

RESEARCH ARTICLE

Novel underwater soundscape: acoustic repertoire of plainfin midshipman fish

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ABSTRACT

Toadfishes are among the best-known groups of sound-producing (vocal) fishes and include species commonly known as toadfish and midshipman. Although midshipman have been the subject of extensive investigation of the neural mechanisms of vocalization, this is the first comprehensive, quantitative analysis of the spectro-temporal characters of their acoustic signals and one of the few for fishes in general. Field recordings of territorial, nest-guarding male midshipman during the breeding season identified a diverse vocal repertoire composed of three basic sound types that varied widely in duration, harmonic structure and degree of amplitude modulation (AM): ‘hum’, ‘grunt’ and ‘growl’. Hum duration varied nearly 1000-fold, lasting for minutes at a time, with stable harmonic stacks and little envelope modulation throughout the sound. By contrast, grunts were brief, ~30–140 ms, broadband signals produced both in isolation and repetitively as a train of up to 200 at intervals of ~0.5–1.0 s. Growls were also produced alone or repetitively, but at variable intervals of the order of seconds with durations between those of grunts and hums, ranging 60-fold from ~200 ms to 12 s. Growls exhibited prominent harmonics with sudden shifts in pulse repetition rate and highly variable AM patterns, unlike the nearly constant AM of grunt trains and flat envelope of hums. Behavioral and neurophysiological studies support the hypothesis that each sound type’s unique acoustic signature contributes to signal recognition mechanisms. Nocturnal production of these sounds against a background chorus dominated constantly for hours by a single sound type, the multi-harmonic hum, reveals a novel underwater soundscape for fish.

KEY WORDS: Vocalization, Advertisement call, Amplitude modulation, Chorus, Hearing

INTRODUCTION

Among tetrapods, the frequency content of a vocalization typically results from an airflow-dependent vibration of variably tensioned membranes at the source (syrinx and larynx) and subsequent post-source filtering (e.g. vocal tract) (Riede and Goller, 2010; Bradbury and Vehrencamp, 2011). However, in many fishes, sounds arise not from air movement but from the vibration of peripheral structures such as bony elements or the swim bladder by a single pair of sonic or vocal muscles (Ladich and Fine, 2006; Parmentier and Diogo, 2006; Bass and Ladich, 2008). To produce signals with divergent

properties, fish can modify the muscles’ contraction strength and rate to vary sound amplitude and pulse repetition rate (PRR), respectively, and the duration of repeated contractions to modify sound duration. For fish, muscle contraction rate, and thus PRR, also sets the fundamental frequency (F_0) of multi-harmonic sounds. While the brain directly determines PRR/ F_0 (Bass and Baker, 1990; Chagnaud et al., 2011), harmonic content is likely determined by the biomechanical properties of the skeletal element(s) to which the muscles attach, e.g. swim bladder, pectoral girdle and the body wall, which vibrate during the transmission of any vibration into the aquatic medium (e.g. Lancey, 1975; Fine et al., 2009).

One of the best-known groups of sound-producing fishes is toadfishes, a single order and family (Batrachoidiformes, Batrachoididae) that include species commonly referred to as midshipman fish and toadfish (Greenfield et al., 2008). These fish have emerged as an important vertebrate model for identifying auditory and vocal mechanisms of acoustic communication (Bass and McKibben, 2003; Bass and Chagnaud, 2012). Despite numerous morphological and physiological studies of neural mechanisms, rigorous quantitative analysis of the spectral and temporal characters of individual sounds among toadfishes has largely been lacking. Here, we focus on the plainfin midshipman fish, *Porichthys notatus* Girard 1854, the subject of extensive investigations of acoustic mechanisms at the behavioral, hormonal and neurobiological level, where the contextual and qualitative analyses of sounds have been conducted (see above reviews).

Midshipman, like other toadfishes, produce acoustic signals by vibrating the swim bladder with a single pair of simultaneously contracting sonic/vocal muscles attached to the outer walls of the gas-filled bladder (Greene, 1924a; Greene, 1924b; Cohen and Winn, 1967). The biomechanical simplicity of sound production that involves one pair of muscles has been key to studies showing how separate neuronal populations comprising a hindbrain central pattern generator determine the amplitude, PRR/ F_0 and duration of natural sounds (Bass and Baker, 1990; Chagnaud et al., 2011; Chagnaud et al., 2012). The analysis of midshipman sounds has been largely qualitative and not presented in a single cohesive framework. Here, we present the first systematic, quantitative analysis of multiple spectral and temporal characters of midshipman sound types based on recordings from nest sites in their natural habitat during the nocturnal breeding season. We focus on the three types of sounds that are known to be produced by midshipman fish: ‘grunts’, ‘hums’ and ‘growls’ (Bass et al., 1999; Brantley and Bass, 1994; Cohen and Winn, 1967; Ibara et al., 1983). This species has two male reproductive morphs known as type I and type II males that are distinguished by a large suite of behavioral, somatic, neural and endocrine characters (Bass, 1996). Our prior studies of the nesting and reproductive behavior of midshipman fish held in captivity show that type I males build and defend nests, acoustically court females with hums, and produce grunts and growls in agonistic contexts (Bass et al., 1999; Brantley and Bass, 1994; Genova et al.,

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2012). Type II males follow a sneak and satellite spawning tactic and, like females, are only known to produce isolated grunts that have a very low amplitude compared with type I male sounds (Brantley and Bass, 1994). We presume that all sounds recorded from nests in the current study (grunt trains, hums, growls) were produced by type I males. To analyze growls, the least reported and yet, as we show, the most complex of their sounds, we developed a new analysis tool to quantify amplitude modulation (AM) rate that provides a metric for comparing the sound types of midshipman and those of other fish species.

As we report, each sound type can be defined by a unique combination of spectral and temporal characters. We consider the significance of these results within the context of prior studies of the behavioral and neural mechanisms for sound discrimination in midshipman fish and toadfishes in general. More broadly, the production of these sounds against a background chorus constantly dominated for hours by a single sound, i.e. multi-harmonic hums, reveals a previously undocumented level of complexity in the acoustic landscape of fish.

RESULTS

The physical attributes of each sound type were quantified on the basis of ~60 h of recordings by one of us (M.A.M.) from 14 nests in their natural habitat in Washington State over the course of 7 days during the 1997 breeding season (5, 6, 7, 20, 22, 23 and 24 June). The study site was one of prior investigations of nesting habitat and spawning success of midshipman fish (DeMartini, 1988; DeMartini, 1991). Figs 1 and 2 show photographs of the site. DeMartini

established nests with roofs of varying size that were made of cement. These same nest covers were still present when we conducted our studies and along with natural rocky coverings were chosen as focal nests with resident type I males for hydrophone recordings (Fig. 1). Each day, a single custom-built hydrophone (Bioacoustics Research Program, Cornell Laboratory of Ornithology) was suspended from an iron stake immediately adjacent to a nest during the morning low tide (Fig. 2).

We could not confidently assign vocal records to focally identified males as individual nests at this site often include more than one type I male (Fig. 1B,C). For the nests included in this study, a census of each nest was taken the morning before a recording session and varied from one (nine nests) to two (eight nests), three (one nest) and four (two nests) type I males (this reflects more than one census taken at some of the 14 nests included in the study). For those cases where recordings were made at the same nest on consecutive evenings, it was found that nest occupants sometimes varied between days. For example, one nest had two occupants one morning and four the next that included the original two. A second nest had three the first morning and two the next that were present the first morning. A third nest had two the first morning and two the next that included only one of the original two. A fourth nest had two the first morning and one the next that was one of the original two. Two nests had a single occupant on each of two consecutive mornings, but the occupants were different each day. One caveat to this analysis is that individual fish were identified only on the basis of standard length, as we wanted to minimize stress for the nest occupants in their natural habitat. In the absence of individual



Fig. 1. Midshipman (*Porichthys notatus*) nests. The study site was first established by E. DeMartini, who made nests with cement roofs of varying size (DeMartini, 1988; DeMartini, 1991). Four of these same nest covers are apparent in a panoramic view of the study site (A) with one of them highlighted (B). These nests, along with natural rocky coverings (round- to ovoid-shaped rocks in A) were chosen as focal nests for hydrophone recordings. The undersides of nest roofs are covered with clusters of fertilized eggs (bright yellow) and larvae (orange). Nests often contained more than one nest-guarding male (white arrows in A,B) that were sometimes found engaged in aggressive interactions during the morning census (C, different nest from B).

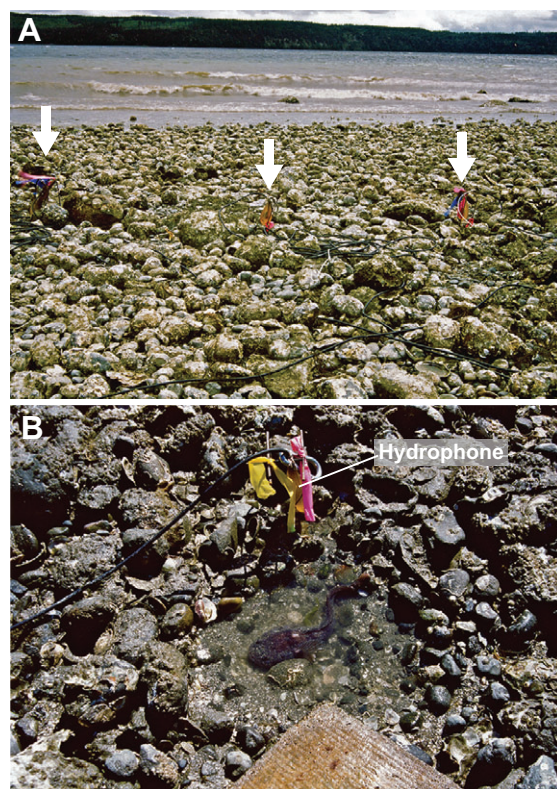


Fig. 2. Hydrophone placement at midshipman nests. Each day, a single hydrophone was suspended from an iron stake immediately adjacent to each nest during the morning low tide. Shown here is a panoramic view of three nests with hydrophones (A, white arrows), with a highlight of one nest (B) from a different morning. Each hydrophone was labeled with different combinations of flagging tape (see B; a yellow disc-shaped hydrophone is between the yellow and pink tape; also see A).

markings, e.g. with tags, it remains possible that occupancy by the same individual was not at all stable from day to day.

To obtain a robust sample size for assessing the relationship of hum F_0 to body size and temperature, we (M.A.M. and A.H.B.) recorded the hums of type I males collected from nest sites in northern California and held at the Bodega Marine Laboratory under semi-natural conditions (see Genova et al., 2012). Individual males were the sole occupants of artificial nests, and were immediately collected following sound recordings and verified as the type I morph based on gonad and swim bladder morphology following euthanasia [see Bass for review of morphological criteria (Bass, 1996)].

Grunts

Our prior study showed that grunts are produced in agonistic contexts (Bass et al., 1999; Brantley and Bass, 1994). Grunts were broadband with most energy concentrated below 500 Hz and produced either singly or serially at regular intervals as a grunt train (Fig. 3A,B). Individual grunts were a repetitive series of spike-like sound pulses (Fig. 3C). Because of their brevity, selected grunts were analyzed manually at regular intervals for PRR by dividing the time difference between the first and final pulse by the number of pulses in the grunt (which ranged from four to 15 pulses). For 26 grunt trains from three nests that ranged from two to 209 grunts (median 33), individual grunt ($N=194$) PRR and duration ranged from 81.3 to 142.8 Hz (mean \pm s.d. 112.7 ± 14.0 Hz) and from 28 to 138 ms (mean 73.8 ± 24 ms), respectively.

To show trends in temporal characteristics within a grunt train, Figs 4 and 5 illustrate a range of measures for a representative sample of 10 of the longer grunt trains varying from 30 to 209 grunts per train. As trains progressed, there was an overall increase in the duration of individual grunts (Fig. 4A). The interval between grunts, or inter-grunt interval (IGI), was fairly stable throughout most of a train, with longer intervals sometimes occurring near the beginning and towards the end (Fig. 4B). Although patterns varied, the PRR was generally higher at the beginning of a train (Fig. 4C). These trends in temporal characteristics were most pronounced in the longest trains (>150 grunts), and a comparison of the first and last 10% of measured grunts within these trains showed a significant decrease in PRR and increase in grunt duration (Mann–Whitney non-parametric t -test, $P<0.05$). The pulse period (PP), the inverse of PRR, within a given grunt became longer the later it occurred in a single grunt (Fig. 5). That is to say, each sound pulse in a grunt was generally more delayed than the previous pulse (see Fig. 5, inset). Hence, as the duration of grunts increased later in the train (Fig. 4A), there was a concomitant decrease in average PRR (Fig. 4C).

We investigated the dependence of grunt PRR on ambient temperature. Given the change in the PRR of individual grunts within a train (Fig. 4C), we assessed the average PRR across grunts in each train and tested its Pearson correlation with water temperature. A train's average PRR showed a significant positive relationship with temperature ($N=26$ trains, $R^2=0.1887$, $P=0.0266$). Individual grunts observed within a train also showed this correlation ($N=28$ grunts, $R^2=0.1866$, $P=0.0217$). Additionally, there was a weak negative relationship between average train IGI and temperature, such that the time between grunts in a train decreased with increased temperature ($N=26$ trains, $R^2=0.1767$, $P=0.0325$).

Hums

Observational and underwater playback studies show that female, type I male and type II male midshipman are attracted to hums (Brantley and Bass, 1994; McKibben and Bass, 1998; McKibben and Bass, 2001). Hums were the longest duration midshipman sound recorded, and exhibited a fairly flat envelope with a stable F_0 and a prominent harmonic stack throughout the entire duration (Fig. 6). Hums can last for more than 1 h (Ibara et al., 1983). Hum duration in the sample studied here ($N=91$ hums, nine nests) ranged nearly 1000-fold from 0.488 to 451.44 s (mean 70.11 ± 88.78 s). Because of the rapidity of sound onset and offset, start and end times were selected by visually determining the time at which the sound's amplitude above the background envelope reached $\sim 50\%$ of its maximum value. Limitations in recording technology at the time of data collection (1997) precluded recording sounds that lasted over an hour, but anecdotal reports suggest that they are not uncommon.

Measures of F_0 were taken at five time points in each hum: at the start; at the first, second, and third energy quartiles; and at the end. Start and end positions are defined above, and energy quartiles are defined as time points that divide the sound into quarters, each containing 25% of the sound's summed energy. If an overlapping growl or grunt coincided with any of these measuring points, the nearest measurable slice was taken instead. The F_0 was measured using a 750 ms spectrogram slice at each of these time points. Because of computer memory limitations, these periodic measurements were taken only on hums under 300 s in duration. For the entire sample size, hum F_0 ranged from 84.0 to 104.1 Hz (mean 96.8 ± 5.4 Hz; $N=91$ hums, nine nests). Temperature variance at recording sites throughout the night (14.24 to 16.32°C) could largely account for the F_0 range (see below). For this same sample, hum F_0

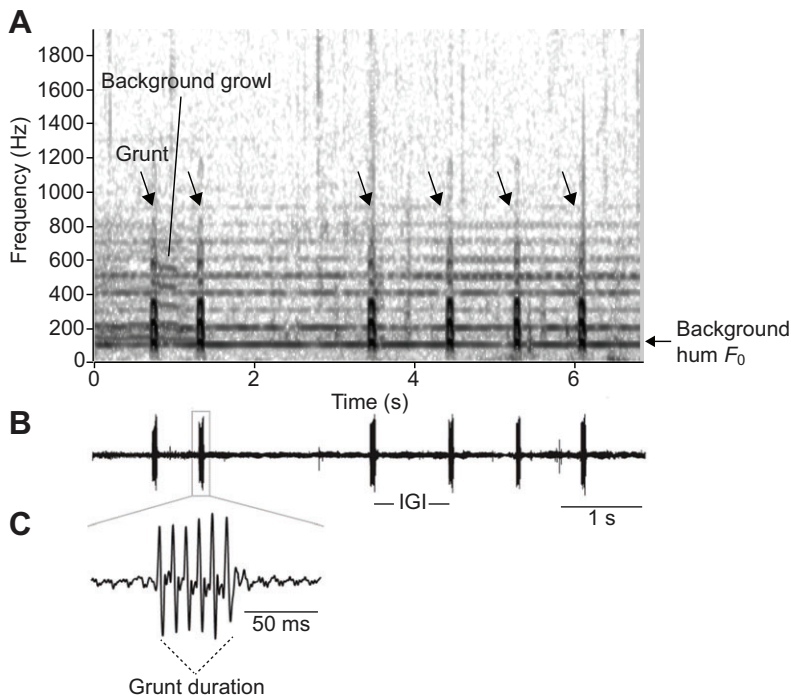


Fig. 3. Midshipman grunt trains. The spectrogram (A) and waveform (B) of the series of grunts show their broadband characteristic and inter-grunt interval (IGI) of ~0.5 to 1 s. As the IGI between the first two grunts and the preceding four is at least 2-fold the IGI of the first two grunts, the two sets were considered separate grunt trains. Also note the longer IGI for the second grunt train. Expansion of a single grunt (C) shows the typical pulsatile waveform of a grunt. As was the case in all of the field recordings analyzed, the harmonic stack from a background hum appeared throughout the recording and is apparent in the spectrogram (the fundamental frequency, F_0 , is indicated). The background hum is also apparent as a baseline oscillation in the oscillogram display in C. A background growl also overlaps the beginning of the first grunt train. For the spectrogram shown here and all subsequent figures, the following settings were used: window size=3000 samples, 3 dB filter bandwidth=21.1, overlap=75%, discrete Fourier transform (dFT) size=65,529, grid spacing=0.673 Hz. Recorded on 22–23 June 1997 at 16.15°C.

measured over the time course of this sample of individual hums was highly stable, only varying by 0–6.0 Hz (mean 1.7 ± 0.95 Hz).

Hum F_0 varied with water temperature, such that an increase in 1°C corresponded with a 5 Hz increase in F_0 ($N=24$ hums sampled

from four nests). We wanted to link hum recordings to a large sample of individually identified fish. For type I males ($N=29$) collected from nest sites in northern California and held in captivity at the Bodega Marine Laboratory (see Genova et al., 2012), we found a strong positive relationship between F_0 and temperature (linear regression, $R^2=0.9748$, $F_{1,27}=1045.607$, $P<0.001$; data not shown). For this same population, F_0 showed a negative relationship with body size ($R^2=0.29042$). However, when the effect of water temperature on F_0 was accounted for (using the residuals of the relationship of F_0 versus temperature), there was no relationship between F_0 and either body length ($R^2=0.0054$, $F_{1,27}=0.1462$, $P=0.7052$) or body mass ($R^2=0.028$, $F_{1,27}=0.7815$, $P=0.385$).

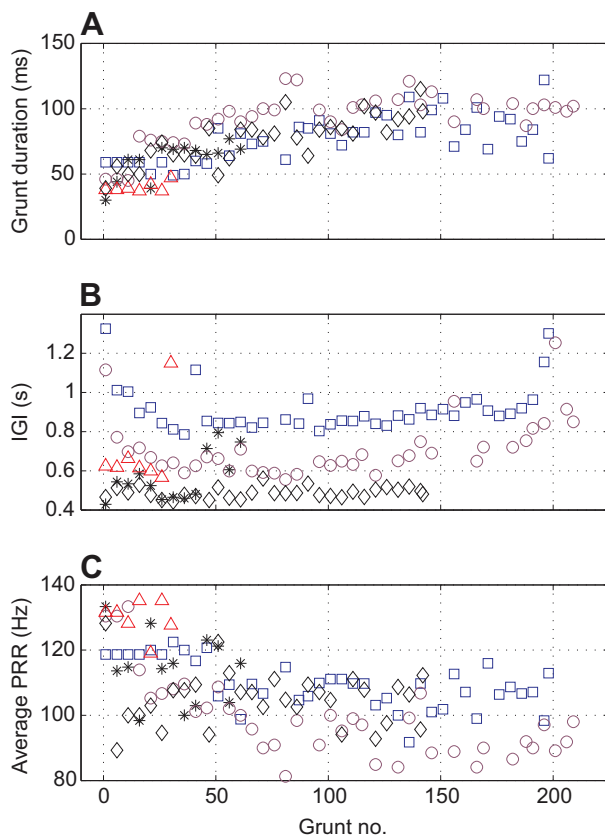


Fig. 4. Temporal characteristics of midshipman grunt trains. (A) Grunt duration. (B) IGI of grunt trains. (C) Average pulse repetition rate (PRR) of individual grunts. Symbols represent grunts from five representative trains.

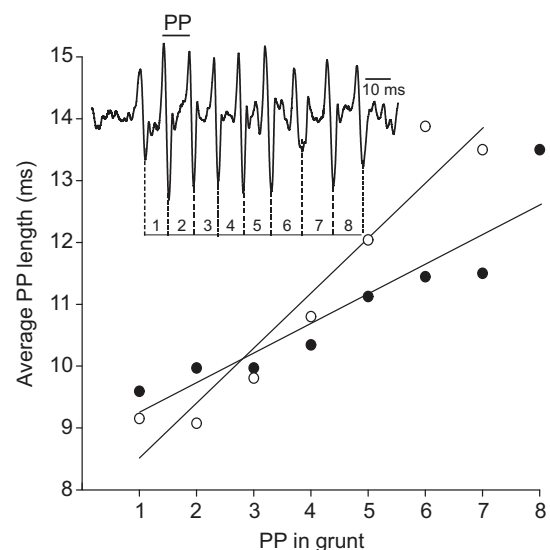


Fig. 5. Temporal characteristics of individual midshipman grunts. A plot of average pulse period (PP) duration for grunts from two grunt trains shows that pulses become farther apart over the course of the grunt. Inset illustrates a sample grunt and PP measurements. Grunt trains were recorded on 5–6 and 22–23 June 1997 at 13.78°C (open circles) and 16.15°C (filled circles).

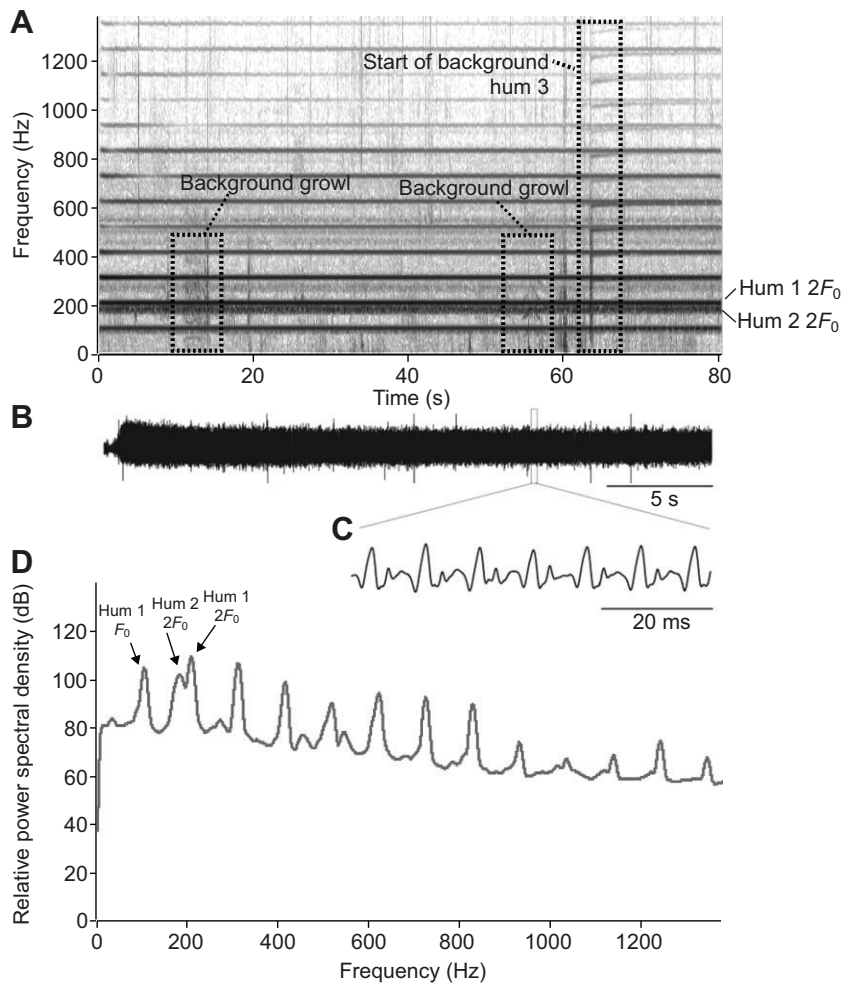


Fig. 6. Midshipman hum. Sonogram (A) and waveform (B) of a hum segment exhibiting nearly constant amplitude and fundamental frequency with a clear harmonic stack. An expansion of the hum (C) shows the uniformity of the waveform. The frequency spectrum (D) of the segment shows clear peaks at the 100 Hz F_0 and subsequent harmonics. Background growls are indicated. Second harmonics ($2F_0$) of overlapping hums that occur throughout the record are indicated. The harmonic stack of a third hum (hum 3) that begins late in the record is also indicated. Recorded on 23–24 June 1997 at 16.14°C.

Growls

Growls are the least studied in a behavioral context but appear to be made by type I males in an agonistic context (Bass et al., 1999). For example, growls were heard (by A.H.B. and M.A.M.) from the nest illustrated in Fig. 1C just prior to overturning the rocky shelter. Growls, like grunts and hums, were also produced repetitively although at more variable and longer intervals (Fig. 7). Duration was intermediate between that of individual grunts and hums, varying nearly 60-fold from 0.197 to 11.62 s (mean 2.76 ± 2.49 s) (e.g. Fig. 7). The complexity of growls (Fig. 8A) became especially apparent in spectrograms that revealed a prominent harmonic structure with abrupt frequency modulation (Fig. 8B). Closer inspection showed that growls could often be separated into initial sections of variable though higher PRR ranging from 74 to 117.1 Hz (mean 106.5 ± 6.37 Hz) (Fig. 9A,B) and a section towards the end of distinctly more variable amplitude and lower PRR ranging from 46.4 to 96.9 Hz (mean 70.9 ± 9.31 Hz) (Fig. 9C, right panel). The majority (67%) of growls analyzed exhibited this high-to-low PRR shift. The others began with a low PRR section, and many alternated back and forth between the two modes, yielding a vast range of sound variability (Figs 7–9). Background hums were always apparent in the spectrograms of growls (Figs 8, 9), as they were for grunts (Fig. 3A) and hums (Fig. 6A); similarly, background growls and grunts were also apparent in focal recordings of other sound types (Fig. 3A, Fig. 6A, Fig. 8).

AM comparisons across sound types

A cursory overview of a repetitive series of growls highlighted the wide variance in AM (Figs 7–9). We sought to develop a method

for quantifying AM complexity. By quantifying the change in amplitude as a function of time, Fig. 10 shows the change in AM over duration in representative examples of nest recordings that were divided into 100 ms time slice selections: growls (Fig. 10A–C), grunt train (Fig. 10D), isolated hum (Fig. 10E), and overlapping hums or beats (Fig. 10F) produced by nearby males (see Bass et al., 1999; Bodnar and Bass, 1997). Growl AM showed instability (Fig. 10A–C) compared with the more stable, cyclical-like change in AM observed for grunt trains and isolated hums (Fig. 10D,E and inset in 10E). Grunt trains showed the greatest magnitude of change because of the sharp rise and fall in amplitude for individual grunts (Fig. 10D). The variable AM amplitude of acoustic beats (Fig. 10F and inset) resembled that of growls.

We quantitatively compared the AM of growls with that of overlapping hums, i.e. acoustic beats, the other acoustic signal with long durations and a comparable magnitude of AM (Fig. 10A–C,F). As all natural habitat recordings included hums in the background (see above), we analyzed growls in cases where background humming was minimal compared with the signal of interest. Individual grunts and hums were not analyzed as they typically did not exhibit significant shifts in AM (Fig. 3C, Fig. 10E). We also did not subject grunt trains to this analysis as they would yield a predictable AM pattern with very high magnitude like that shown in Fig. 10D that is set by the IGI (Fig. 4B). Growls ($N=26$) and beats ($N=12$) from five midshipman nests had a mean cumulative change in amplitude of $11.9 \pm 0.7\%$ and $10.4 \pm 1.1\%$, respectively. Comparison of the cumulative amount of AM showed no significant

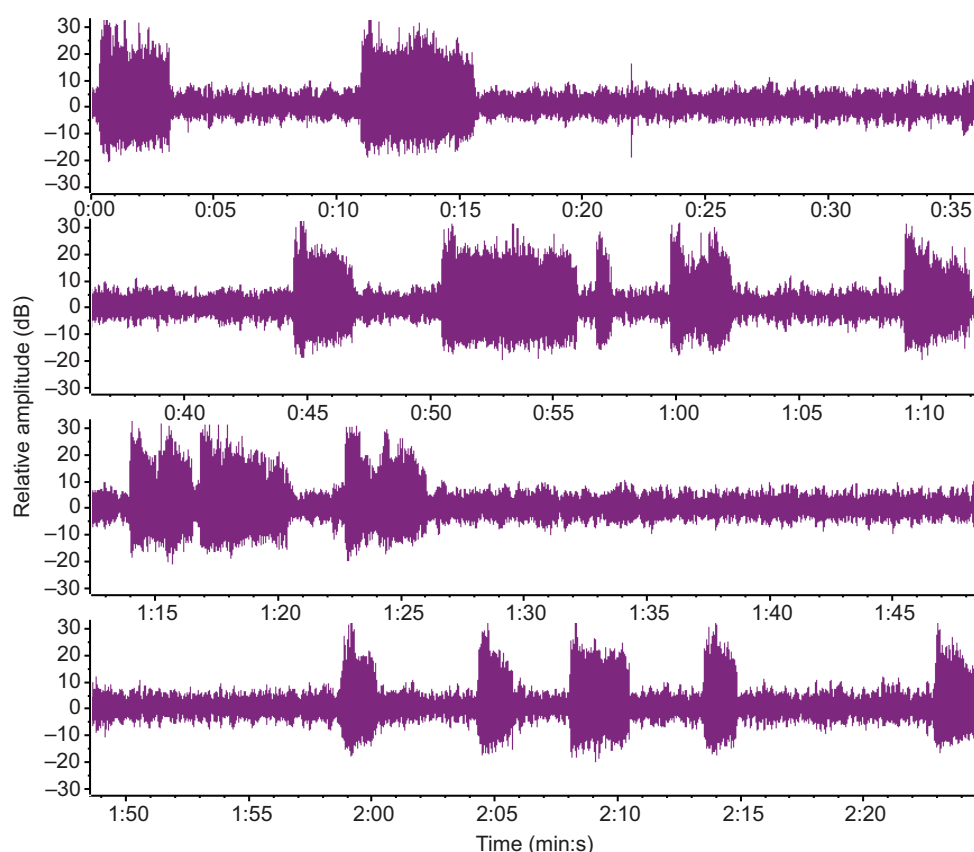


Fig. 7. Repetitive series of midshipman growls. An example waveform of a 2 min 25 s duration continuous recording from a single type I male midshipman nest showing the variability in duration, amplitude and amplitude modulation (AM) of 15 serially repeated growls. The sound was bandpass filtered from 15 to 500 Hz to remove incidental background noise from the waveform. Recorded on 22–23 June 1997 at 16.14°C.

difference between growls and beats (nested ANOVA effects test, $F_{1,36}=1.4189$, $P=0.2414$). The mean AM frequency for growls ($N=31$) and beats ($N=11$) from five nests was 9.51 ± 0.1 and 10.50 ± 0.04 Hz, respectively. There was also no significant statistical difference in AM frequency between the two sound types ($F_{6,35}=0.88$, $P=0.5190$).

Bandwidth and dominant frequency of sound types

When accounting for the nest from which the sound was recorded, grunts had a significantly larger bandwidth than either growls or hums, but growls and hums were not significantly different from each other (ANOVA, $F_{2,89}=19.669$, $P<0.0001$) (Fig. 11A). Hums had a significantly lower dominant frequency than grunts or growls, but grunts and growls were not significantly different from each other (ANOVA, $F_{2,85}=9.573$, $P<0.0002$) (Fig. 11B).

Spectrographic cross-correlation and principal coordinates (SCC-PCo) analysis of sound types

SCC-PCo analysis of the three sounds showed that the first three principal coordinates accounted for 26.47% of the overall variation in the data (PCo1: 11.49%, PCo2: 8.8%, PCo3: 6.15%). Each sound type formed distinct clusters along PCo1 and PCo2 (Fig. 12). Growls had the highest level of variability compared with hums and grunts as shown by the wide dispersion of points in the PCo scatterplot (Fig. 12). Consistent with dramatic differences in individual acoustic characters (see above), SCC-PCo revealed a clear divergence in the acoustic structure of sound types.

Soundscape

As noted earlier, background hums are always observed during focal recordings of grunts, hums and growls (Fig. 3, Fig. 6A, Fig. 8,

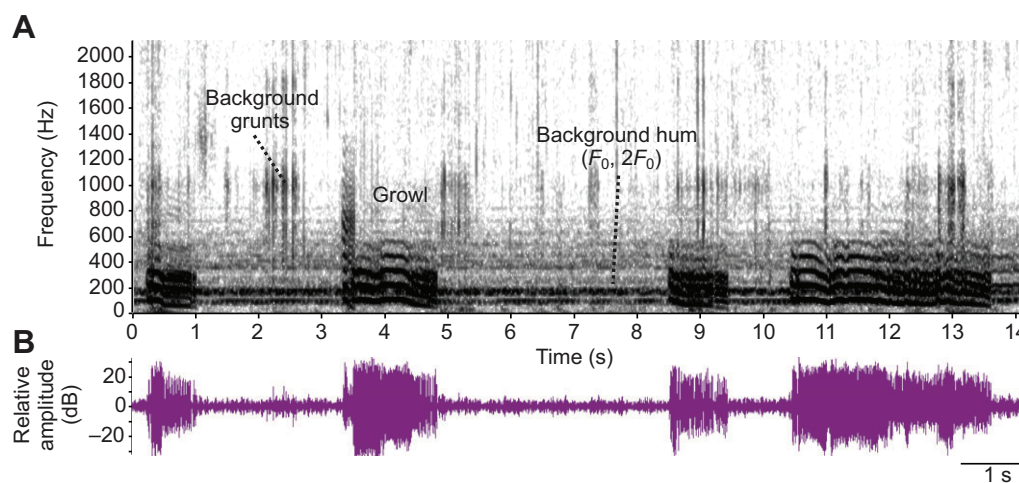


Fig. 8. Temporal and spectral characteristics of midshipman growls. Shown here is the spectrogram (A) and waveform (B) of a sequence of type I male midshipman growls. For the waveform, the sound was bandpass filtered between 15 and 1500 Hz to reduce the incidence of background noise. Background grunt train, growl and hum (F_0 , $2F_0$) are indicated. Recorded on 22–23 June 1997 at 16.29°C.

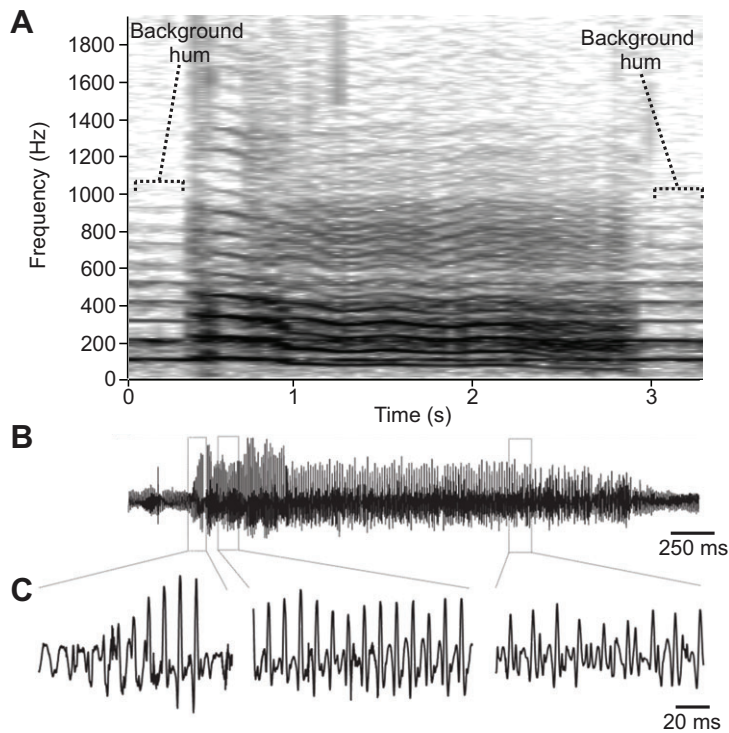


Fig. 9. Harmonic quality of midshipman growl. The spectrogram (A) shows prominent harmonics throughout a single growl exhibiting an early segment with a stable harmonic stack followed by an abrupt drop in frequency (at 1 s), and a final segment of variable frequency. In the waveform (B), the amplitude difference between the initial high-frequency (C, left and middle panels) and subsequent low-frequency (C, right panel) segments is readily apparent, as the amplitude decreases visibly with the drop in frequency. As with all analyzed field recordings, the harmonic stack of a background hum is visible in the spectrogram (arrow in A). Recorded on 22–23 June 1997 at 16.14°C.

Fig. 9). During a recording session, one is always aware of background humming the entire night (M.A.M. and A.H.B., unpublished). To visually portray this constant background humming, Fig. 13A shows a 6 h recording from a single type I male's nest. Fig. 13B,C expands segments of this recording to show the grunt and growl signaling originating from the focal nest where the hydrophone was positioned.

DISCUSSION

We show that widely divergent patterns of duration, harmonic structure and AM distinguish acoustic signals in midshipman fish. The results are significant in several regards. First, together with prior behavioral, neurobiological and neuro-hormonal studies (see Introduction), this report provides the essential, and until now missing, complement to place midshipman fish as the most comprehensively studied species of sound-producing/vocal fish. Second, the nocturnal production of sounds against a background chorus dominated without pause for hours by a single multi-harmonic sound type reveals a novel underwater soundscape. Third, building upon recent reports of the Lusitanian toadfish, *Halobatrachus didactylus* (Amorim, 2006; Vasconcelos et al., 2012), the three-spined toadfish, *Batrachomoeus trispinosus* (Rice and Bass, 2009; Rice et al., 2011), and two toadfish species from Belize, *Sanopus astrifer* and *Batrachoides gilbert* (see Mosharo and Lobel, 2012), the current study begins to reveal the breadth of intraspecific and interspecific diversity in the spectro-temporal properties of toadfish calls beyond that of species within the genus *Opsanus* that have predominated the literature for nearly six decades (see below). Fourth, together with our earlier studies of the three-spined toadfish (Rice and Bass, 2009; Rice et al., 2011), we looked in detail at the structure of individual sounds themselves. This includes an in-depth analysis of growls, the least studied but acoustically most variable in structure of midshipman sounds and perhaps toadfish sounds in general (see further discussion in the following section). Fifth, the analysis presents a quantitatively driven toolbox for the comprehensive analysis of one species' spectral and temporal

characters that can serve as a template for future comparative studies of closely related (e.g. toadfishes) and more distantly related fishes.

Acoustic repertoire of toadfishes

The earliest reports for toadfishes, showing spectrograms and oscillograms of *Opsanus tau* and *Opsanus beta* sounds, present examples of advertisement boatwhistles and agonistic grunts (Fish, 1954; Fish and Mowbray, 1970; Gray and Winn, 1961; Tavalga, 1958; Tavalga, 1960). Boatwhistles have been the most extensively reported, and along with hum and hoot analogs are now documented for eight species of toadfishes (reviewed in Amorim, 2006; Mosharo and Lobel, 2012; Rice and Bass, 2009). Grunt-like calls are typical of toadfishes and many fish species (Amorim, 2006; Bass and McKibben, 2003; Fine and Thorson, 2008; Ladich and Myrberg, 2006; Myrberg and Lugli, 2006). The longer duration grunts of midshipman and other toadfishes (Brantley and Bass, 1994; Maruska and Mensinger, 2009) (this report) resemble the croaks of Lusitanian toadfish (Amorim et al., 2006; Dos Santos et al., 2000).

Like other toadfishes, the plainfin midshipman produces several types of sounds in agonistic and courtship contexts. The vocal abilities of this species of midshipman have long been known, earning it common names such as the 'California singing fish' and 'canary bird fish' (Greene, 1924b; MacGinitie, 1935). Holder and Jordan comment on the 'musical clicking' of midshipman (Holder and Jordan, 1909). Cohen and Winn first illustrated midshipman sounds, identifying grunts and buzzes that likely correspond to the growls described in the current report (Cohen and Winn, 1967). Hubbs (Hubbs, 1920) notes the humming sound of midshipman that is later described, but not illustrated, in more detail by Ibara et al. (Ibara et al., 1983). Brantley and Bass (Brantley and Bass, 1994), followed by Lee (Lee, 1996) and Bass et al. (Bass et al., 1999), provide qualitative descriptions of midshipman sounds that include spectrograms and oscillograms of representative hum, growl and grunt sounds of type I males. The quantitative analyses presented here confirm these earlier observations, but expand upon them for a much larger and hence more representative sample size.

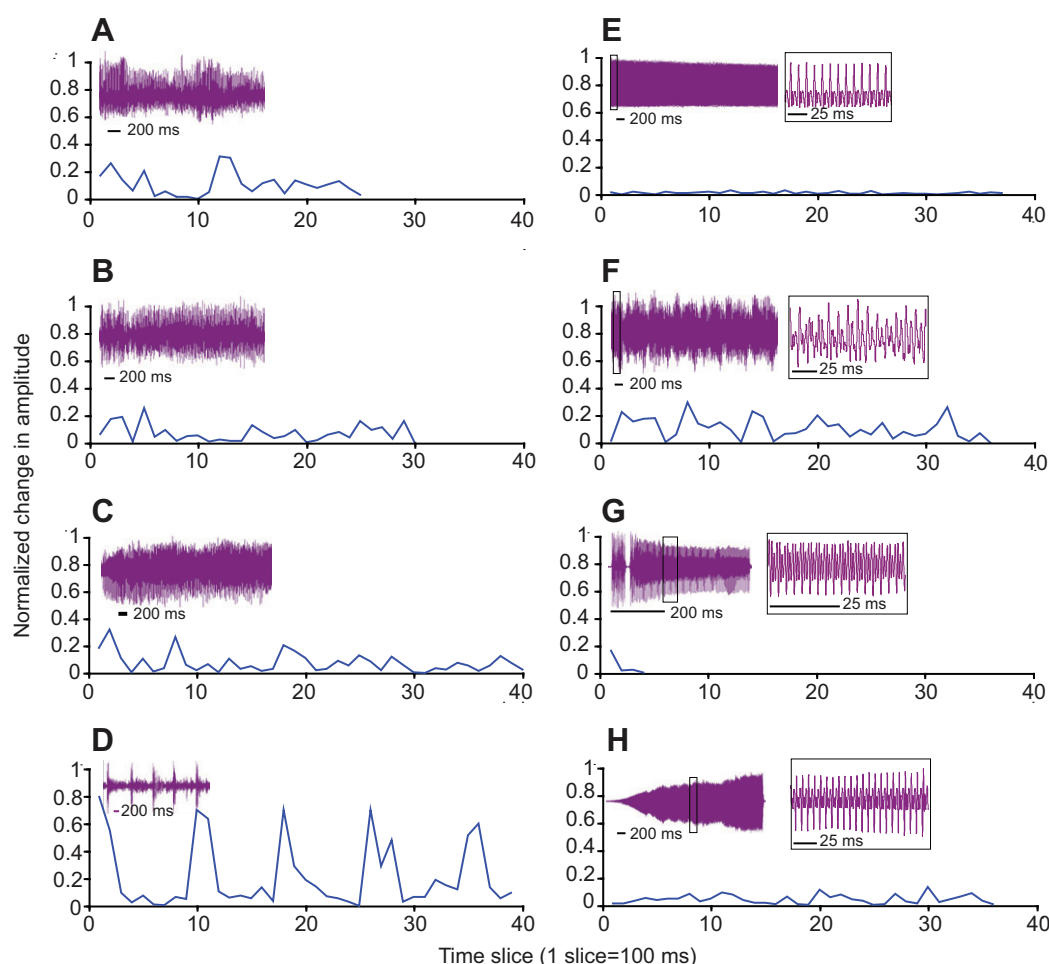


Fig. 10. Change in AM for representative sounds of midshipman and toadfish. Waveforms (purple) and normalized changes in AM (blue) from representative growls (A, nest F; B, nest J; C, nest D), grunt train (D, nest F), isolated hum (E, nest F), and two overlapping hums (acoustic beat; F, nest J) of type I male midshipman along with representative sounds from Gulf toadfish, *Opsanus beta* (boatwhistle; G) and the three-spined toadfish, *Batrachomoeus trispinosus* (hoot; H). Sounds were bandpass filtered between 15 and 1500 Hz, and divided into 100 ms time slice selections.

Growls are the most complex calls produced by midshipman, and perhaps toadfishes in general, given their variable mix of broadband energy and prominent harmonics along with variable FM and AM patterns. We are not aware of growls being explicitly described in the repertoire of other toadfishes, with one exception. Fish and Mowbray (Fish and Mowbray, 1970) note growls in the spectrograms for *O. tau*, but it is not possible to discern their structure from the illustrations, nor do later reports of *O. tau* appear to mention growls. In sharp contrast to midshipman grunts and hums, the spectro-temporal patterning of no two growls seems to be alike (Figs 7, 8).

As regards the mix of broadband and harmonic elements, growls share this character with the boatwhistles of other toadfish species that display a grunt-hoot or a grunt-hoot-grunt sequence (e.g. Edds-Walton et al., 2002; Thorson and Fine, 2002; Amorim, 2006; Mosharo and Lobel, 2012). However, distinctly unlike midshipman growls, these sequences of broadband (grunt) and harmonic (hoot) elements appear to be relatively stable for any one species. Using the AM analysis approach we developed for growls, we carried out a cursory overview of AM patterns in representative boatwhistles and hoot sounds from our prior studies of the Gulf toadfish, *O. beta* (from Ramage-Healey and Bass, 2006), and the three-spined toadfish, *B. trispinosus* (from Rice and Bass, 2009). *Opsanus beta* boatwhistles exhibit a brief grunt-like segment followed by a nearly constant PRR segment with a gradual decline in amplitude that becomes flat (Fig. 10G and inset); the early rapid decline in AM reflects the transition between its two segments. The hoots of *B. trispinosus* show a nearly constant PRR like midshipman hums and

the hum/hoot-like segment of *O. beta* boatwhistles, but variable AM like midshipman growls and beats (Fig. 10H and inset).

Together, comparative studies highlight diverse patterns of spectro-temporal complexity among the sounds of toadfishes that might contribute to the evolution of social context-dependent vocalizations. In this broader context, we next discuss each of the three main characters that together contribute to the distinct acoustic signature of each midshipman sound – duration, PRR and AM.

Sound duration

While the long duration hum of midshipman that can last for more than 1 h (Ibara et al., 1983) may be a rare acoustic character among fishes [but see Hawkins and Amorim for a 20 min hum-like signal in haddock (Hawkins and Amorim, 2000)], it draws attention to the significance of sound duration as a salient acoustic feature during social interactions. Underwater playback studies with midshipman show that females carrying mature eggs exhibit positive phonotaxis to pure tones mimicking the nearly constant F_0 and essentially flat envelope of natural hums; females that have released their eggs are not responsive to playbacks (McKibben and Bass, 1998; McKibben and Bass, 2001). Different combinations of tone duration and silent gaps between repetitive tones show gradual increases in positive phonotaxis as duration increases and/or gap duration decreases (McKibben and Bass, 2001). Male Gulf toadfish increase the duration of their calls in response to tone playbacks that mimic conspecific boatwhistles (Ramage-Healey and Bass, 2005). Male Lusitanian toadfish exhibit a positive relationship between body condition and boatwhistle duration (Amorim et al., 2010). However,

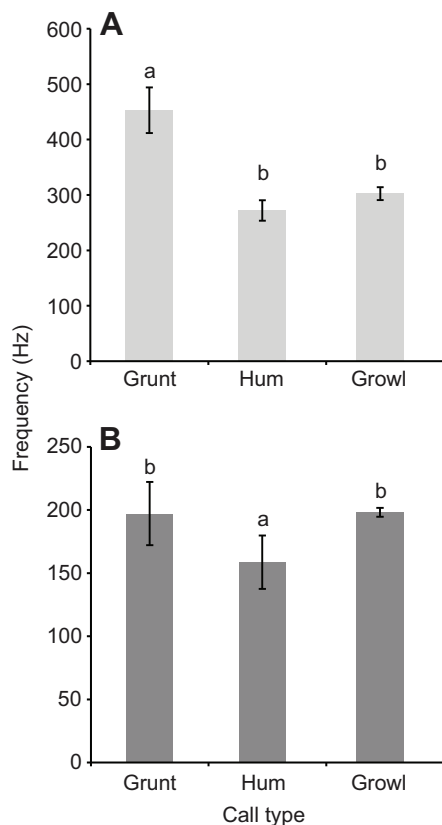


Fig. 11. Spectral characters of sound types of midshipman. Shown here is mean \pm s.e. bandwidth (A) and dominant frequency (B) of grunts, hums and growls. Different letters above call types indicate significant differences in *post hoc* analysis.

call rate and the relative amount of time spent calling, but not boatwhistle duration, are strong predictors of reproductive success in the Lusitanian toadfish (Vasconcelos et al., 2012). Duration may yet prove to be most important during male–male interactions as inferred from the playback studies with Gulf toadfish males (above).

PRR and F_0

Underwater playbacks show a wide acceptance threshold for tone PRR by gravid female midshipman; females approach a single speaker broadcasting tones that vary by as much as 20 Hz (McKibben and Bass, 1998). For a representative sample of acoustic beats produced by the overlapping hums of two neighboring males from the same population studied here, most (41 of 56) F_0 differences between the hums were below 4 Hz (Bodnar and Bass, 1997). When given the opportunity to choose between two sound sources from separate speakers, gravid females show robust tone preferences when PRR differs by 10 Hz, but they do not show a preference when PRR differs by only 5 or 2 Hz (McKibben and Bass, 1998), which is well within the range of most F_0 differences between hums (see above). After taking ambient temperature into account, we found no significant relationship between body mass and F_0 . This, together with the playback studies, makes it unlikely that small individual differences in F_0 play an important role in female choice. Like duration (see above), PRR/ F_0 may yet prove important during male–male interactions or be indicative of general reproductive state. For example, Vasconcelos et al. report lower dominant frequency for the boatwhistles of nesting toadfish males that have eggs in their nest versus those without eggs (Vasconcelos et al., 2012).

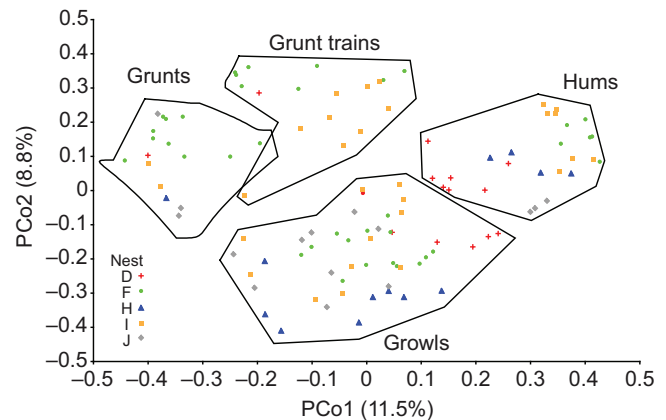


Fig. 12. Spectrographic cross-correlation and principal coordinates (SCC-PCo) analysis of midshipman sound types. Analysis of grunts, grunt trains, hums and growls produced by male midshipman from five different nests. The polygons cluster the sounds by type, and the different symbols correspond to sounds produced from within the same nest.

The increase of hum F_0 by about 5 Hz $^{\circ}\text{C}^{-1}$ matches our earlier (Brantley and Bass, 1994; McKibben and Bass, 1998) and current observations for a California population of type I male midshipman. A number of measures also showed positive relationships between temperature and the PRR of grunts, although temperature only explained a small amount of the variation. The temperature dependency of PRR has been observed for the grunts of type II male and female midshipman (Brantley and Bass, 1994) along with the sounds of other toadfish species (Amorim et al., 2006; Fine, 1978; Fine and Thorson, 2008; Maruska and Mensinger, 2009), and fishes in general (e.g. Crawford et al., 1997; Papes and Ladich, 2011). This correlation across many species is consistent with the temperature dependency of the firing rate of the central vocal pattern generator that determines PRR/ F_0 in fishes (Bass and Baker, 1991). The weak relationship between type I male grunt PRR and temperature was surprising given the strong relationship observed for the PRR/ F_0 of type I male hums (Brantley and Bass, 1994) (this report) and the grunts of type II males and females (Brantley and Bass, 1994). This may reflect a greater variance in the output of the type I male's vocal pattern generator when they are in a grunt behavioral state that might further relate to type I male growls that exhibit dramatic shifts in PRR during a single sound.

Amplitude modulation

The likely significance of AM to sound discrimination by midshipman fish first became apparent with the report of overlapping hums that lead to acoustic beats in the midshipman's acoustic habitat during the breeding season (Bass et al., 1999; Bodnar and Bass, 1997) and in phonotaxis responses to playbacks of tone-generated beats from a single underwater speaker (McKibben and Bass, 1998). Single neuron recordings from the eighth nerve and midbrain demonstrated robust encoding of AM (along with duration and PRR/ F_0), further supporting the likely role of AM in acoustic discrimination (reviewed in Bass and McKibben, 2003). Recent studies of toadfish boatwhistles draw further attention to a role for AM in acoustic discrimination tasks. Lusitanian toadfish boatwhistles of similar duration and harmonic structure, but different degrees of AM, may function as either a courtship or an agonistic signal, with agonistic calls showing less AM (Vasconcelos et al., 2011).

As noted earlier, gravid females readily choose between tones when faced with simultaneous playbacks intended to mimic

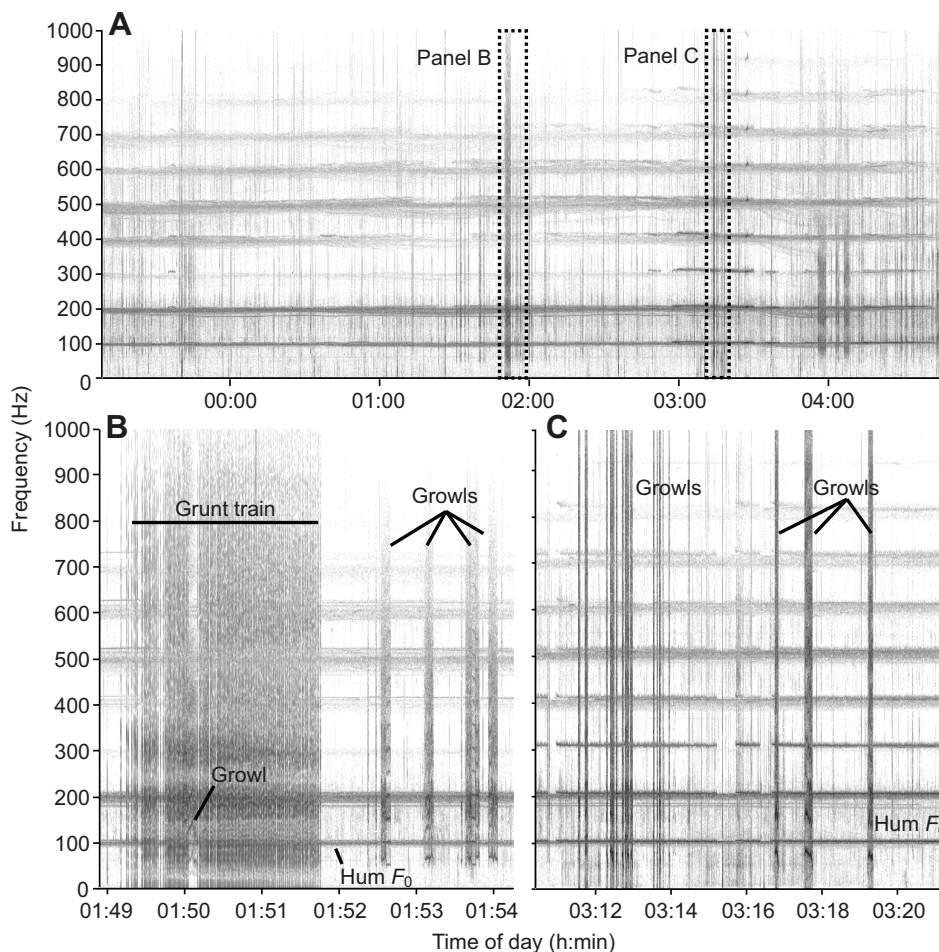


Fig. 13. Night-time soundscape of midshipman fish during the breeding season. Shown here is a continuous record of close to 6 h of sound recording from a single hydrophone during one night of the summer breeding season with the readily apparent background, multi-harmonic stack of type I male hums (A). Selected segments of this recording show grunt and growl signaling originating from the focal nest where the hydrophone was positioned (B,C).

concurrent hums originating from the nests of neighboring males (McKibben and Bass, 1998). However, beat stimuli originating from a single speaker become increasingly unattractive as they become less ‘hum-like’ with increasing AM amplitude (McKibben and Bass, 1998; McKibben and Bass, 2001). Grunt trains and growls also do not elicit positive phonotaxis (McKibben and Bass, 1998) (A.H.B., J. R. McKibben and M.A.M., unpublished observations). The shared unattractiveness of signals with prominent AM (single source beats, growls, grunt trains) versus the strong attractiveness of individual hums with an essentially flat envelope shape supports our earlier proposal that ‘perceptual limits’ in midshipman may be established by ‘generalization across modulation types and envelope shapes’ (McKibben and Bass, 2001). The results presented here reveal similar AM patterns between beats and growls. How might signals with prominent AM be distinguished from each other? At some yet to be defined distance, the concurrent hums of neighboring males may be perceived as a single source beat and hence unattractive, like the growls from one male. However, the resolution of beats into separable hums as an individual approaches the nests of neighboring males likely underlies the distinction of growls from beats (McKibben and Bass, 1998). The discrimination of grunt trains from growls might depend predominantly on the AM stability of grunt trains compared with the relative instability of growls (see Fig. 10).

Concluding comments

During the nocturnal breeding season, midshipman fish are faced with the essential listening task of distinguishing hums that advertise a male’s readiness to spawn from grunts and growls that indicate

ongoing agonistic encounters (Bass et al., 1999; Brantley and Bass, 1994). We propose for midshipman, and fishes in general, that each acoustic signal’s distinct combination of spectral and temporal characters allows for the neural-dependent discrimination of these sounds that differ in social valence. Midshipman are apparently distinct among the toadfishes so far studied in having two sound types, growls and hums, lasting of the order of seconds (and even longer for hums), but distinguished by unique combinatorial patterns of duration (up to 40-fold greater for the hums analyzed here), PRR (constant for hums, but variable for growls) and AM (essentially flat for isolated hums, but variable for growls).

Although we only analyzed individual hums with durations of up to about 450 s, they can last for 1 h or more (Ibara et al., 1983). Fig. 13 shows how the nocturnal soundscape of midshipman is dominated by humming during one evening of the breeding season for a close to 6 h recording from a single hydrophone at a type I male’s nest. Chorusing by fish has been reported for open-ocean and near-shore populations (e.g. McCauley and Cato, 2000; Mann and Grothues, 2009). Most reports describe intensity levels with some examples of individual calls dominating the chorus at intervals dispersed across the time period of the recording (Mann and Grothues, 2009; Wall et al., 2013). Advertisement-like calling throughout the evening by toadfish is well known (e.g. Thorson and Fine, 2002; Fine and Thorson, 2008; Rice and Bass, 2009; Wall et al., 2013), but like the other reports cited above showing long-term records, the calls are produced at intervals throughout an evening (see Wall et al., 2013). The midshipman soundscape adds a level of acoustic complexity that appears to be previously undocumented for

a single species of fish. Individual males call against a background that is dominated without pause for hours by one conspecific multi-harmonic sound type, hums, rather than a background of intermittent calling by one or more species (see above) or abiotic noise from either natural (e.g. Lugli, 2010) or anthropogenic (e.g. Vasconcelos et al., 2007; Popper and Hastings, 2009) sources.

Background noise can enhance the detection of auditory stimuli through a mechanism known as stochastic resonance (Jaramillo and Wiesenfeld, 1998). Recent studies of katydids show that the long-lasting background trill of one species enhances the detection of conspecific chirps by a closely related, sympatric species (Siebert et al., 2013). In the case of the midshipman soundscape, the most prominent background noise is the hum of male conspecifics. Though primary auditory afferents in midshipman show spike adaptation to tonal stimuli of up to 10 s in duration that mimic hum F_0 , action potential firing remains highly synchronized throughout the duration of the stimulus (McKibben and Bass, 1999). Duration is also robustly encoded in the midbrain auditory nucleus (Bodnar and Bass, 2001). Neurophysiological evidence further suggests that background harmonics may enhance peripheral encoding (McKibben and Bass, 2001). Given the likely stable encoding of the hum soundscape throughout the night-long chorus, it remains to be shown how this acoustic landscape might impact the encoding of acoustic signals by either a non-calling or calling individual.

MATERIALS AND METHODS

Sound recordings

During the breeding season of June 1997, acoustic activity in nests of midshipman fish on a private beach in Brinnon Bay, WA, USA, was recorded between 22:00 h and 04:00 h local time, when type I males are most actively vocalizing (Brantley and Bass, 1994; Ibara et al., 1983). Sample recordings from this same site have appeared in a qualitative context elsewhere (Bass et al., 1999; Bass and Clark, 2003; Bodnar and Bass, 1997; Lee, 1996; Rice and Bass, 2009; Rice et al., 2011). The inhabitants of each nest were weighed and measured (standard length), and identified as type I male, type II male or female on the basis of size and coloration (see Bass, 1996; Brantley and Bass, 1994). Sounds were recorded at a sampling rate of 44.1 kHz on a Digital Audio Tape-corder (Sony DAT Walkman, TCD-D8). Temperature DataLoggers (Onset Computer Corp., Pocasset, MA, USA) attached to the same stake as the hydrophone recorded the water temperature at 1 h (4–7 June) or 10 min (20–23 June) intervals.

All sounds comprising the analyses presented here are archived by the Macaulay Library of the Cornell Lab of Ornithology (macaulaylibrary.org/using-the-archive). All recordings from a single nest during one night have a single catalog number (190000–190027).

Sound analyses

Sounds were analyzed on the basis of bandwidth (the PRR/F_0 range at which 90% of the energy in the signal is contained), dominant frequency, duration, PRR/F_0 , harmonic content and AM pattern. All sounds were analyzed in Raven Pro 1.4 (Bioacoustics Research Program, 2012) using a Hann window with 50% overlap and FFT 2400 samples. Dominant frequency and bandwidth were calculated using the robust measurements in Raven Pro (Charif et al., 2008).

Recognizing the wide variance in AM, we developed a quantitative methodology for measuring and comparing the changes in the magnitude of AM. Representative growls and hums, along with representative sounds from other toadfish species, were bandpass filtered between 15 and 1500 Hz (to decrease the influence of environmental background noise), and divided into 100 ms time slice selections in Raven Pro. Using the contour of the filtered waveform envelope, the maximum amplitude was calculated for each 100 ms slice. Given the signals analyzed were recorded at an unknown distance to the hydrophone, all of the calls have different receive levels in the recording, and the source level of the call is unknown. Thus, it is inappropriate to compare amplitude values across signals using absolute

numbers. For the purposes of this analysis, the most relevant component of the signal was the pattern of AM, and not the amplitude itself. To account for differences between sounds, maximum amplitudes for each slice were normalized as follows. We identified the maximum peak in each call with the highest amplitude, and measured the other pulses in the waveform relative to the amplitude of the maximum peak. This process represented each peak in the waveform as a proportion relative to the call's maximum amplitude, and consequently rendered the patterns of AM comparable across different calls. Normalized amplitude differences were summed and divided by the number of slices in the sound to calculate the total amount of amplitude change in the sound relative to sound duration.

The frequency of AM of growls and hums was also evaluated using the 'ama' time wave AM analysis routine in the Seewave acoustic analysis package in R (Sueur et al., 2008). Sounds were analyzed using a Hilbert amplitude envelope, and a window length of 16,192 points, and the AM frequency was taken from the resulting peak in the envelope spectrum. Differences in the degree of amplitude change and AM frequency, and the associated variability (using the coefficient of variation) of these parameters between growls and hums were evaluated with a nested-ANOVA using the JMP 10 statistical package (SAS Inc., Cary, NC, USA).

Sequential, 1 h recordings covering an entire nocturnal calling period were selected for a night-long analysis. Sounds were decimated from 44.1 kHz sampling rate to a 2 kHz sampling rate (using SoX, <http://sox.sourceforge.net/>). Decimated sounds were then visualized in Raven Pro as a continuous, 6 h long spectrogram with a window size of 7000 points, discrete Fourier transform (dFT)=8192 samples and 95% overlap.

SCC-PCo analysis

Representative examples of different sounds were taken from each nest, bandpass filtered from 15 to 1500 Hz, and quantitatively compared using SCC-PCO (see Cortopassi and Bradbury, 2000; Rice and Bass, 2009). SCC was performed using the batch correlator function in Raven, with the following spectrogram settings: Hann window, window size=3000 samples, overlap 75%, dFT=4096. All sounds used in the analysis ranged from 0.1 s (grunts) to 7 s (sub-sampled hums) in duration; differences in sound duration (of the order of seconds) did not bias the cross-correlation results (data not shown). A total of 110 sounds (49 growls, 17 grunts, 18 grunt trains, 26 hums) were included in the SCC analysis resulting in 12,100 sound comparisons. The resulting output from the 12,100 correlations is a similarity matrix, consisting of the similarity score between all possible pair-wise comparisons of sounds. This matrix was converted to a distance matrix (distance=1–similarity) and analyzed with a PCo analysis using the PCoord script in the R Package (Casgrain and Legendre, 2004) following a previously used method (Cortopassi and Bradbury, 2000; Rice and Bass, 2009).

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Competing interests

The authors declare no competing financial interests.

Author contributions

A.H.B. and M.A.M. designed and conducted the experiments; E.L.M., A.N.R. and A.H.B. analyzed the data and wrote the paper.

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