capable of detecting these amplitudes and were motivated to respond. This is further supported by audiograms of *O. tau*, a species in the same sub-family as *A. cryptocentrus*. Audiograms made using behavioral conditioning and responses to acoustic stimuli in the field (Fish and Offutt 1972) both suggest that the sound levels at the second harmonic were likely detectable by the focal fish. Our study supports the likelihood of this more sensitive hearing over thresholds predicted by audio brainstem response technique for *O. tau* (Yan et al. 2000), which predicts thresholds of approximately 122 dB re 1μPa at about 230 Hz. If the fish instead were responding to other frequency content, or multiple frequencies, they would have been required to have higher hearing sensitivity than predicted for responding to the second harmonic alone. We stress that the levels to which we propose the fish were responsive are not hearing thresholds, but rather levels that appear to have been both audible and of sufficient amplitude to elicit a tagging response.

The role of the spatial distribution of toadfish males in the species chorus

One of our primary interests was to consider how social factors that influence calling behavior at the individual level might scale up to influence the toadfish chorus and its role in a habitat's soundscape. The impact of social factors on the vocal behavior of an individual male is well supported in toadfish, and is seen again here in *A. cryptocentrus*. In addition to the evidence provided by the group of fish we recorded, the unique behavior of Fish 2 provides a specific case example. This fish was exposed to the lowest amplitude background calls as evidenced by the lowest amplitude harmonics in Fig. 5 and the lower amplitudes randomly selected in the recording for the null model (Fig. 6). The production of both calls and tags suggests this fish detected the calls being produced around him, but the distinctly different calling behavior suggests the amplitudes were below a certain level required for a fully motivated response (Fish and Offutt 1972). Calls were produced at the lowest rate of the fish that called (Table 1), the rate was nearly constant through time (Fig. 3), and he produced the highest percentage of double note calls (Table 1). In turn, we predict that the background calls were of insufficient amplitude to motivate a high call rate, to respond to calls of other males as they varied their rates in time (Jordão et al. 2012), and more double note calls were produced because the social environment did not necessitate avoidance of call overlap. Fish 10 provides another unique case, as this fish produced only tags and no calls despite exposure to an acoustic background similar to the fish with high call and tag rates (Fig. 6). We predict that individual factors, such as condition, may have contributed to the lack of call production (Mitchell et al. 2008). We do not believe that this fish was a female (which do not produce boatwhistle calls in other toadfish species) since it is unlikely that a female would compete acoustically with males using tagging behavior.

The vocalizations of many *A. cryptocentrus* males composes the species chorus and how this chorus contributes to the soundscape of the habitat will likely depend on if fish engage in pair-wise acoustic interactions or if they are influenced by multiple fish. We investigated if the focal fish responded to a) audible calls of different amplitudes, thus representing different neighbors (Fig. 8), or instead to b) only the calls perceived as loudest, thus representing the greatest source of competition. If toadfish males are motivated to respond to calls from more than one neighboring male, we predict that the patterns of overlap of active spaces of males will influence their acoustic behavior. Active space is the area over which reliable detection of a signal occurs (e.g. Marten and Marler 1977; Janik 2000) and in the present context is considered the space over which a male's call will remain at a sufficient amplitude to be detected by another fish in that space. This distance is determined by several factors that influence the transmission loss of the sound as it travels away from the fish that produced the call. These factors include water depth, sediment type, and water temperature and density, which influence the sound speed profile. These factors vary over space and time, making predictions of the active space subject to considerable uncertainty. As such, we do not attempt to predict the distances between the focal fish and the fish to which they responded. Our goal here is to support the hypothesis that individual males responded acoustically to multiple neighboring fish. Given these fish are distributed over some area, we can support the notion that the density and distribution of toadfish will influence the calling of individuals and thus the characteristics of the toadfish chorus. If a male just responds to its most intense (loudest) competitor, then the chorus will be mainly influenced by pair-wise interactions over space.

We support the idea that focal fish were responding to multiple neighbors with two lines of evidence. First, given low variation in the amplitudes of the focal fish calls, we predicted that if the focal fish were responding to only a single neighbor, we would also see low variation in the acoustic stimuli to which they presumably responded with tags. However, we observed that the amplitude variation in the portion of the background calls that preceded the tags was greater than the amplitude variation observed in the calls of the focal fish. An example of this is shown in Fig. 8, where Fish 10 tagged background calls that we predict were produced by three different neighboring males. Thus, Fish 10 was in the active spaces of at least three other males, and these males influenced the vocal behavior of Fish 10. Our second line of evidence is the strong positive correlation between call and tag rates. We argue that tag rate can serve as a proxy for the rate of toadfish-generated sounds that satisfied the amplitude requirements for being both detectable and motivating a response from the focal fish. This provides the advantage of using the vocal behavior of the fish itself to inform what background call levels satisfied the requirements for motivating a response. Thus, we propose that call rate increased with the rate of vocal activity of the surrounding fish, as reflected in tagging behavior. Since tag rate was greater than call rate for all fish but Fish 2, we argue that the focal fish were responding to more than one individual in its acoustic environment. This is supported by both the mean call and tag rates (Table 1) and the time series of these rates (Fig. 4). However, in the time series there are times when the call and tag rates are similar, suggesting the possibility of call rate matching with a specific neighbor (Jordão et al. 2012). While all of the isolated grunts we selected for analysis did not overlap a distinct calling event, the relationship between call and tag rate remains robust even if these are excluded.

These results support that a male is likely to be acoustically responsive to more than just the highest amplitude neighbor if he is in the active space of

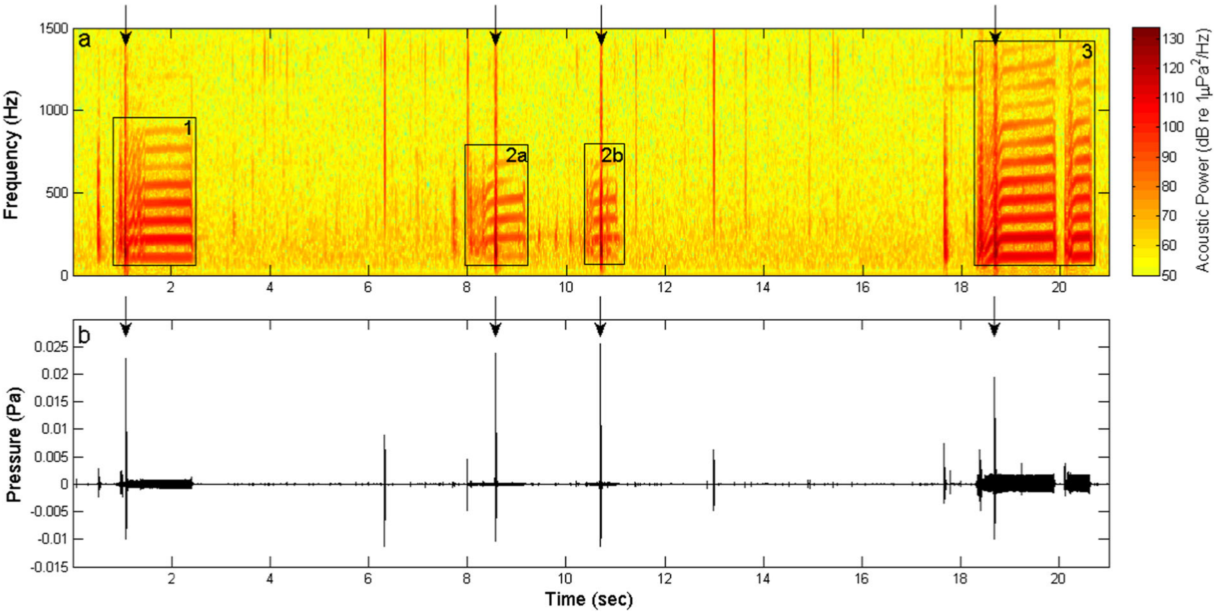

Fig. 8 Example of tagging behavior. A spectrogram of 21 s recorded from Fish 3 is shown in (**a**) and the corresponding waveform is shown in (**b**). During this time the fish produced four tags (black arrows) in response to calls from presumably three neighboring fish. The maximum spectral amplitudes (in dB re $1\mu Pa^2/Hz$) of the sections of these background calls that occurred *before* the tags are: 113 dB at 215 Hz (box 1), 100 dB at 199 Hz (box 2a), 96 dB at 198 Hz (box 2b), and 117 dB at 184 Hz (box 3). The amplitude variation and differences in harmonic structure suggests that these four calls were produced by at least three different fish neighboring Fish 3. We predict that calls 2a and 2b were produced by the same fish. Hence, Fish 10 was responding to multiple background fish, and not just the highest amplitude neighbor (box 3)

multiple males. This observation suggests that the acoustic behavior of this species could produce an amplifying effect dependent on the patterns of overlap of active spaces. For example, calling of one fish stimulates increased calling and tagging behavior by fish that occupy burrows in that fish's active space. Increased vocal behavior by these fish will in turn stimulate increased calling in any fish that are within the active spaces of their calls. How such a pattern will unfold will depend on how burrows are distributed relative to one another and their occupancy, dependent on habitat level characteristics and population size. Thus, we propose that differences in the distribution of toadfish can contribute to the spatial variation observed in soundscapes.

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Compliance with ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. University of Texas at Austin IACUC protocol AUP -2015-00070.

Conflict of interest The authors declare that they have no conflict of interest.

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