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Acoustic characteristics and variations in grunt vocalizations in the oyster toadfish *Opsanus tau*

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Abstract Acoustic communication is critical for reproductive success in the oyster toadfish *Opsanus tau.* While previous studies have examined the acoustic characteristics, behavioral context, geographical variation, and seasonality of advertisement boatwhistle sound production, there is limited information on the grunt or other non-advertisement vocalizations in this species. This study continuously monitored sound production in toadfish maintained in an outdoor habitat for four months to identify and characterize grunt vocalizations, compare them with boatwhistles, and test for relationships between the incidence of grunt vocalizations, sound characteristics and environmental parameters. Oyster toadfish produced grunts in response to handling, and spontaneous single (70% of all grunts), doublet (10%), and trains of grunts (20%) throughout the May to September study period. Grunt types varied in pulse structure, duration, and frequency components, and were shorter and of lower fundamental frequency than the pulse repetition rate of boatwhistles. Higher water temperatures were correlated with a greater number of grunt emissions, higher fundamental frequencies, and shorter sound durations. The number of grunts per day was also positively correlated with daylength and maximum tidal amplitude differences (previously entrained) associated with full and new moons, thus providing the first demonstration of semilunar vocalization rhythms in the oyster toadfish. These data provide new information on the acoustic repertoire and the environmental factors correlated with sound production in the toadfish, and have important implications for seasonal acoustic communication in this model vocal fish.

Keywords Acoustic communication · Agonistic behavior · Grunt · Semilunar · Sound production · Temperature effects

Introduction

Acoustic communication is an important component of inter and intraspecific social interactions among many fishes. Batrachoidid fishes (toadfish and midshipman) produce sounds through contractions of sexually dimorphic sonic muscles attached to the swimbladder, and are some of the best studied vocal fishes (see Bass and McKibben 2003; Amorim 2006

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for reviews). At the start of the breeding season, male oyster toadfish *Opsanus tau* (Linneaus) establish a territory where they produce an advertisement boatwhistle call to attract females to the nest site (Gudger 1910; Gray and Winn 1961; Winn 1972; Fish 1972). Females then attach up to hundreds of eggs to the nest substrate where the male fertilizes them. Males are polygynous and remain at the nest to fan and guard the eggs and embryos until they are free-swimming, and may continue to produce boatwhistles and accept additional females during the parental care period (Gray and Winn 1961; Mensinger et al. 2003; Mensinger and Tubbs 2006). Only sexually mature male toadfish produce the tonal boatwhistle advertisement call, while both males and females produce shorter broadband grunts during putative agonistic interactions (Fish 1954; Gray and Winn 1961). While several studies examine the acoustic characteristics, behavioral context, geographical variation, and seasonality of advertisement boatwhistle production in the oyster toadfish (Gray and Winn 1961; Fish 1972; Fine 1978a; Edds-Walton et al. 2002), there are only limited investigations of the grunt vocalizations in this species (e.g. Fish 1954; Tavolga 1958; Fine 1978b; Barimo and Fine 1998).

Agonistic sounds in fishes are emitted in numerous contexts such as distress or disturbance situations, as well as during competition for space, food, or mates (Ladich and Myrberg 2006). In general, agonistic sounds are shorter in duration and have broadband frequency components compared to the longer duration tonal advertisement calls (e.g. boatwhistle of toadfish and hum of midshipman). Toadfish grunts have been described historically as non-harmonic pulsed sounds that are produced by both males and females in presumably agonistic contexts throughout the year (Fish 1954; Gray and Winn 1961; Winn 1967). Many soniferous species produce several different types of agonistic sounds that may provide specific information on behavioral situation or sender status and condition (Ladich 1997). However, the functional significance of these diverse sounds is often difficult to discern in the presence of concomitant visual stimuli, and therefore relatively little is known about different signal content during encounters within single species. To categorize all such sounds as “agonistic” requires behavioral and functional confirmation to justify this label. Acoustic repertoires vary among batrachoidid fishes, from as many as four putative agonistic sound types (i.e. grunt, croak, double croak, knock) described in the Lusitanian toadfish *Halobatrachus didactylus* (Bloch and Schneider) (dos Santos et al. 2000), to only a single one commonly mentioned in studies on *Opsanus* species (i.e. grunt). Most previous studies on *O. tau* concentrate on the advertisement boatwhistle call, and while early literature eludes to possible variations in grunts (including ‘incomplete boatwhistles’ and the ‘growl’ described as single grunts produced almost continuously; Winn 1972) (Fish 1954; Winn 1967; Fish 1972), there is little information on the type, structure, acoustic characteristics, and behavioral contexts of grunts in this species. Thus it is possible that the oyster toadfish produces multiple types of non-advertisement sounds, as seen in other vocal fishes (Ladich and Myrberg 2006).

Sound production in toadfish is mediated by contractions of the intrinsic sonic muscles on the swimbladder (Skoglund 1961). Thus, factors such as temperature that influence muscle contraction or pattern generator activity in the brain are predicted to modify sound characteristics (Bass and Baker 1991; McKibben and Bass 1998; Feher et al. 1998; Rome 2006). Increased temperatures were associated with an increase in the fundamental frequency of boatwhistles of oyster toadfish, while boatwhistle duration was independent of water temperature (Fine 1978a; Edds-Walton et al. 2002). A similar relationship may be expected for grunt vocalizations, but temperature or other environmental and seasonal effects on grunt production have not been examined in the toadfish.

The purpose of this study was to 1) identify and characterize the grunts produced by oyster toadfish and compare them with the advertisement boatwhistle, and 2) test for relationships between grunt vocalization characteristics and environmental parameters such as water temperature, light levels, daylength, and tidal and lunar cycles in a group of toadfish maintained in an outdoor habitat. This study identifies several different grunt vocalizations in the oyster toadfish that vary in pulse structure, duration, and frequency components. In addition, the duration and frequency of vocalizations showed negative and positive relationships to water temperature, respectively. The number of grunt vocalizations was also positively correlated with water temperature, daylength, and lunar phase over the May to September study period. These data provide new information on the acoustic repertoire and the relationships between environmental factors, vocal behavior, and sound characteristics in the toadfish, and have important implications for seasonal acoustic communication in this species.

Materials and methods

Animals and experimental setup

Sound recordings were made from a group of toadfish housed in an outdoor concrete rectangular raceway tank (1230 long x 120 wide x 150 high cm) at the Quissett campus of the Woods Hole Oceanographic Institute (WHOI), Woods Hole, MA. Freshly captured oyster toadfish *Opsanus tau* L. ($\overline{x}$standard length=26.7±4.3 SD cm; range=22 to 33 cm) were transported from holding tanks in the Marine Resources Center at the Marine Biological Laboratory and placed into 3 separate adjacent regions within the raceway tank. Region 1 was a 227 cm long x 120 cm wide area within the larger raceway tank partitioned at each end by mesh dividers and contained 9 fish (5 males; 4 females). Regions 2 and 3 were 1 x 1 x 1 m plastic mesh cages positioned within the larger raceway tank that each contained 2–3 fish (region 2: 1 male, 1 female; region 3: 1 male, 2 females). Water flow was contiguous between the regions and fish could presumably interact visually, chemically, and acoustically. Two fish died prior to the end of the study (1 male on Aug. 2; 1 female on Sept. 4), but all other fish remained active, in good health, and were feeding at the end of the experiment. Each region contained adequate concrete shelters (1 standard cinderblock per male fish) that could also serve as spawning substrates. In fact, nests with fertilized eggs were found in region 1 on June 16 and in region 3 on July 2

Toadfish were fed frozen squid or bait fish 1–2 times per week. Fresh seawater was continuously pumped into the tank and was drained via a standpipe at the opposite end. The tank was uncovered and therefore exposed to similar ambient light levels and weather conditions as the local wild population. Water depth (70 cm) and salinity (29 ± 1 ppt; measured periodically from July-Sept) remained constant throughout the study, thus there was no physical change in water depth, current direction, or hydrostatic pressure cues from tides. Daily sunrise and sunset times, lunar phase, and high and low tide heights in feet were obtained from Woods Hole tide tables (N 41° 31.4′ W 70° 40.3′) to test whether toadfish vocalizations were correlated with daylength, lunar phase or previously entrained daily tidal rhythms. Rhythms associated with lunar (i.e. cycles of approximately 29.5 days) or semilunar (14–15 days) cycles and daylength were tested by examination of the number of grunts per day, while circatidal rhythms (i.e. cycles of approximately 12.5 h) were tested by examination of the number of grunts per hour over several 72 h time periods around the full and new moons (8 times). Circatidal rhythms were examined around full and new moons because tidal amplitude differences were large and more likely to reveal a pattern if one existed. Temperature and light levels were also recorded every 15 min from May 23 to September 21, 2007 by a HOBO® Pendant Temperature/Light Data Logger (#UA-002-08; Onset Computer Corp., MA.; resolution=0.10°C) submerged in the raceway tank and positioned about 10 cm off the substrate. This fine-scale monitoring allowed the temperature and light level to be recorded within 7.5 min of each individual vocalization over the 121 day study period. All monitoring and maintenance procedures for fish used in this study were approved by the Marine Biological Laboratory Institutional Animal Care and Use Committee.

Vocalization recordings and analysis

Toadfish vocalizations were recorded continuously (24 h / day) for 121 days from May 23 to September 21, 2007 with 3 hydrophones (Brüel and Kjær; Nærum, Denmark; model #8103; sensitivity -211 dB re: 1V / µPa; frequency response 0.1 Hz-180 kHz) suspended in the tank. There were intermittent periods without acoustic monitoring due to power or equipment failures, but all were less than 24 h and represented under 5% of the total collection time. Focal behavioral observations and video-monitoring were not feasible in this study because water visibility was limited and the total time that fish emitted the short duration grunts was low and unpredictable relative to the total acoustic monitoring time. Further, vocalizing toadfish are not readily discernable from non-vocalizing toadfish especially when situated inside their shelter or in motion. A hydrophone was placed in the center of each of the 3 regions so that all fish were located ≤ 1 m from a hydrophone, and each hydrophone was separated by about 2 m. Thus an individual vocalization was often recorded on more than one hydrophone and differences in the amplitude of each vocalization on the three spatially separated hydrophones allowed an approximation of both the location and number of vocalizing fish. All sounds were amplified (DC amplifier FC-23B; World Precision Instruments, Inc.), recorded at a sampling rate of 4 kHz, digitized on a PowerLab running Chart 5 software (AD Instruments, Inc.) and stored on computer. Background noise in the tank was minimal, no other sound-producing animals were present, and artifacts due to physical displacement of the hydrophone by fish or other phenomena could be distinguished easily from vocalizations. Blood samples were also taken from all of the fish maintained at the raceway as part of a separate study (sampled ~12 pm on June 3 and 16, July 2 and 27, Aug 19, and Sept 13), and thus it is possible that there were short-term effects on vocalization behavior on these dates. However, the latency to resume vocalization after blood sampling ranged from 3 to 18 h, which was consistent with other quiescent periods during the study. This sampling also allowed the recordings of the net grunts, which were obtained by briefly holding individual fish in a dipnet underwater near the hydrophone prior to blood sampling.

The total number of vocalizations was determined from the recorded waveforms. Vocalizations were divided *a posteriori* into 5 different types based on pulse structure: 1) single grunts: an individual grunt that was not part of a train or doublet, 2) grunt trains: a series of 3 or more individual grunts in succession with either fixed or variable intervals, 3) doublet grunts: a series of 2 consecutive grunts separated by < 800 ms where the first grunt was longer than the second grunt, 4) net grunts: individual grunts produced while the fish was held within a net underwater, and 5) boatwhistles: sounds with an intital grunt-like segment 1 followed by a tonal segment 2 as described by previous studies (e.g. Gray and Winn 1961; Winn 1972; Edds-Walton et al. 2002). In addition, the following measurements were determined from the recorded waveforms on a subset of each sound type: number of grunts in a train, total grunt train duration (s), individual grunt duration (ms) (all types), and interpulse interval (ms) (doublet grunts). Subsets of each sound type were chosen by analyzing every other sound (or every 3rd sound during periods of high sound production and every sound during periods of low sound production) in the continuous recordings. However, only sounds with clear waveforms that could be distinguished easily above the background were used. Frequency components for each sound were calculated with a 1024-point fast fourier transform or FFT (Hamming window). Grunts were broadband with frequency components from ~30–500 Hz. However, since the FFT calculations of all grunt sounds showed clear multiple peaks (i.e. harmonic-like intervals), the frequency for the first two most dominant peaks was determined for each grunt (F1 = lower frequency peak and F2 = higher frequency peak). A subset of boatwhistle sounds from the same group of fish was also analyzed for comparison with grunt characteristics. For boatwhistles, the pulse repetition rate (PRR; or fundamental frequency) was measured from 20 consecutive pulses in segment 2 of the tonal portion of the call, and duration was measured from the beginning of the call when the initial segment 1 appeared above background to the end of segment 2 (see Edds-Walton et al. 2002). Sound source levels were not determined because the distance between the hydrophone and vocalizing fish was not known.

Comparison with grunt vocalizations from previous years

In order to test whether the grunt vocalizations recorded from the single group of toadfish held in the raceway habitat were representative of *O. tau*, we compared the sound types and acoustic characteristics from this population with a subset of vocalizations from other groups of oyster toadfish that were held and monitored in an outdoor pond habitat (20 x 20 x 1 m) at WHOI in the summers of 2004 and 2006.

Statistical analyses

Statistical comparisons among sound types were performed with non-parametric Mann-Whitney Rank Sum tests or Kruskal-Wallis one-way analysis of variance (ANOVA) on Ranks with subsequent Dunn's test for pairwise comparisons because data failed either the test for normality (Kolmogorov-Smirnov test) or equal variance (Levene median test). Vocalizations for these comparisons were grouped by grunt type and pooled among all vocalizing individuals. We were unable to consistently identify individual vocalizing fish from the recordings, but based on amplitude differences among the three spatially separated hydrophones, at least 8 different fish were found to produce some grunts over the study period. The percentage of each different grunt type recorded in the three tank regions was also examined separately to test whether group composition was related to the number or type of grunt vocalizations. Linear regression was used to test for relationships between sound characteristics (e.g. duration and frequency) and water temperature, while Spearman Rank Order Correlation analysis was used to test for correlations between the number of grunts produced per day and water temperature, daylength and tidal height across the study period, as well as the correlation between the number of grunt vocalizations and the number of boatwhistles per day. Mann-Whitney Rank Sum tests were also used to test for differences in acoustic characteristics between the population monitored in 2007 and populations monitored in 2004 and 2006. Statistical analyses were performed with SigmaStat 3.1 (Systat Software, Inc., San Jose, CA., USA).

Results

Temperature and light levels

Water temperature and light levels showed clear daily fluctuations across the study period. Temperatures varied by ≤ 2.5°C over a 24 h period and ranged from 14°C early in the study period (May) to over 25°C in early August. Light intensities ranged from 0 to 760 lum/ft^2 over a 24 h period, but any temporal variations were difficult to interpret due to confounding factors such as cloud cover and tank shadows. As a result, daylength (hours of daylight between sunrise and sunset) was determined from local tide charts and also used to test for correlations between daylength and vocalization behavior (see below).

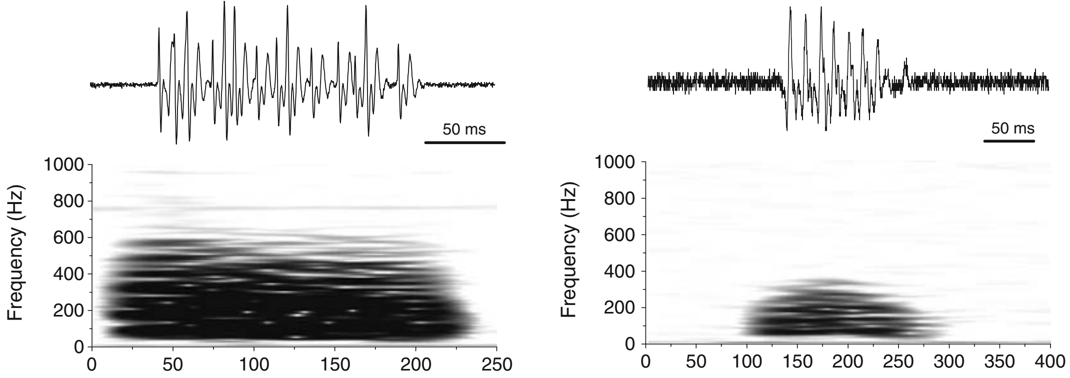
Vocalizations

A total of 3040 grunts were recorded from the group of 14 toadfish on 108 of the 121 days of the study period between May 23 and September 21, 2007. The majority of grunts (61%) were recorded from region 1 that contained 9 fish, followed by region 3 (26%; 3 fish) and region 2 (13%; 2 fish). Grunts were produced both day and night, and there was no difference in the number of grunts produced by time of day across the study period (data compared in 3 h time blocks; Kruskal-Wallis 1-way ANOVA on ranks, $p>0.05$). However, the number of grunts produced during hours of darkness (average rate=142.5 grunts/hr) was higher than during hours of light (average rate=102.4 grunts/hr) (Mann-Whitney Rank Sum test, $p=0.02$).

There was also a positive correlation between the number of grunt vocalizations and the number of boatwhistle vocalizations per day (Spearman Rank Order Correlation, $r=0.36$; $p<0.001$). Boatwhistle production was maximal at the end of June and beginning of July. Periods of boatwhistle production were accompanied primarily by grunt trains from fish within the same region as the calling individual.

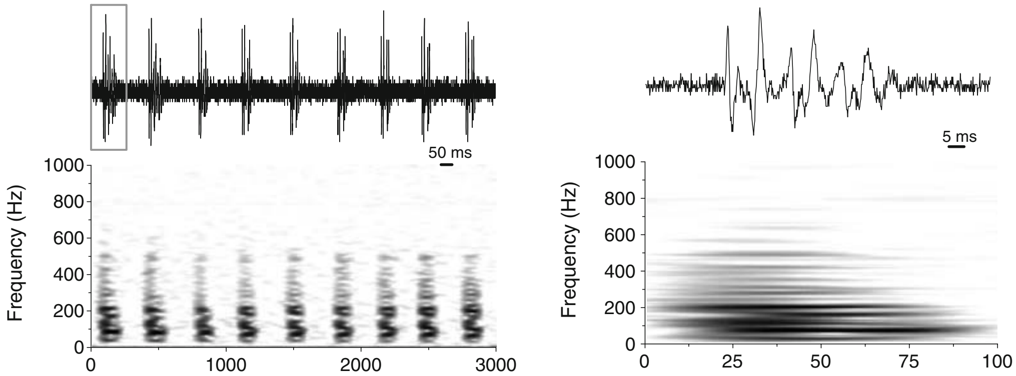
Grunts were classified into one of 4 different types based on their pulse structure: single grunts, net grunts, doublet grunts, and grunt trains (Fig. 1). A single grunt was defined as an individual grunt that was not part of a train or doublet and was the most common grunt type recorded (70% of all grunts). Single grunts had durations of 46–269 ms ($\overline{x}=111.2\pm34.3$ SD ms; $n=327$ analyzed) and broadband frequency components of ~40–250 Hz (Fig. 2). Single grunts were similar in duration to the first doublet grunt, but were longer than both the second doublet grunt and individual grunts within a train, and shorter than both net grunts and boatwhistles (Kruskal-Wallis 1-way ANOVA on ranks, $p<0.001$; Dunn's tests, $p<0.05$) (Fig. 2). Frequencies of single grunts did not differ from the first or second doublet grunts, but were higher than grunts within a train and lower than boatwhistles (Kruskal-Wallis 1-way ANOVA on ranks, $p<0.001$; Dunn's tests, $p<0.05$) (Fig. 2).

Grunt trains were a series of 3 or more individual grunts in succession, accounted for 20% of all grunts recorded, and were most common during times of boatwhistle production. Both fixed-interval and variable-interval grunt trains were produced throughout the study period. Grunt trains contained 3 to 22 grunts ($\overline{x}=6.9\pm5.4$ SD; $n=64$ trains analyzed) that lasted 0.5–13.4 sec ($\overline{x}$ train duration=4.3±3.1 SD sec) and in most cases the first grunt in a train was longer than the subsequent grunts. Individual grunts within a train were 36–249 ms in duration ($\overline{x}=81.1\pm29.7$ SD ms;



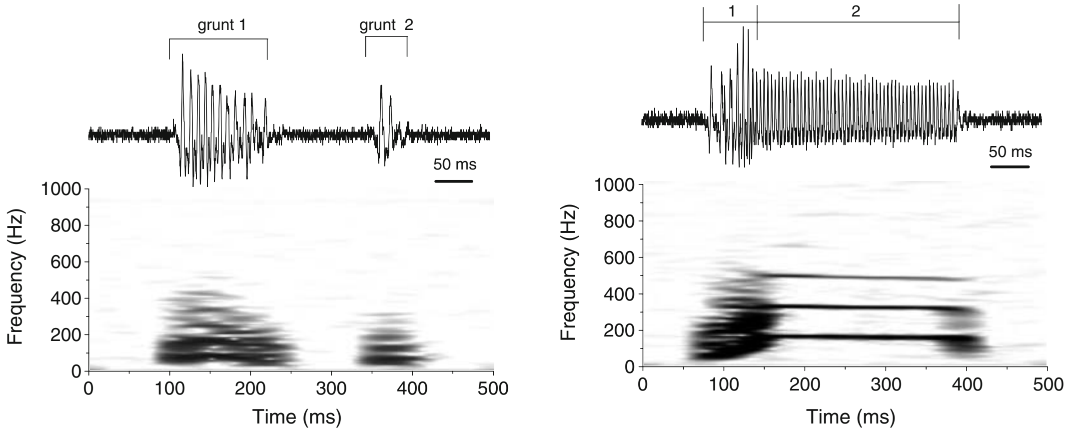
(a) Net grunt

(b) Single grunt



(c) Grunt train

(d) Individual grunt within train



(e) Doublet grunt

(f) Boatwhistle

Fig. 1 Sounds produced by the oyster toadfish *Opsanus tau*. Oscillograms (*top*) and sonograms (*bottom*) of each vocalization type are shown. **a** Net grunts were long duration broadband grunts produced during handling or restraint. **b** Single grunts were individual broadband grunts of variable duration. **c** Grunt trains were a series of individual broadband short duration grunts, with either variable or fixed-intervals (*shown*). The first grunt outlined in the gray box is expanded and shown in **d** (*individual grunt within train*). **e** Doublet grunts were an initial longer duration grunt (*grunt 1*) followed by a second shorter grunt (*grunt 2*), both with similar broadband frequency components. **f** Boatwhistles were long duration calls with an initial broadband grunt-like segment (1) followed by a tonal segment (2). Sonogram shows the pulse repetition rate at ~156 Hz and 2 harmonic intervals. Spectral display settings are Hanning window, 256 point, 100% window width

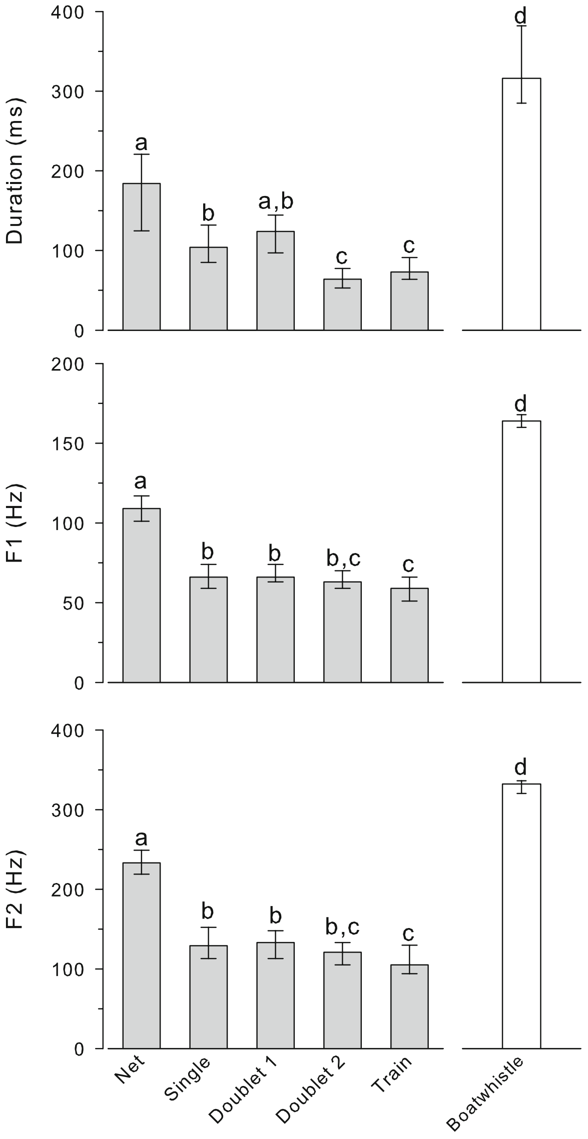


Fig. 2 Comparison of duration and frequency components of individual grunts among the different grunt types and boatwhistle vocalizations produced by the oyster toadfish *Opsanus tau*. Boatwhistles (*open bars*) were longer and of higher fundamental frequency than individual grunts among all grunt types (*gray bars*). Grunt types were also distinguished from each other based on duration, frequency components, and number of individual grunts. Data are plotted as medians (bars) and 25th and 75th quartiles (*error bars*). See text for explanation of sound types. F1 and F2 indicate the two most dominant frequency peaks from FFT analyses, where F1 is the lower frequency and F2 is the higher frequency. Bars with different letters indicate statistical differences (Kruskal-Wallis one-way ANOVA on ranks, $p<0.001$; Dunn's tests, $p<0.05$), while bars with same letters indicate no difference ($p>0.05$) [number of sounds analyzed: 80 net; 327 single; 65 doublet 1; 65 doublet 2; 242 grunts within 64 trains (*train*); 143 boatwhistle]

$n=242$ grunts in 64 trains) and had frequencies of 40–250 Hz (Figs. 1, 2). These individual grunts within a train were similar in duration and fundamental frequency to the 2nd doublet grunt, but were shorter and had lower frequency components than all other grunt types and boatwhistles (Kruskal-Wallis 1-way ANOVA on ranks, $p<0.001$; Dunn's tests, $p<0.05$) (Fig. 2).

Doublet grunts were a series of 2 adjacent grunts separated by < 800 ms ($\overline{x}$ interpulse interval=267.7±202.1 SD ms; $n=65$ analyzed) where the first grunt was longer ($\overline{x}$ duration=123.8±30.4 SD ms; $n=65$) than the second grunt ($\overline{x}$ duration=65.7±13.1 SD ms; $n=65$), but both grunts had similar frequency components (40–200 Hz) (Figs. 1, 2). Doublet grunts, which composed 10% of all grunts, were recorded throughout the study period and from multiple individuals.

Net grunts were individual grunts produced while the fish was restrained within a net underwater, and were made by both males and females in response to handling, but were not observed at any other time. Net grunts were longer ($\overline{x}$ duration=176.8±66.4 SD ms; $n=80$ analyzed) and had higher frequency components (78–277 Hz) than all other grunt types, but were shorter and had lower fundamental frequencies than boatwhistles (Kruskal-Wallis 1-way ANOVA on ranks, $p<0.001$; Dunn's tests, $p<0.05$) (Figs. 1, 2).

Single grunts, doublet grunts and trains of grunts (grunt trains) were all produced throughout the study period from late May to late September, with a peak in production during July for all types (Fig. 3). While the percentage of doublet grunts from the whole population remained relatively constant over the study period (8–13%), the percentage of single grunts decreased by ~17–40% and the percentage of grunt trains increased by ~16% from early in the season (May-June) to late in the season (Sept) (Fig. 3). When the three regions were examined separately, all grunt types were produced in each of the three regions, and the greatest percentage of each grunt type was related to the number of fish within each region (i.e. region 1 > region 3 > region 2) regardless of month. The relative percentages of each grunt type recorded from regions 1 (9 fish) and 2 (3 fish) across the May to September recording period was similar to that seen for the population as a whole (e.g. as in Fig. 3). In contrast, recordings from region 3

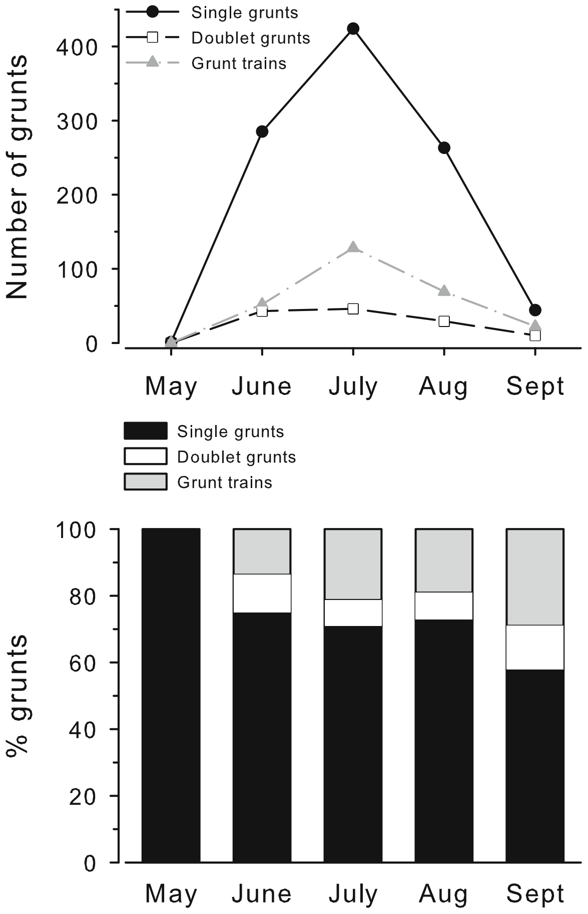


Fig. 3 Temporal distribution of different grunt types produced by oyster toadfish *Opsanus tau* across the May to September study period. Single grunts were the most common grunt type followed by trains of grunts (*grunt trains*) and doublet grunts. The temporal pattern of the total number of each grunt type per month of single grunts, doublet grunts, and grunt trains was similar, with each type showing a peak in July (*top graph*). However, while the percentage of doublet grunts remained relatively constant from June to September, the percentage of single grunts decreased and the percentage of grunt trains increased from early to late in the season (*bottom graph*)

(1 male and 1 female fish) showed a peak in the percentage of single, doublet, and trains of grunts in early July with reduced vocalizations in all preceding and subsequent months.

Inter-year comparisons

Oyster toadfish (n = at least 6 different individual vocalizing fish) maintained in pond habitats at WHOI during previous years also produced net grunts when caught or handled, and spontaneous single, doublet, and trains of grunts (both fixed and variable interval) similar to those recorded in 2007. While recordings from 2004 and 2006 showed several longer grunt trains (e.g. trains of 3–63 grunts; train durations of 4–60 s) compared to those recorded in 2007, there was no difference in duration or fundamental frequency of single, doublet, or individual grunts within trains among the years (Kruskal-Wallis 1-way ANOVA on ranks, $p>0.05$).

Relationships between environmental parameters, vocalization behavior, and sound characteristics

Vocalizations were produced across the range of temperatures measured (14–25°C) and the total number of grunts produced per day was correlated with average daily water temperature (Spearman Rank Order Correlation, $r=0.36$, $p<0.001$) (Fig. 4). In contrast, the total number of grunt vocalizations was not correlated with average daily light levels measured by the light probe positioned in the habitat (Spearman Rank Order Correlation, $p=0.98$). However, there was a positive correlation between the number of grunts per day and the total hours of daylight per day (i.e. daylength: sunrise to sunset) (Spearman Order Rank Correlation, $r=0.29$, $p=0.001$) (Fig. 4).

The total number of grunts recorded from toadfish within the outdoor raceway tank also increased during maximum tidal amplitude differences around the full and new moons (Fig. 5). When all vocalizations were standardized to a day of the lunar month, they were not uniformly distributed across the lunar cycle and showed an increase around the full and new moons (Rayleigh's test, $Z=85.8$, $p<0.001$). The number of grunt vocalizations per day was also positively correlated with maximum tidal amplitude differences associated with the full and new moons (i.e. maximum high tide minus minimum low tide) (Spearman Rank Order Correlation, $r=0.46$, $p<0.001$) (Fig. 5). This semilunar rhythm appeared to shift in phase towards the end of July (arrow in Fig. 5), such that vocalizations preceded the full moon and maximum tidal amplitude by ~5 days. In contrast to this semilunar cycle (i.e. every 14–15 days), there was no correlation between grunt vocalizations and daily tidal rhythms (i.e. associated with one tidal cycle or 12.5 h) around the full and new moons (Spearman Rank Order Correlations, $p>0.05$).

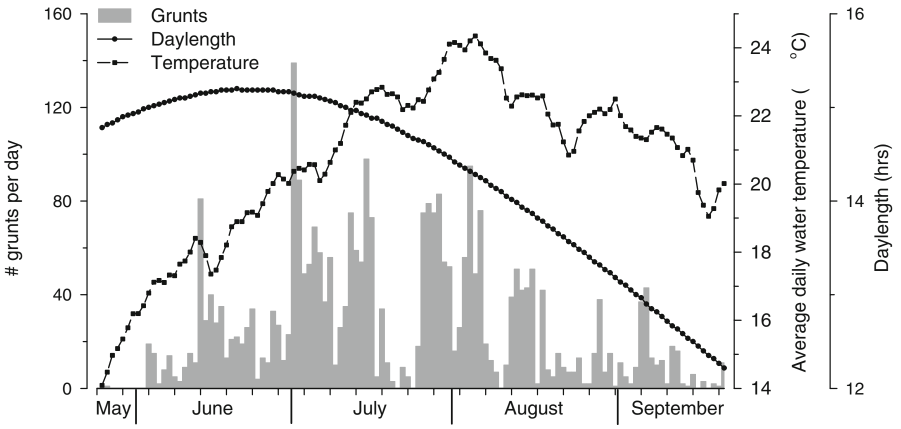


Fig. 4 Relationship among the number of toadfish grunt vocalizations, daily water temperature, and daylength from May to September 2007. The total number of grunts per day (*gray bars*) was correlated with both average daily water temperature (*black squares, dashed line*) and hours of daylight or daylength (*black circles, solid line*) over the study period (Spearman Rank Order Correlations, $p < 0.001$). Ticks on x-axis represent 5 day intervals

There was a negative relationship between duration and water temperature for net grunts, single grunts and individual grunts within a train, but not for either doublet grunt (Table 1). There was also a negative relationship between total grunt train duration and the number of grunts per train and water temperature (Linear Regressions; train duration, $p<0.001$; number of grunts per train, $p=0.006$). In contrast, there was a positive relationship between fundamental frequency and water temperature for all grunt types (Table 1).

To test whether the correlations between water temperature and grunt duration and fundamental frequency may have been related to time within the seasonal study period rather than actual water temperature, we also compared the acoustic characteristics of each grunt type (single, doublet, and individual grunts within trains) produced during two temporally separated times (July 14 –22 and August 9–17) that had similar water temperatures (22–23°C). This analysis showed there was no difference in either fundamental frequency or duration between the two time periods for any of the grunt types (Mann-Whitney Rank Sum tests, $p>0.05$).

Discussion

Distress or disturbance sounds are widespread among fishes, and although most are recorded in non-biologically relevant situations (e.g. in response to electrical shock, net restraint or handling), they may still serve a communicative function by deterring predators or warning conspecifics of predator presence (Ladich and Myrberg 2006). Toadfish net grunts differed in duration, frequency components, and structure compared to spontaneously produced grunts and boatwhistles. These net grunts also differed in fundamental frequency (*O. tau*, < 125 Hz; *H. didactylus*, ~260 Hz) and structure and duration (*O. tau*, single sound ~177 ms duration; *H. didactylus*, pair of sounds ~73 ms duration) from the handling-induced ‘knocks’ described in the Lusitanian toadfish (dos Santos et al. 2000), but this may be due in part to the difference in swim bladder morphology between these two species. Net grunts in the present study were only recorded during restraint and handling, and not during natural interactions, but the behavioral context for this sound type may have been absent (e.g. predator presence).

In addition to net grunts, oyster toadfish produced single grunts, doublet grunts, and grunt trains. The similarity in pulse structure among grunt types indicates that they are all based on a stereotyped pulse, whose temporal patterns or rate of production can be varied to produce different sounds. This is also the case for the different agonistic sounds of the Lusitanian toadfish, however their sounds have much higher frequency components (~ 250–650 Hz) compared to *O. tau* (dos Santos et al. 2000), which may be due to either swim bladder-sonic muscle morphological variations or differences in propagation of high versus low frequencies in shallow water (Fine

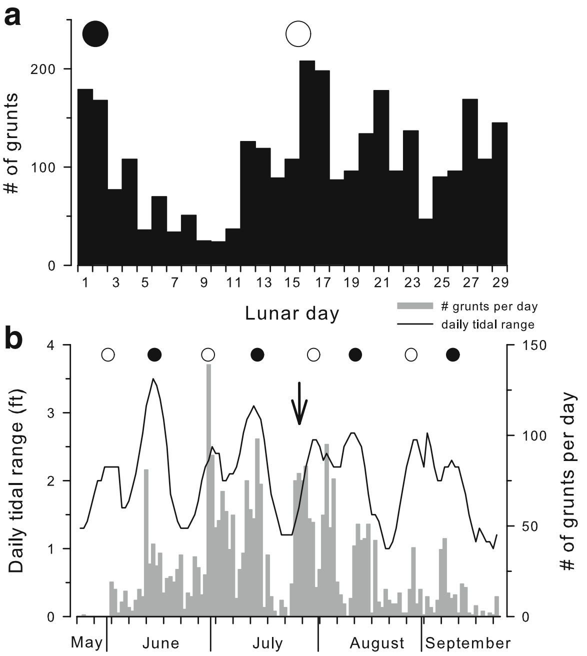


Fig. 5 Relationship between the lunar cycle and the number of grunt vocalizations produced by oyster toadfish *Opsanus tau*. **a** Total number of grunt vocalizations recorded on each day of the lunar month in the toadfish raceway tank from May to September 2007. Vocalizations were not uniformly distributed across the lunar cycle and showed an increase around the full and new moons (Rayleigh's test, $p<0.001$). The total number of grunts per lunar day was summed across four lunar cycles and is a combination of all grunt types. Day 1 is the new moon. **b** Total number of grunts per day (*gray bars*) in relation to the daily difference in tidal height (*ft*) (*solid line*) between the maximum high tide and minimum low tide from May to September 2007. Grunt vocalizations increased during periods of maximum tidal amplitude differences (Spearman Rank Order Correlation, $p<0.001$). Arrow indicates possible shift in rhythm. Ticks on x-axis represent 5 day intervals. For both **a** and **b**: filled circles = new moon, open circles = full moon

and Lenhardt 1983). While our experimental design did not allow identification of the behavioral context of different sound types, it can be assumed that all were made either spontaneously (e.g. related to some external environmental or internal physiological cue) or during intraspecific interactions because there were no other animals or predators within the habitat. A previous study in the toadfish demonstrated a high percentage of grunts produced during intraspecific interactions (e.g. nesting males almost always grunted towards active males) (Gray and Winn 1961). In the present study, the highest percentage of grunts was recorded from region 1, which was larger in size and contained 9 toadfish, including multiple large males (32–33 cm SL). Thus the greater number of individuals, space, and diversity of shelters may have increased the chance for encounters. The incidence of grunt production was also higher at night when toadfish are known to be more active (Phillips and Swears 1981), which lends support to the idea of increased encounters.

Grunts were also hypothesized to function in agonistic dominance displays in the related gulf toadfish *Opsanus beta* (Goode and Bean) (Thorson and Fine 2002). However, without behavioral confirmation of context, it is also possible that some of the sounds produced by toadfish in the present study were not agonistic. For example, Gray and Winn (1961) described an occasion where many grunts were produced by several individual oyster toadfish after an underwater explosion occurred several miles away. Toadfish grunts have been described as agonistic vocalizations in the literature for decades, but studies that experimentally demonstrate the agonistic behavioral context are limited (e.g. Gray and Winn 1961). The characterization of all non-boatwhistle vocal-

Table 1 Relationship between duration and fundamental frequency of each grunt type in *Opsanus tau* and water temperature. *n*, number of grunts analyzed; r^2, coefficient of determination

	Duration			Frequency	
	n	r^2	*p*	r^2	*p*
Single grunts	327	0.03	**0.001**	0.09	**< 0.001**
Net grunts	80	0.27	**< 0.001**	0.05	**0.04**
Doublet grunt 1	65	0.03	0.19	0.37	**< 0.001**
Doublet grunt 2	65	0.005	0.58	0.36	**< 0.001**
Grunts within trains	242	0.02	**0.04**	0.12	**< 0.001**

Bold values indicate $p<0.05$ from tests of linear regression. Relationships between duration and water temperature were negative, while those between fundamental frequency and water temperature were positive.

izations as agonistic [definition from Miriam-Webster Dictionary 2008: of, relating to, or being aggressive or defensive social interaction (as fighting, fleeing, or submitting) between individuals usually of the same species] may be premature. The grunt trains in particular have been noted numerous times throughout the day when toadfish remained in their habitats and were not physically interacting (Mensinger unpublished observations). As this is the first paper to characterize the larger repertoire of grunts in *O. tau*, further investigation of non-advertisement sounds is warranted before classifying them as agonistic.

Water temperatures can have profound effects on sound production in fishes (e.g. Bass and Baker 1991; McKibben and Bass 1998; Connaughton et al. 2000; Amorim et al. 2006; Amorim 2006). In the present study, the incidence of grunt vocalizations in *O. tau* was positively correlated with water temperature. Increased production of agonistic sounds was also associated with increased water temperatures at the start of the breeding season in the Lusitanian toadfish and may be related to changes in activity levels or behaviors associated with establishment of a territory and nest site in preparation for spawning (Amorim et al. 2006).

The pulse repetition rate or fundamental frequency of advertisement calls is directly correlated with water temperatures in all batrachoidid fishes examined to date (Fine 1978a; Bass and Baker 1991; Edds-Walton et al. 2002; Amorim et al. 2006). Higher temperatures were also associated with higher fundamental frequencies of agonistic grunts in the midshipman fish *Porichthys notatus* Girard (Brantley and Bass 1994) and Lusitanian toadfish (Amorim et al. 2006). Results from the present study show the same positive relationship for the grunt vocalizations in the oyster toadfish that appear to be independent of time within the breeding season. Both sound types (grunts and boatwhistles) are produced by intrinsic sonic muscles on the swimbladder, and the muscle contraction rate determines the sound frequency (Skoglund 1961; Fine et al. 2001; Rome 2006). Thus the variations in frequency components of both grunts and boatwhistle sounds are likely attributed to temperature effects on sonic motor neuron discharge rates and muscle physiology (Bass and Baker 1991; Feher et al. 1998; Rome 2006).

Single grunts and grunts within trains also decreased in duration with increasing temperatures. The correlation between duration and temperature is similar to that shown for sounds of other species (Connaughton et al. 2000) and indicates the sonic muscles contract with a faster velocity at higher temperatures, which is supported by the faster reuptake of Ca^{2+} by sarcoplasmic reticulum in toadfish sonic muscles at higher temperatures (Feher et al. 1998; Rome and Lindstedt 1998; Rome 2006). The differences in duration among grunt types and boatwhistles indicates that sound duration could denote the type of sound produced, providing important information on behavioral context or possibly some attribute of the callers condition or status (i.e. dominance). The relatively weak but significant relationships between water temperature and duration and fundamental frequency of grunts recorded in this study may also reflect individual variations (Edds-Walton et al. 2002; Fine and Thorson 2008).

Reproductive activities in many marine organisms, including demersal spawning fishes, occur on circalunar, semilunar or circatidal cycles, and adaptive explanations for this phenomenon include benefits to the eggs or larvae, spawning adults, or brooding males (Robertson et al. 1990; Robertson 1991). The semilunar periodicity of sound production in the toadfish has not been previously described for this species, but a similar pattern was evident from toadfish housed in the pond habitat in previous years (Mensinger unpublished observations). Sound production associated with lunar cycles is also observed in other fish species (Breder 1968). Semilunar sound production in the toadfish may result from general increased activity during full and new moons that would increase intraspecific encounters (e.g. foraging, exploratory, or reproductive behaviors). Playbacks of natural sounds were shown to facilitate vocalization behavior in listening neighbor toadfish (Winn 1967; 1972; Fish 1972; Remage-Healey and Bass 2005), and thus a few fish that start to vocalize based on some external or internal cue may induce others to follow. It is also possible that the observed increase in vocalizations around the full and new moon helps to identify individuals and synchronize the population during the breeding season. The reproductive season for northern populations of *O. tau* is generally from May through July (although it may vary from year-to-year and be temperature dependent; Gray and Winn 1961; Mensinger et al. 2003), but it is not known whether there is increased spawning activity around the full or new moon over this time period. Fluctuations in plasma steroid levels according to lunar and semilunar spawning cycles are evident in many fishes and are often correlated with gonadal recrudescence and the reproductive cycle (e.g. Rahman et al. 2000; 2001; Wang et al. 2008). Circulating steroid levels in oyster toadfish were also positively correlated with water temperature, daylength and lunar phase (Maruska et al. 2009), suggesting some relationship among vocalization behavior, reproductive physiology and behavior, and environmental parameters.

The correlation between grunt vocalizations and tidal amplitudes associated with the semilunar cycle in the absence of the physical cues of hydrostatic pressure, changing water depth, and current directions also suggests that toadfish have an endogenous semilunar rhythm or clock. Free-running rhythms under laboratory conditions have been demonstrated in several other fishes (Hsiao and Meier 1989; Cummings and Morgan 2001; Pisingan and Takemura 2007). Endogenous rhythms result from the interaction between internal clocks (timers) and external entraining agents (*zeitgebers*), and are commonly related to movement patterns, foraging activity, and reproduction in many estuarine, subtidal, and intertidal invertebrates (e.g. Palmer 1974; Barlow et al. 1986) as well as fishes (Hsiao and Meier 1989; 1992; Cummings and Morgan 2001; Pisingan and Takemura 2007). The apparent phase shift between toadfish grunts and lunar phase towards the end of July (see Fig. 5) is a phenomenon commonly seen in circalunar and semilunar organisms held under laboratory conditions because the external stimulus (or entraining cue) is not present to synchronize the internal clock, and small differences between the lunar cycle and the activity cycle result in gradual advances or delays in phase over time (Palmer 1974; Hsiao and Meier 1989; 1992).

In conclusion, these data on variations in grunt vocalizations provide new information on the acoustic repertoire and the environmental parameters related to sound production in the toadfish. Future studies are needed to identify the context and biological function of different sound types, as well as which sound characteristics (e.g. duration, frequency, number of pulses/grunts, etc.) are important cues for social interactions in this species.

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