Does the mating call of the male plainfin midshipman, *Porichthys notatus*, reflect mate quality?

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ABSTRACT

For signals in nature to be honest, they have to provide information about the sender's body condition, size, or reproductive quality. The plainfin midshipman (*Porichthys notatus*) is a model for the study of acoustic signals due to their importance in its social and reproductive behavior. In order to test whether larger males produce louder and lower frequency calls, males were recorded at night and subsequently measured. Overall, loudness of the mate call was significantly positively correlated with body size and swim bladder volume. Body size and swim bladder volume were also significantly correlated with frequency of the first five harmonics; however this correlation was positive. Loudness of the second and the third harmonic relative to the first harmonic also increase with body size and tend to be louder than or the same as the fundamental frequency in larger males. Based on preliminary data, the plainfin midshipman does seem to exhibit honest signals that provide information about the sender's size, especially in regards to amplitude.

INTRODUCTION

Vocalizations are common throughout the animal kingdom and can serve several purposes. One of its more well studied purposes is its use in the context of communication and social behavior, which include courtship and reproduction. In several species, females select mates based on condition-dependent traits of male vocalizations and mate choice decisions via sexual selection may ultimately shape these traits (Amorim et al., 2009). As a result, acoustic signals should convey honest information about the sender to help the receiver to decide and respond appropriately to the signal. Such an exchange of information is adaptive for both sender and receiver in social acoustic communication.

Vocalizations can evolve through sexual selection and these acoustic signals are said to be “honest” if they bear a cost to the sender, either because of costs to produce the vocalization or because of increased vulnerability to predation or attacks (Amorim et al., 2010). Other reliable signals cannot be faked due to physical or physiological constraints that force the signal to reveal honest information about the sender (Amorim et al., 2010). Some examples of honest signals are high calling rates, which are energetically costly to maintain and depend on the sender’s physical condition, and the fundamental frequency of the signal, which may be dependent on the size of the sender (Amorim et al., 2010).

In fish, calling activity is limited by physiological constraints, and some acoustic parameters are closely related to larger fish sizes, such as lower dominant frequency, higher amplitude, and increased pulse duration (Connaughton et al. 2000, Amorim et al., 2009). In the family Batrachoididae (midshipman and toadfishes), vocalizations are commonly used in mating behavior and agonistic displays. Signals are produced through the rhythmical vibration of the swim bladder by means of rapid contractions of the sonic muscles, which show sexual dimorphism and hypertrophy during the mating season, with males having heavier sonic muscles and a higher number of muscle fibers (Amorim et al., 2009). The use of vocalizations in mating behaviors has been extensively studied in several members of this family. Amorim et al. (2009, 2010) have shown that Lusitanian toadfish (*Halobatrachus didactylus*) mating calls may send honest signals about male quality. In one study, body length and condition were both correlated with sonic muscle mass (Amorim et al., 2009), which in a following study was shown to have a significant positive effect on call amplitude modulation (Amorim et al., 2010). In another study, McKibben and Bass (1998) showed that female plainfin midshipman (*Porichthys notatus*) (Girard 1854) preferred the mate calls of males that were more intense and longer in duration. While the relationship between male quality and call characteristics has been extensively studied in the Lusitanian toadfish, such relationships of the call characteristics and male quality in the midshipman are unknown. In the type I, or nesting, male midshipman, body condition has been observed to increase prior to the nesting period, which then peaks at the beginning of the nesting cycle followed by a gradual decline (Sisneros et al., 2009). Furthermore, Sisneros et al. (2009) found that body condition and fecundity of the type I males were positively correlated with body mass at the end of the breeding season. In order to build on this information and determine whether plainfin midshipman type I males exhibit honest signals during the breeding season, we compared different call characteristics, namely fundamental frequency and amplitude, with different aspects of male size and body condition.

METHODS

Experimental Species

For this experiment we used a highly vocal fish species, the plainfin midshipman (*Porichthys notatus*). Its range covers much of the west coast of North America, extending from northern Mexico to Canada (Walker & Rosenblatt, 1988), with some having been reported as far north as Alaska (Walker & Rosenblatt, 1988; Arora, 1948). Its habitat can extend from the intertidal zone to depths as great as 145 fathoms (Arora, 1948). While found in deeper waters throughout most of the year, the plainfin midshipman enters pools in the intertidal zone during the breeding season from late spring to early summer, with the peak in June (Arora, 1948). Parental males, also called ‘type I’ males, establish nests under rocks in the intertidal zone and emit long-duration, multi-harmonic hums to attract females (McKibben & Bass, 1998). Type I males can hum continuously for minutes up to an hour or more (McKibben & Bass, 1998). Spawning begins after a female enters the nest, and eggs are affixed to the under surface of the rock (Arora, 1948; McKibben & Bass, 1998). Soon after, the female departs the nest, returning to deeper waters, while the male remains to guard and clean the fertilized eggs until the larvae are free swimming (Arora, 1948; McKibben & Bass, 1998). Males continue to attract multiple mates during the breeding season whereas females only spawn once (McKibben & Bass, 1998). During the breeding season, most of the males are starved from continuously guarding their nest (Arora, 1948; Sisneros et al., 2009), which could lead to poorer body conditions and increased physiological costs to calling. Besides females and the type I males, there is also a ‘type II’ male morph, which utilizes sneak spawning strategies (McKibben & Bass, 1998). The type II males are similar to females in that they do not produce the hum-like mating vocalization and instead produce agonistic grunts, and they do not have large sonic muscles like the type I males (McKibben & Bass, 1998). Both males and females were collected on June 10, 2013 and June 23, 2013 from Seal Rock near Brinnon, WA, in the Hood Canal.

Experimental Setup

Type I male plainfin midshipman (N = 13; 6 calling and 7 non-calling) were set up in two indoor circular cement tanks (diameter = 2.43 m), with six males present in each tank (Fig. 1). Six ‘nests’ (20 cm x 20 cm) were set up using bricks and were evenly spaced apart within the tanks. Three to four females were added to each tank to stimulate male calling behavior. Three B&K mini-hydrophones (model 8103) were arranged to hang down from the top of the tank through a set of PVC pipes and sit directly in front of the face of three different occupied nests. The hydrophones were connected to a B&K multi-channel conditioning amplifier (model 2692) and to an oscilloscope.

Symbol Identification	
DR	Door
D	Drain
W	Water input
O1-O6	Out tank nests
I1-I6	Inner tank nests

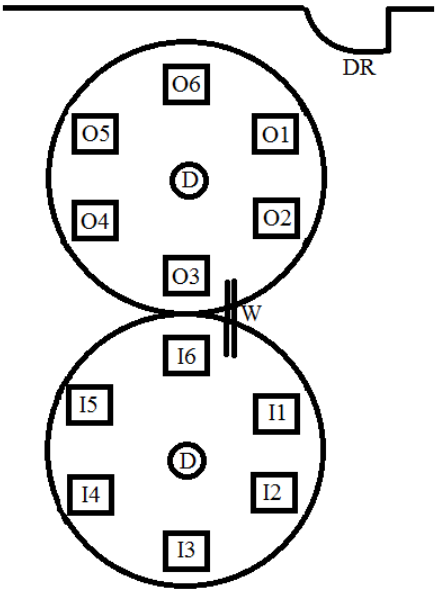


Figure 1. Diagram of experimental tank setup

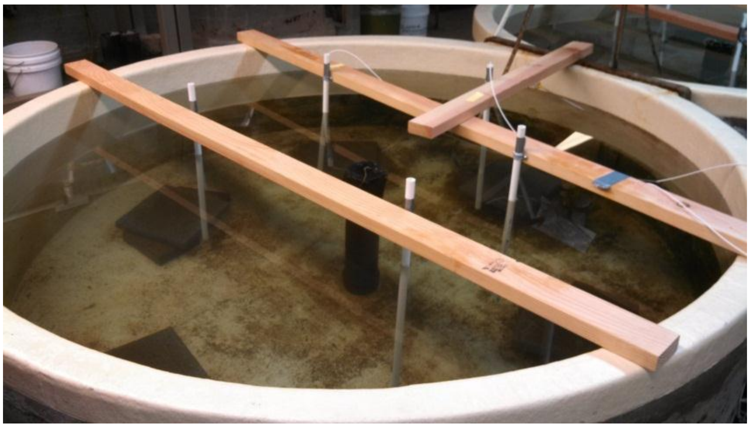


Figure 2. Photograph of experimental tank setup, specifically showing the hydrophone and male nest setup

Figure 3. From left to right: B&K multi-channel conditioning amplifier, oscilloscope, and two portable speakers. The oscilloscope was used to measure peak-to-peak amplitude, wave period, and frequency at any given time.

Experimental Protocol

In order to determine which male was calling at any particular time, each channel was separately connected to the oscilloscope and the channel with the highest amplitude was determined to be the calling male and was used for further analysis on that night of recording. Each male in the sample was recorded for up to ten minutes on a ZOOM recorder. Water flow to the tanks was turned off prior to recording. To measure peak-to-peak amplitude, wave period, and frequency, up to six different measurements were taken from the oscilloscope during the time that the male was calling. Water temperature was also measured during this time since it has been shown that water temperature has an effect on the frequency of hums preferred by female midshipman (McKibben & Bass, 1998) and may have an effect on the frequency of hums produced by males. Since amplitude and frequency varied slightly between measurements, these measurements were averaged to obtain an average peak-to-peak amplitude and average frequency for each male in the sample. After at least an hour of calling, that male would be removed from the tank and euthanized using 2 mL of benzocaine. Standard length (SL), weight, swim bladder volume, sonic muscle index, and gonadosomatic index (GSI) were measured following dissection. Weight and SL were used to calculate the condition factor (K) using the formula $K = (W/SL^3) \times 100$. The recordings were analyzed using the software program Audition (v.1.0) to determine call characteristics such as fundamental frequency, harmonic frequencies, and relative amplitudes of the different harmonics compared to the fundamental. The midshipman call is made up of a series of overlapping harmonics of increasing frequency. The Audition software provides FFT power spectra that allow us to observe which harmonics make up the majority of the call in terms of loudness. At the end of our experiment, we sacrificed and dissected the remaining males and labeled them as ‘non-callers’ to compare to the morphometric data we gathered for the males that did call over the course of the experiment.

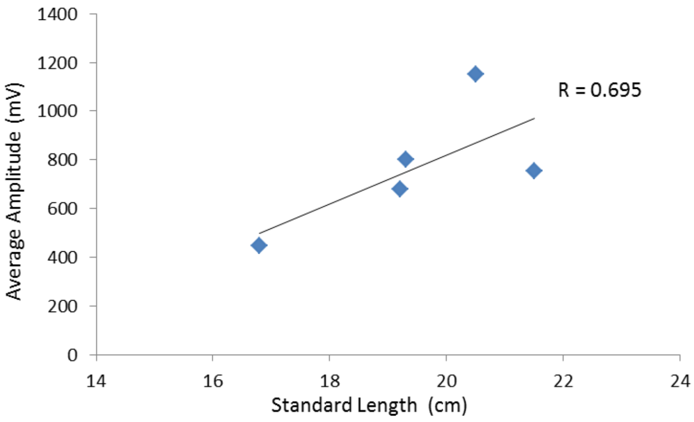
Statistical Analyses

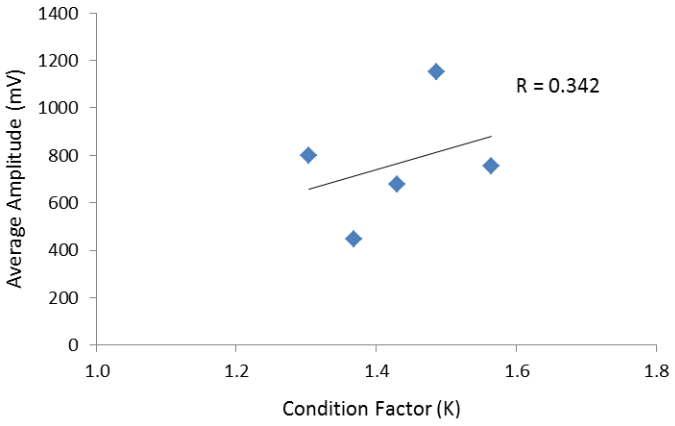
Type I male call characteristics (average peak-to-peak amplitude, fundamental frequency, harmonic frequencies, amplitude of harmonics relative to the fundamental) were tested for correlation with each of the different morphometric measurements (SL, condition factor, swim bladder volume, sonic muscle index). Correlations were tested using paired student t-tests on KaleidaGraph software (v.4.1.0), and correlations were considered significant if the p-value was less than 0.05. The correlation coefficient (R) was also recorded for each test. For non-caller versus caller comparisons, unpaired student t-tests with unequal variance (KaleidaGraph v.4.1.0) were used to compare average values for SL, condition factor, swim bladder volume, sonic muscle index, and GSI.

RESULTS

We compared five different size and condition measurements to the loudness of the hum produced (average peak-to-peak amplitude), the frequency of the harmonics produced, and the amplitude of the harmonics relative to the fundamental frequency (H_1).

The average amplitude was correlated with several of the different size and condition measurements (Fig.4). All correlations were considered significant, with $P<0.05$. The highest correlation occurred with swim bladder volume (Fig. 4, Table 1) with R = 0.738. Standard length also had a relatively high correlation coefficient, with R = 0.695. All correlations with average amplitude were positive except for sonic muscle index, which exhibited a negative correlation.





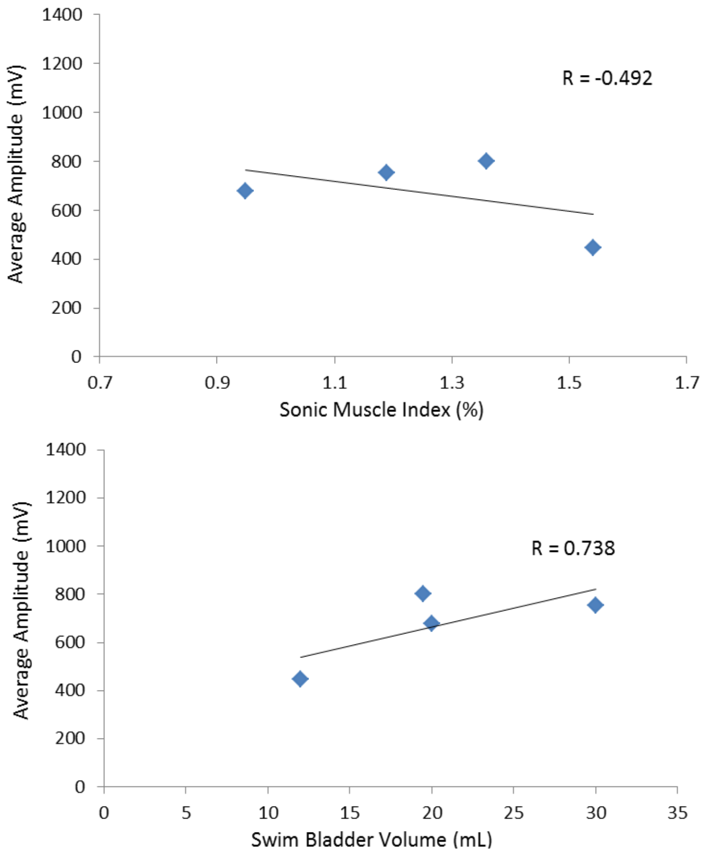


Figure 4. Relationship of average peak-to-peak amplitude with standard length (SL), condition factor (K), sonic muscle index, and swim bladder volume. All correlations were significant with P<0.05.

Table 1. Relationship between male body and call characteristics (t-test correlation)

		Average Amplitude	Frequency					Amplitude Relative to H1			
			H1	H2	H3	H4	H5	H2	H3	H4	H5
SL	R	0.695*	0.224*	0.685*	0.709*	0.665*	0.719*	0.535*	0.367*	0.514*	0.115*
	N	5	10	9	9	9	9	9	9	9	9
K	R	0.342*	0.395*	0.512*	0.499*	0.512*	0.493*	**0.507**	0.438*	0.416*	-0.056*
	N	5	10	9	9	9	9	9	9	9	9
SMI	R	-0.492*	0.036*	0.182*	0.140*	0.214*	0.133*	**-0.957**	-0.912*	-0.981*	-0.884*
	N	4	9	8	8	8	8	8	8	8	8
SBV	R	0.738*	0.179*	0.759*	0.775*	0.728*	0.771*	0.567*	0.475*	0.533*	0.043*
	N	4	9	8	8	8	8	8	8	8	8

Table 1. Significant differences are indicated by asterisks, i.e. *P<0.05; Bold values indicate non-significant differences. SL – standard length; K – condition factor; SMI – sonic muscle index; SBV – swim bladder volume.

When we looked at harmonic frequency versus water temperature, we discovered that there is a significant positive correlation between the frequency of the first five harmonics and water temperature during sampling (P<0.05). Because of this, we standardized all of our frequency values at a chosen temperature in order to analyze variation based on size and not temperature. To do this we first plotted harmonic frequency versus water temperature and generated the trend lines for each of the five harmonics (Fig. 5). Then, using the equation for the regression of trend lines, we determined what the frequency would have been at the sampled temperature based on this equation. We subtracted this value from the original frequency value we recorded in order to obtain the change in frequency (Δfreq). Then we calculated what the frequency would be if the temperature was set at 14 °C. This value was chosen because most of our recordings were taken at or around 14 °C. Finally, we added the frequency at 14 °C to the Δfreq we calculated for that specific recording, and this final value was our standardized frequency that we used for further analysis.

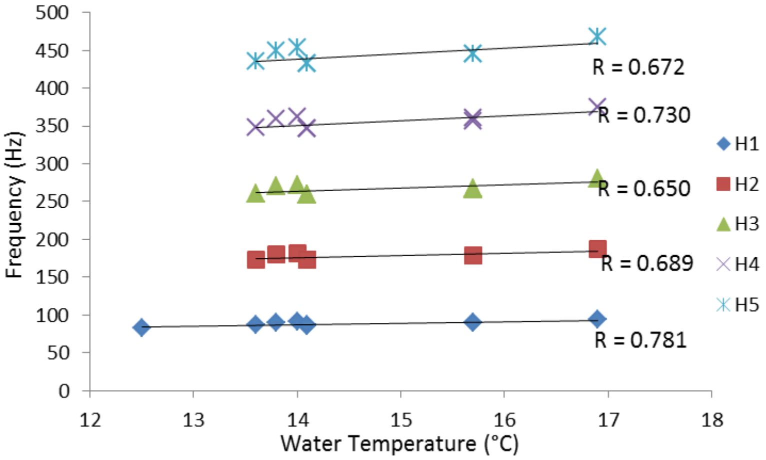
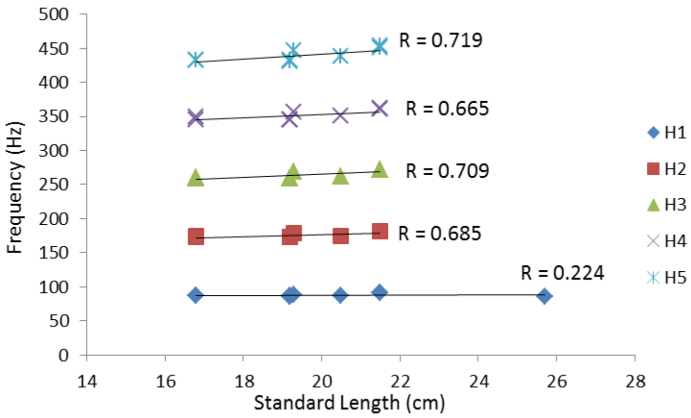
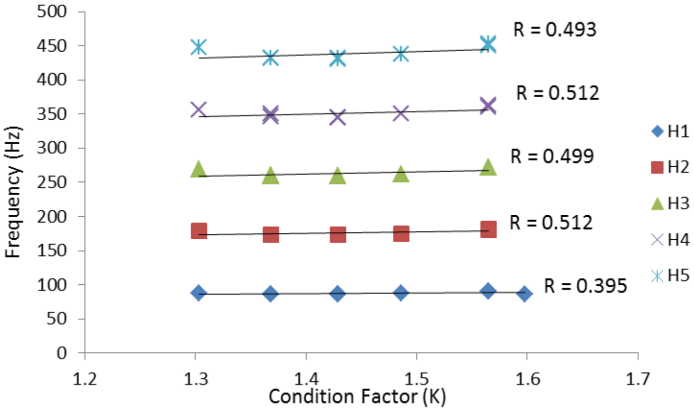


Figure 5. Frequency of the hum produced by type I males was found to be temperature dependent, increasing as water temperature increased.

For our harmonic frequency analysis, we analyzed how frequency changed with size and condition for only the first five harmonics (Fig. 6, Table 1). These harmonics are well within the determined range of hearing for female midshipman during the breeding season, which show robust temporal encoding of up to 340 Hz, therefore making them biologically relevant in this study (Sisneros & Bass, 2003). All size and condition relationships with the five harmonic frequencies were statistically significant with $P<0.05$. The relationships with the highest correlation coefficients were frequency with standard length ($R > 0.6$) and frequency with swim bladder volume ($R > 0.7$). However, the correlation coefficients for all size measurements with the first harmonic, or fundamental frequency, were relatively low (<0.4) compared to those of the higher harmonics.





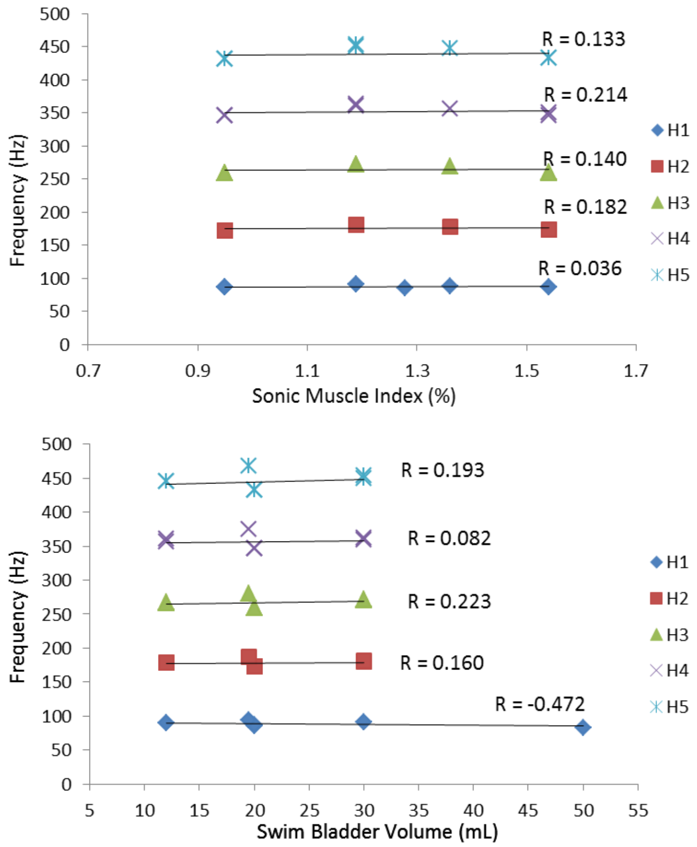


Figure 6. Relationship of harmonic frequency with standard length (SL), condition factor (K), sonic muscle index, and swim bladder volume. All correlations were significant with P<0.05.

After looking at how frequency of the first five harmonics varied with body size and body condition, we analyzed the different amplitudes of the harmonics. However, when using the Audition (v.1.0) software, the amplitudes of the different harmonics are only given in relative values that could not be compared across different recordings. In order to make those comparisons, we calculated the difference in amplitude between each harmonic and the fundamental frequency, or H_1 (Fig.7, Fig.8, Table 1). All morphometric comparisons with amplitude of the harmonics relative to H_1 were statistically significant ($P<0.05$) except in two instances: the condition factor compared to the second harmonic, and the sonic muscle index compared to the second harmonic. All relationships with amplitude relative to the fundamental were positive, except with respect to sonic muscle index for all harmonics and condition factor for the fifth harmonic only. The only high correlations are from the relationship of amplitude relative to the fundamental with sonic muscle index, which has correlation coefficients of R > -0.8 for all harmonics. What is interesting to note is, that as size increases, it is more likely for the second, and sometimes third, harmonic to be louder than the first harmonic. The third, fourth, and fifth harmonics were never louder than the first harmonic. Also, the fifth harmonic was commonly louder than the fourth harmonic in the smallest males sampled.

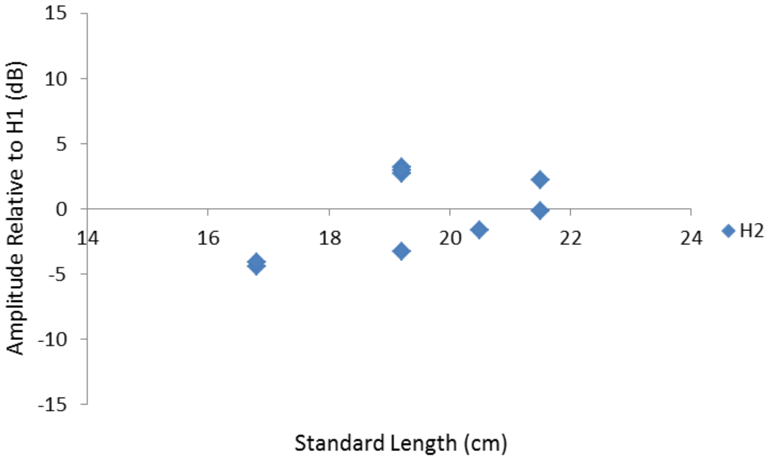
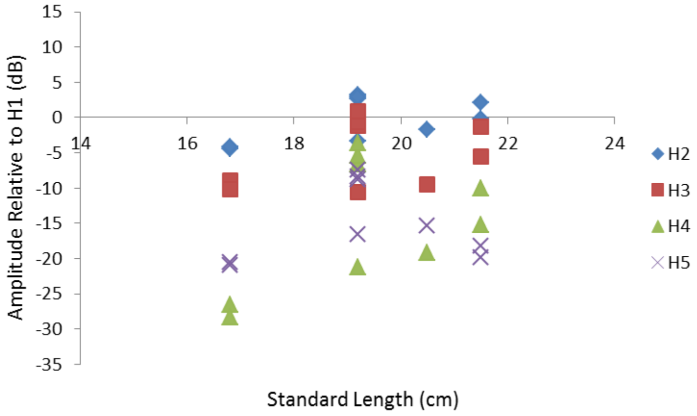
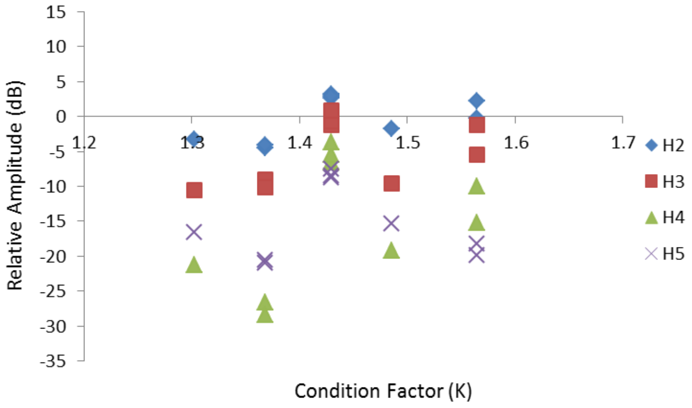
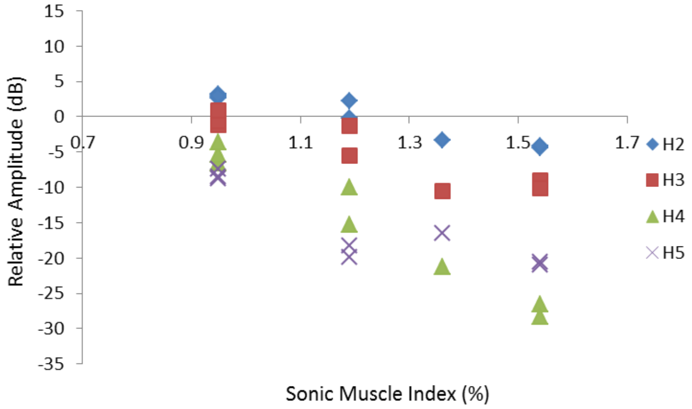


Figure 7. Scatter plot showing the relationship between standard length and the amplitude of the second harmonic relative to the fundamental frequency. What is interesting to note is, as length increases, there are more instances of the second harmonic being higher in amplitude than the fundamental frequency.


Amplitude Relative to H1 (dB)
15
10
5
0
-5
-10
-15
-20
-25
-30
-35
14
16
18
20
22
24
Standard Length (cm)
H2
H3
H4
H5


Relative Amplitude (dB)
15
10
5
0
-5
-10
-15
-20
-25
-30
-35
1.2
1.3
1.4
1.5
1.6
1.7
Condition Factor (K)
H2
H3
H4
H5


Relative Amplitude (dB)
15
10
5
0
-5
-10
-15
-20
-25
-30
-35
0.7
0.9
1.1
1.3
1.5
1.7
Sonic Muscle Index (%)
H2
H3
H4
H5

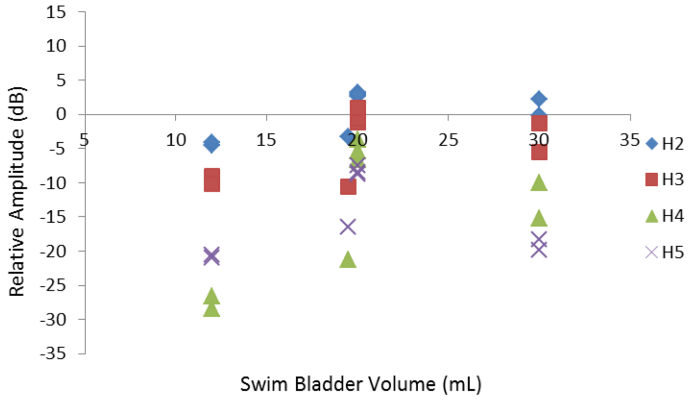
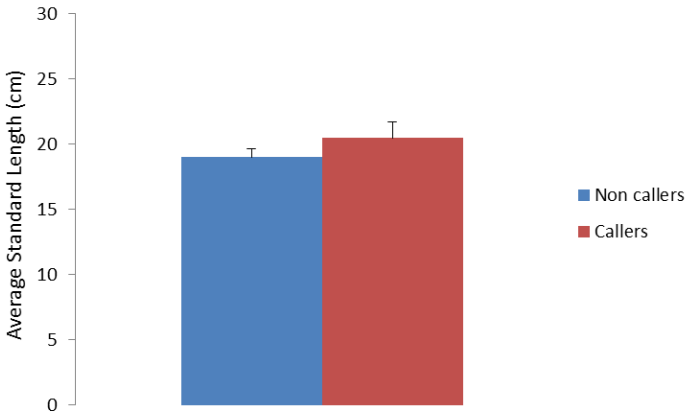
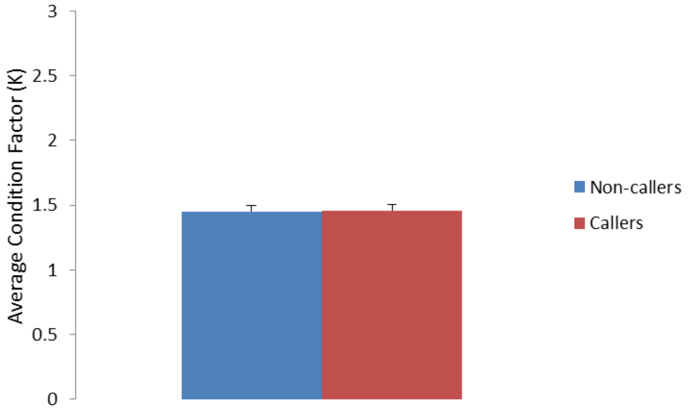
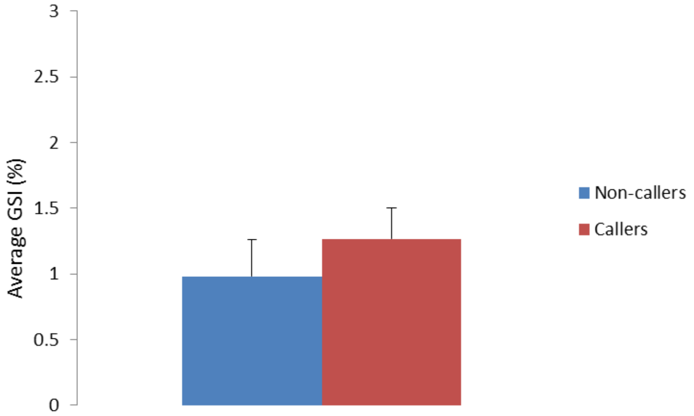


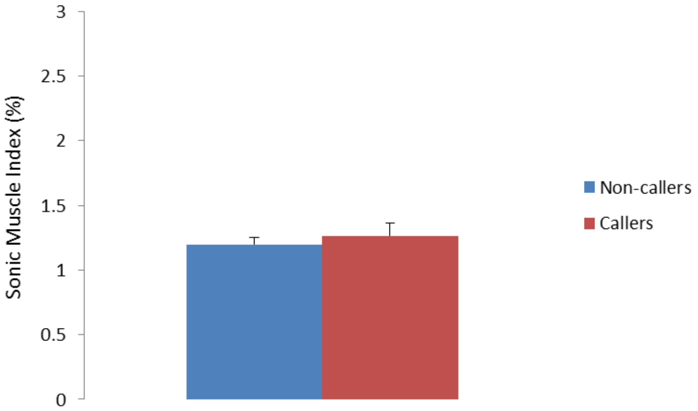
Figure 8. Plots showing the relationship between amplitude relative to H_1 and four different size and condition measurements. As size of the male increases, it is more likely for H_2, and sometimes H_3, to be louder than H_1. Also, in the smallest males, H_5 was found to be louder than H_4.

Lastly, we compared the different morphometric measurements between the males who were calling and those that were not by the end of the experiment to see if there were any differences. There were no statistical differences between non-callers and callers for any of the measurements taken, except for swim bladder volume ($P<0.05$) (Fig. 9).


Average Standard Length (cm)
30
25
20
15
10
5
0
Non callers
Callers


Average Condition Factor (K)
3
2.5
2
1.5
1
0.5
0
Non-callers
Callers


Average GSI (%)
3
2.5
2
1.5
1
0.5
0
Non-callers
Callers


Sonic Muscle Index (%)
3
2.5
2
1.5
1
0.5
0
Non-callers
Callers

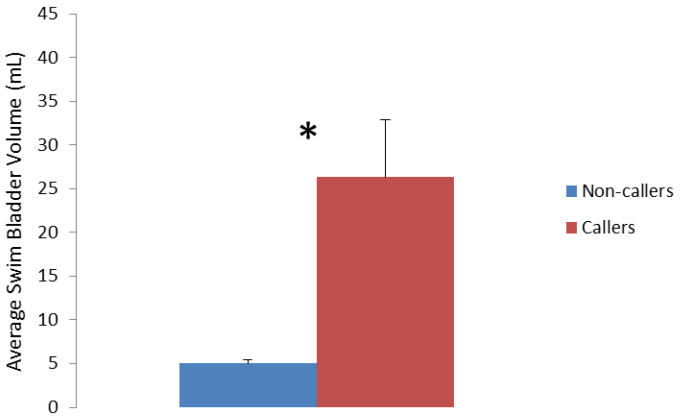


Figure 9. Differences between size characteristics in non-calling and calling males. There were no differences in morphometric measurements between non-calling males and calling males except for in average swim bladder volume, indicated by the asterisk (P<0.05).

DISCUSSION

We looked first at average peak-to-peak amplitude to characterize how different size and condition measurements of male plainfin midshipman affect the loudness of the hum they produce. While all correlations were considered statistically significant (P<0.05), standard length and swim bladder volume had the highest correlation coefficients and were therefore most correlated with average amplitude. This supports the hypothesis that larger males produce a louder hum and that females could potentially use hum amplitude as an honest indicator of male size. Males of a larger body size were more likely to produce louder calls. While larger males are also likely to have larger swim bladders, the more the swim bladder inflates, the more likely it is to change the characteristics of the call. It has been hypothesized that a more inflated swim bladder will create greater resonance and amplify or affect the frequency of the sound produced. Louder calls are likely to be important in attracting females to their respective nest, especially in the noisy intertidal environment, surrounded by other calling males.

While it’s not a strong correlation, sonic muscle index was negatively correlated with size, although it is not initially intuitive. Sisneros et al. (2009) showed that sonic muscle index is actually negatively correlated with standard length in midshipman males. This may be because sonic muscles reach a certain mass over time and stop growing while the male continues to grow to a certain size. This would result in larger males having a smaller sonic muscles index while smaller males would have a larger sonic muscle index, which leads to a negative correlation. However, sonic muscle index is not as highly correlated with average amplitude (R = -0.492), making it a poor indicator of body size through call loudness.

Once we realized that frequency of the mating hum was temperature dependent, we developed a way of standardizing all of our frequency measurements at a chosen temperature. The fact that calling frequency is actually temperature dependent has been suggested in other studies. McKibben and Bass (1998) showed that female preference for certain frequencies changed with water temperature, and that this preference also exhibited a positive correlation. Since the tanks that were used to run the experiment and house the fish were flow-through systems, it was difficult to control for temperature, making this mathematical method of standardization particularly useful for analyzing how frequency correlated with size without being affected by water temperature as well. Amorim et al. (2010) performed a similar study on the Lusitanian toadfish (*H. didactylus*) in the field and had to deal with a water temperature range much greater than the one in this study. Although their temperature ranged from 19.5-28 °C, they did not apply any correction factor in their frequency analysis. Further studies analyzing the temperature dependence of hum frequency, as well as looking at other ways of standardizing for temperature, would prove beneficial for these types of future studies.

Again, standard length and swim bladder volume were the most correlated with harmonic frequency. However, the correlation was positive, not negative, which is what we originally expected. Lower call frequency has been documented several times as an indicator of larger body size in several different species (Connaughton et al., 2000; Mager et al., 2007), but we are seeing an opposite trend in male midshipman. This could be due to several reasons. It has been shown that females have an increased sensitivity to higher frequencies in the breeding season, which has been thought to help with picking out some of the other harmonics (Sisneros & Bass, 2003). It also may help in picking up the higher fundamental frequencies we are seeing in these larger males. However, there could be discrepancies due to our standardization technique in order to account for the different sampling temperatures, as well as our small sample size. More data on a larger amount of males in a wide size range, while controlling for temperature, would be ideal to resolve this relationship. In regards to swim bladder volume, again, a more inflated swim bladder will result in resonance and may contribute to different frequencies, in this case higher frequencies.

While previous studies have analyzed the loudness and fundamental frequency of the call, few studies have focused on the different harmonics. We studied the first five harmonics since they were within the range that can be detected and localized by gravid females during the breeding season (Sisneros and Bass, 2003). Not only did we analyze the frequency of these harmonics, but we also looked at the amplitude of the harmonics relative to the fundamental frequency. The correlations for all of these relationships were rather low, and several were not even significant ($P>0.05$). However, what is interesting to note is that in some cases, the second and sometimes the third harmonic were as loud as or louder than the first harmonic. This seemed to happen more likely in larger males than smaller males (see Figs. 7 & 8), and was true regarding all four of the different size measurements. Another interesting feature is that the fifth harmonic was almost always louder than the fourth harmonic in all four size measurements. Coupled together with females' increased sensitivity to higher frequencies in the breeding season, this harmonic information could inform female midshipman about caller's size, maybe more so than just average amplitude. Sisneros and Bass (2003) suggested that honing in on these harmonics may increase the probability of mate detection and localization, especially in shallow and noisy environments. This study has built on that information in showing that these harmonics may convey honest information about sender size, and that females could potentially use this information in determining potential mates.

Lastly, we compared different measurements of size and body condition between calling males and males that did not call during the course of our experiment. There were no statistical differences between non-calling males and calling males for any of the measurements, except for swim bladder volume. However, this is likely due to how the swim bladder inflates after long periods of calling. Some of the largest males in our study called continuously for a duration of up to two hours, while others called off and on for only a total of ten minutes. However, there wasn't a statistically significant difference in size between the males in this study, so differences in size are not likely to contribute to this difference in hum duration. On the other hand, four out of the seven males that were left at the end of the study had noticeable lesions along the body. This decline in body condition may have contributed to the lack of calling we noticed near the end of our study, as well as the approach of the end of the breeding season for this species.

In conclusion, standard length and swim bladder volume are the most correlated factors with call loudness and call frequency, with larger males producing louder and higher frequency calls in this study. This data supports the hypothesis that plainfin midshipman males exhibit honest signals and that these signals are adaptive for acoustic communication. Larger males were also more likely to produce louder second and third harmonics than other males, and it may be possible that females can detect these differences in harmonics during the summer breeding season and that these harmonics are even more important than the fundamental frequency in attracting mates and providing mate choice information about the sender. Finally, since harmonic frequency seems to be dependent on water temperature, further studies into developing ways to standardize for recordings of these animals taken at different temperatures will prove beneficial, especially for studies conducted in the field in which temperature cannot be controlled.

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