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Acoustic Communication in the Atlantic Mudskipper, Periopthalmus barbarus

Seth Hoffman
Western Kentucky University, seth.hoffman600@topper.wku.edu

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ACOUSTIC COMMUNICATION IN THE ATLANTIC MUDSKIPPER, *PERIOPHTHALMUS BARBARUS*

A Capstone Experience/Thesis Project Presented in Partial Fulfillment

of the Requirements for the Degree Bachelor of Science

with Mahurin Honors College Graduate Distinction

at Western Kentucky University

By

Seth B. Hoffman

May 2021

*****

CE/T Committee:

Dr. Michael Smith, Chair

Dr. Steve Huskey

Dr. Gianluca Polgar

ABSTRACT

Mudskippers are intertidal fishes that can survive both in and out of the water. They are territorial and exhibit behaviors to attract mates and defend against competitors. Recently, it has been shown that vibrations are produced and transmitted through the mud during such displays in one species, *Periophthalmodon septemradiatus*. To see if similar vibrations are produced in other species of mudskippers, I recorded the behaviorial interactions of pairs of the mudskipper *Periophthalmus barbarus* via digital video and acoustic signals via accelerometer. Comparative analysis of fish mass, sex, contest length, and contest outcome coupled with acoustic characteristics within dyadic pairings focused on the bioacoustic patterns and behavioral context of sound production. For example, resident and larger fish almost always won contests between pairs. Audio recordings of the contests revealed a variety of charateristics in sound quality and quantity of the call characteristics. Three different types of acoustic signals were recorded- tones and grunts (with mean peak frequencies of approximately 72 Hz, and mean durations of approximately 0.3 s), and pulse trains (with a mean peak frequency of approximately 69 Hz, and a mean duration of approximately 3 s). These sounds were generally produced immediately before and after aggressive behaviors. Grunts were the most common sound type detected. Contest winners were the only ones to produce pulse trains, and these generally occurred after a contest was won (18 out of 25 pulse trains). Sound signals appear to augment visual signaling in mudskippers. Future research is needed to understand the mechanisms of producing and receiving these acoustic signals.

I dedicate this thesis to my parents, Christie and Chris Hoffman, who have been my foundation in all that I have accomplished.

ACKNOWLEDGEMENTS

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VITA

EDUCATION

Western Kentucky University, Bowling Green, KY — May 2021
B.S. in Biology – Mahurin Honors College Graduate
Honors CE/T: *Acoustic Communication in the Atlantic Mudskipper,* Periophthalmus barbarus

South Warren High School, Bowling Green, KY — May 2017

PROFESSIONAL EXPERIENCE

Immunology Lab Teaching Assistant, WKU — Jan. 2021-Present
Student Worker

Dr. Michael Smith Neurobiology Lab, WKU — May 2018-Present
Research Assistant

AWARDS & HONORS

Summa Cum Laude, WKU, May 2021
Outstanding Biodiversity Student, WKU, May 2021
KAS Zoology Student Presentation Top 3, March 2021
Presidents/ Dean’s List, WKU, Fall 2017-Spring 2021

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INTRODUCTION

Mudskippers are intertidal fishes that spend a large portion of their daily cycle out of water in soft, anoxic, mud environments in which they build reproductive burrows and exhibit a variety of intraspecific behaviors [1], [2]. Studies on these social behaviors have shown that the genus *Periophthalmus* is territorial, using various visual and acoustic signaling during interactions [3]-[5]. Acoustic signals have only been characterized in one mudskipper species, *Periophthalmus septemradiatus*, which transmits vibrations through the mud substrate through a yet undetermined mechanism [6]. As many gobioid fishes exhibit acoustic communication underwater [7], it is likely that acoustic communication through the mud is a modification of their ancestors' ability to communicate underwater. During the Devonian period, the transition of vertebrates from water to land occurred in shallow aquatic intertidal habitats of tropical deltas and flooding plains [8]-[11]. These habitats are remarkably like the environments mudskippers currently occupy making them a potential model in explaining the transition from water to land when considering the physiological characteristics of these fishes [6].

Mechanisms of general fish communication are highly variable and include drumming of the swim bladder, stridulation of skeletal elements, and tendon vibration [12]; however, many sonic mechanisms have yet to be described in an appropriate behavioral context, including that of mudskippers [6]. Lack of data on mudskipper communication could be credited to the unique low frequency signals produced by the group via vibrations through the mud, while most bioacoustics examination focuses on signals that humans can hear through the air or water [13].

The mudskipper, *Pn. septemradiatus*, produces tonal pulses and pulse train calls through the mud through an unknown physical mechanism [6]. There are many solely aquatic mechanisms of acoustic signaling in fishes with similar acoustic characteristics to that of pulse-train mudskipper calls which have been described [6]. For example, the channel catfish, *Ictalurus punctatus*, produces a pulse-train like sound using the ridges on the lateroventral surface of the dorsal process at the base of the pectoral fin spine [14]. Other species, produce single pulses instead of pulse trains. For example, the damselfish, *Chromis chromis,* is known to produce single pulse "pop" sounds through the rapid contact of teeth [15]. The structural similarity between *Pn. septemradiatus* pulses and these strictly aquatic species does not imply a similarity in the mechanism of sound production. Goby species such as *Gobius paganellus* and *Pomatoschistus pictus* have been found to produce sounds using a sonic muscle attached to bony elements of the pectoral fins, that would transmit vibrations to the water medium [16], [17]. Understanding the differences and similarities in sounds produced in strictly aquatic fish species to that of mudskippers will help describe the acoustic adaptations reserved in intertidal species from aquatic gobies.

The purpose of my research was to examine and describe potential acoustic communication in the Atlantic mudskipper, *Periophthalmus barbarus*, and to understand the behavioral contexts under which acoustic signals are produced. Digital video cameras and accelerometers were used to record territorial interactions of dyadic contest pairs of *P. barbarus*. My study focused on three areas of investigation: (i) the acoustic characteristics of the mudskipper calls including frequency and duration and how that relates to the morphological features of size, mass, and sex; (ii) the calls' behavioral context; and (iii) total number of calls and call types in relation to contest time-frame and outcome.

MATERIALS AND METHODS

Experimental Setup and Design

All protocols were compliant with guidelines established by the Institutional Animal Care and Use Committee (IACUC; Protocol 19-11) of Western Kentucky University (Animal Welfare Assurance #A3448-01). A total of 12 *P. barbarus* individuals were obtained, recorded for sex, marked for identification, and acclimated to their own individual 80 L aquarium simulating natural but less dynamic intertidal environments. Aquaria were maintained between 29-33° C and water salinity at 10 ppt. Lights cycled on and off on a 12:12 light:dark cycle. Each fish was fed a diet of live crickets, aquarium fish flakes, and blood warm larvae. Fish were maintained individually where interaction with any other individual was never initiated outside of experimentation. Experimentation was performed in the experimental tank which exhibited similar environmental characteristics as the maintenance tanks, but also featured a caged contest area for the desired dyadic interactions to record via hydrophone (GRAS Type 10CT) and accelerometers (PCB Piezotronics, Inc. Model 394C06), and through video via camera (Logitech C920 Pro Webcam). Hydrophones and accelerometers were positioned 2-3 cm below the mud surface directly below the center edge of the caged contest area while the video camera was positioned outside the tank directly above the contest area (Figure 1). The experimental tank was placed inside of a WhisperRoom, Inc. Sound Isolation Enclosure (SE 2000 series) for clearer audio recordings.

Of the 12 experimental fish (mass range of 3.7-19.1 g, size range of 7.4-12.7 cm total length, 7 males and 5 females; Table 1), an individual fish was selected to become the temporary “resident” of the experimental tank based on desired dyadic pairing (pairings determined by size and history as a “resident” or “intruder”) and allowed to acclimate and take territorial ownership of the tank. Once the resident was given at least 2-3 days to establish a territory within the tank, another individual’s mass and length was recorded, and the fish was placed in the tank within the contest area as an “intruder”. Dyadic pairs were categorized as LRG R (Intruder:Resident mass < 0.77) , LRG I (Intruder:Resident mass > 1.23), or Equal (Intruder:Resident mass > 0.77 and < 1.23). The resident, within its discretion, was then able to approach the cage and be let in via an experimenter-controlled gate to confront the intruder. This confrontation was intended to simulate a natural territorial dispute which typically results in antagonistic acoustic communication. Vibration and video recordings were initiated from the moment the intruder was placed in the cage. The audio and video recordings were then saved for later editing and analysis. All individuals were given an opportunity to behave as the resident or intruder in the experimental tanks, over the course of several days. Various size and sex combinations allowed me to analyze the effect of these variables on the contest outcome; however, no exceedingly large individual was allowed to interact with an exceedingly small individual (Tables 1, 2).

Sound/Data Editing and Analysis

Once dyadic interactions were recorded, I digitized signals at 44.1 kHz (16 bit resolution) and calls were visualized and analyzed via oscillograms, spectrograms, and power spectra using the sound analysis software Audacity (v2.4.1) and Raven Pro (v1.5.23). Audacity was used to amplify the recorded sounds to increase gain by +36 dB consistently. Temporal features were measured from oscillograms and frequency parameters were obtained by power spectra (3 dB filter bandwidth 248 Hz, FFT size 256 points, time overlap 50%, and a Hanning, low pass window below 800 Hz). The new amplified audio files were then synchronized with the existing video files (utilizing VideoPad video editing software to align amplified audio to original video via audio-visual cues such as tapping on the experimental tank) to allow for analysis of the behavioral context of each acoustic signal as well as to identify the caller ("resident" or "intruder") of any signal. Calls were assigned to intruder or resident based on behavioral context (dorsal display, nudging, attacking before or subsequently, etc.) and visual evidence of vibration production via rapid vibration-propagated oscillations of the body.

The edited videos were then observed to find the acoustic signals within the timeline of the dyadic contest, with notes on the behavioral context and outcome. A preliminary analysis of these signals was conducted at normal speed in Audacity. Three different types of acoustic signal were described: grunts, tones, and pulse trains. Grunts were typically more broadband than tones, and were not frequency-modulated, having an intermediate structure between tones and single pulses (Figure 2A). Tones were typically more narrowband than grunts, and were frequently frequency- and amplitude-modulated (Figure 2B). Pulse trains were made of a rapid succession of pulses; the trains had a variable duration of a few seconds (Figure 2C). Peak frequency and duration of the calls were also recorded (duration measured as the time elapsed from start to finish of a single vibration emission). Measured fish variables include size [standard length SL (cm, to the nearest mm), total length TL (cm)], mass (g, to the nearest 0.1 g), and sex (male/female). Acoustic variables were measured in Audactiy; they include sound type (grunt, tone, or pulse train), peak frequency (Hz), and duration. Measured "context variables" include gender pairing (male/male, female/female, or male/female combinations), contest winner (resident or intruder), resident size and mass (SL, TL, mass), size ratio (intruder mass/resident mass), size ratio category (large intruder, large resident, or equal), and total contest time (s) defined as the time elapsed from the first attack of the attacker (intruder or resident) to the surrender/evasion of the loser (intruder or resident).

All statistical analysis was performed with SYSTAT Version 13.1. Analysis of variance (both one-way and two-way ANOVAs) were performed to test the effects of acoustic variables against themselves (e.g., the effect of call type on peak frequency), and against context variables (e.g., the effect of gender pairing on peak frequency). Linear regression analysis was used to test the relationships between number of pulses in a pulse train and duration of the call bout, between total length and peak frequency, and between intruder: resident size ratio and peak frequency.

Table 1. Individual fish measurements for mass, total length (linear distance from the anteriormost tip of snout to the posteriormost tip of caudal fin), and sex. Because each fish was measured and recorded before each trial as an intruder, each recorded mass and length measurement in the table is the last recorded measurement made for that fish's last trial as an intruder.

Name	sex	TL (cm)	M (g)
Small 4	F	7.4	3.7
Small 2	M	8.3	5.1
Small 3	M	9.2	6.9
Small 1	M	10.0	8.4
Med 2	F	9.4	7.4
Med 1	M	10.0	8.9
R3/3	M	11.5	14.1
L3/3	F	11.9	14.7
L1/1	F	12.0	15.9
R2/4	F	11.9	17.7
L2/2	F	12.4	18.4
BIG	M	12.7	19.1

Table 2. Matrix of fishes used for dyadic pairing for experimental trials. Key for fish naming code: 1=Small4; 2=Small2; 3=Small3; 4=Small1; 5=Med2; 6=Med1; 7=R3/3; 8=L3/3; 9=L1/1; 10=R2/4; 11=L2/2; 12=Big.

Fish	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	**0**	0	2	1	1	1	0	0	0	0	0	0
2	0	**0**	1	2	1	1	0	0	0	0	0	0
3	2	1	**0**	3	1	2	0	0	0	0	0	0
4	1	2	3	**0**	2	2	2	1	0	0	0	0
5	1	1	1	2	**0**	2	0	0	0	0	0	0
6	1	1	2	2	2	**0**	0	0	0	0	0	0
7	0	0	0	2	0	0	**0**	1	2	5	3	2
8	0	0	0	1	0	0	1	**0**	4	2	2	2
9	0	0	0	0	0	0	2	4	**0**	2	3	2
10	0	0	0	0	0	0	5	2	2	**0**	2	1
11	0	0	0	0	0	0	3	2	3	2	**0**	1
12	0	0	0	0	0	0	2	2	2	1	1	**0**
Total	**5**	**5**	**9**	**13**	**7**	**8**	**15**	**12**	**13**	**12**	**11**	**8**
Trials												**59**

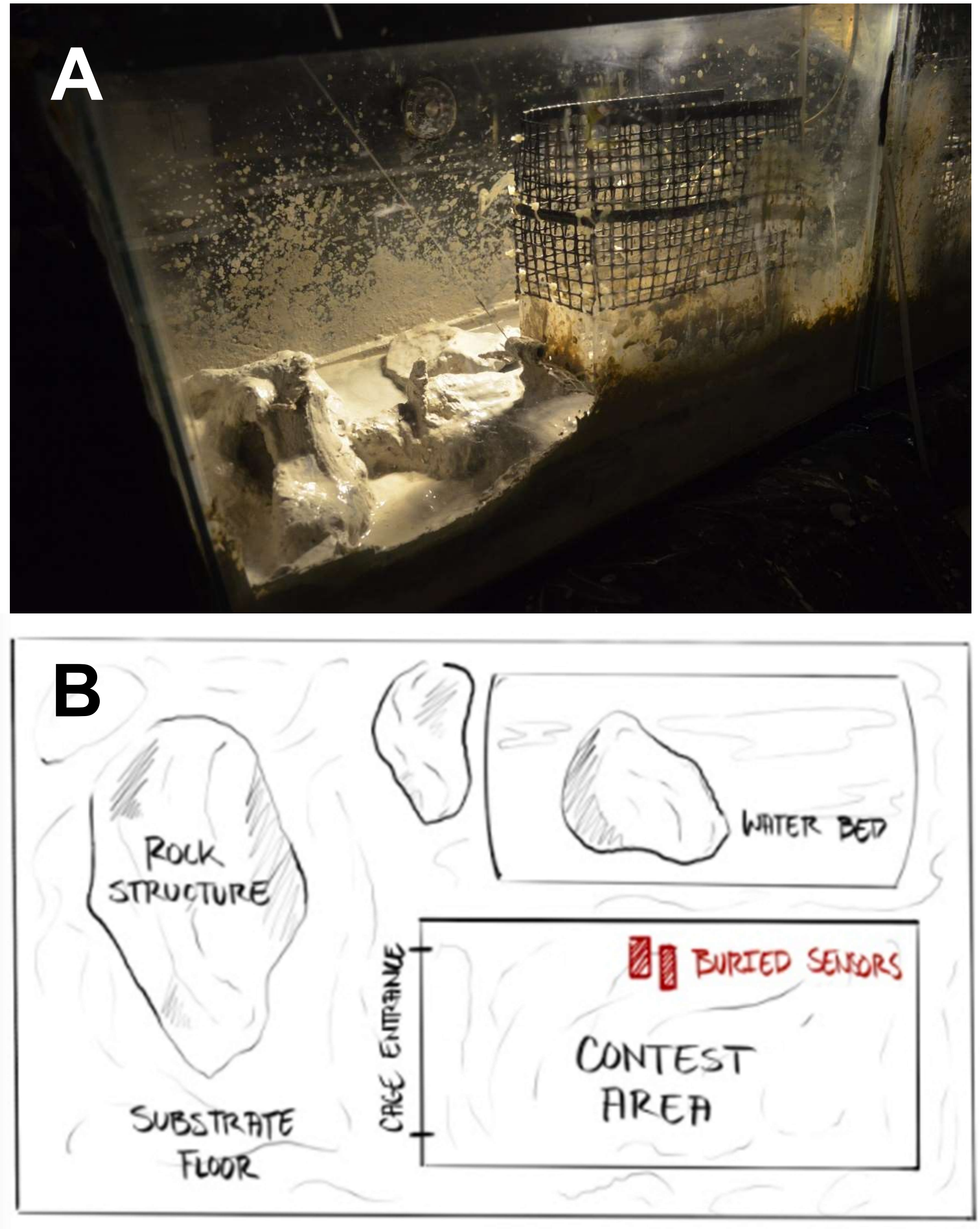
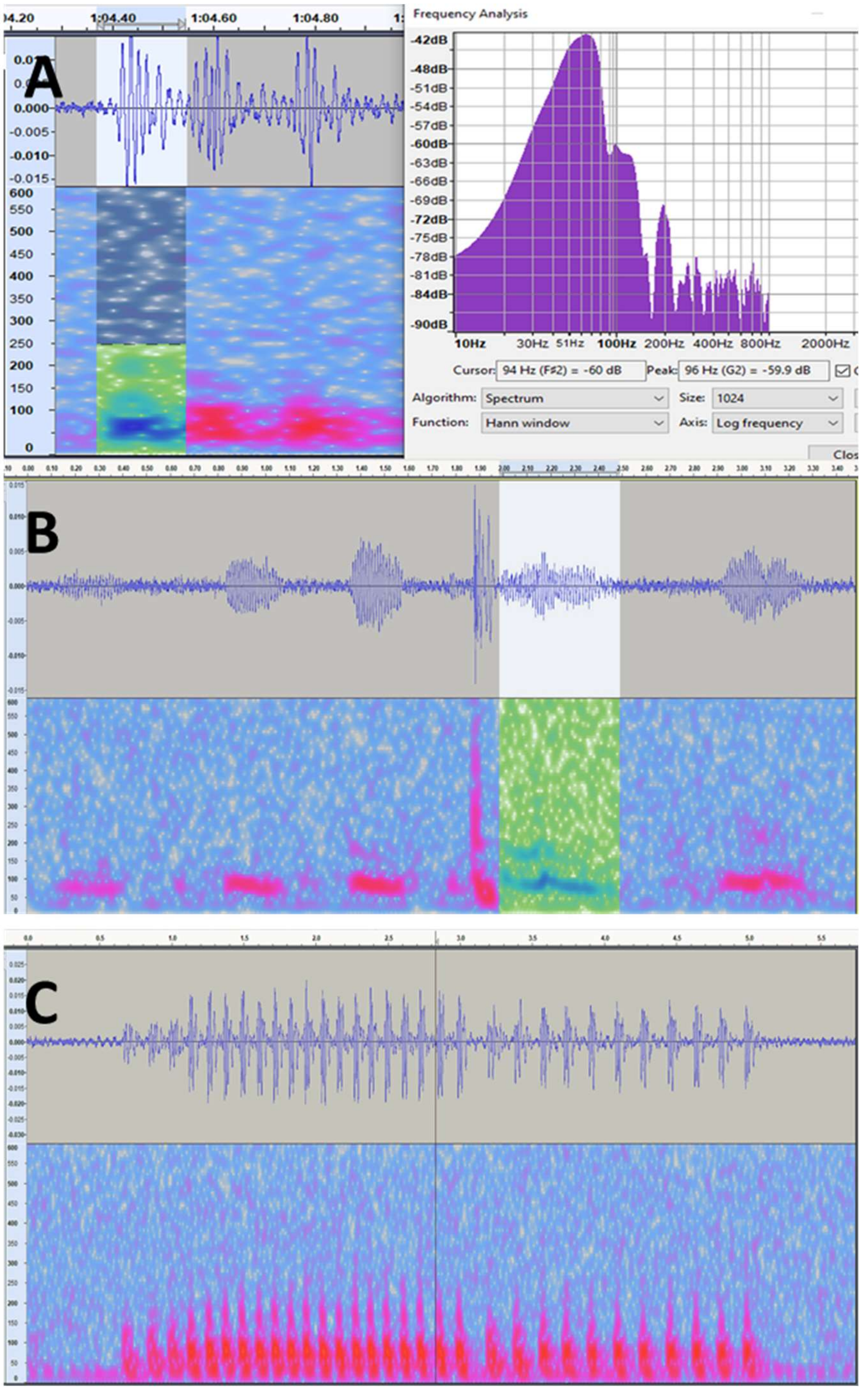


Figure 1. A) Photograph of one of the experimental tanks in which resident mudskippers set up territories. An intruder fish was placed in the Plexiglas and mesh cage, referred to as the contest area. B) Top view of the experimental tank, showing locations of buried accelerometers which recorded their vibrations.



Cursor: 94 Hz (F#2) = -60 dB
Peak: 96 Hz (G2) = -59.9 dB
Algorithm: Spectrum
Size: 1024
Function: Hann window
Axis: Log frequency

Figure 2. Example oscillogram (top) and spectrogram (bottom) of each classified call type. A) Grunt (highlighted) with associated power spectrum showing peak frequency shown on the right side. B) Tone (highlighted). C) Pulse train.

RESULTS

A total of 59 experimental trials were performed, representing the dyadic combinations for the 12 fish that were used in these trials (Tables 1 and 2). From these trials, 173 total calls were recorded and sorted according to call type. Mean, standard deviation, and standard error of peak frequencies and durations of each call were calculated to describe the recorded vibrations (Table 3). Grunts were by far the most common sound type detected (134 out of 173 calls). While both grunts and tones were short, usually less than 0.5 s, pulse trains were sets of multiple pulses that could last for greater than 4 s (Figure 2). There were no differences in call peak frequency between the three call types.

Residents won a majority of contests (33 out of 37, or 89 %). Contest outcome was also associated with which individual produced most of the vibrations, with the winner being the caller the majority of the time (166 out of 173 calls or 96%; Figure 3). Whether a resident fish won or lost a contest, or never entered the contest area, was dependent upon the ratio of the mass of the intruder to the resident (P=0.002; Figure 4). When this ratio was positive (i.e., intruders were larger than residents), residents generally lost. When this ratio was negative (i.e., intruders were smaller than residents), residents generally won if they entered the contest area. Winners were the only ones to produce pulse trains, and these generally occurred after a contest was won (18 out of 25 pulse trains). Additionally, no statistical difference was observed between males and females in either vibration production or contest outcome.

Intruders produced calls with a significantly higher mean peak frequency ($P< 0.001$; Figure 6), however, this result could be biased by the lower number of vibrations emitted by intruders relative to residents. Across all call types, there was a significant negative relationship between fish TL and call peak frequency ($P< 0.001$; Figure 7A). However, a significant inverse relationship was found between peak frequency and intruder: resident size ratio ($P< 0.005$; Figure 7B). Duration of the calls did not significantly differ between sexes, between different sex contexts, by fish size, or by frequency of the call. Duration of pulse trains did provide an indication of the number of pulses within a pulse train, suggesting that the interpulse intervals were fairly constant within a pulse train (Figure 8).

Finally, fish behavior was recorded in terms of territorial contest situation (e.g., which fish won the contest, relative size and sex of intruder and resident) and outcome. Each vibration recorded was contextualized with the accompanied behavior before and after the vibration was made. Specific observed behaviors were not categorized due to complexities among each fish and their respective behaviors. Instead, qualitative behaviors were described thoroughly with each call and recorded to look for potential patterns. Among all recorded behaviors, the noticed patterns included the following trends: greater aggression from resident fishes, submissive/evasive behavior of losing fish (retreating to the corner or clinging to the wall of the contest area), dorsal fin displays during aggressive contests from both fish, dorsal fin displays from winning fish, and greater occurance of pulse train production from winning fish. No significant differences in behavior were noticed between sexes and aggressive behavior was more frequent the larger the fish was relative to the opponent. These qualitative observations aided in understanding vibration patterns in terms of the contexts under which calls are generally made by *P. barbarus*.

Table 3. Descriptive statistics of frequency and duration of the three vibration call types- grunts, pulse trains, and tones.

		Call Type		
Data		**Grunt**	**Pulse Train**	**Tone**
N		134	25	14
Frequency (Hz)	Minimum	44	41	50
	Maximum	107	104	102
	Avg.	72.69	69.28	72.14
	Std. Error	1.02	3.33	4.13
	Std. Dev.	11.8	16.65	15.5
Duration (s)	Minimum	0.16	0.86	0.2
	Maximum	0.77	4.49	0.36
	Avg.	0.32	2.64	0.26
	Std. Error	0.01	0.22	0.02
	Std. Dev.	0.107	1.12	0.06

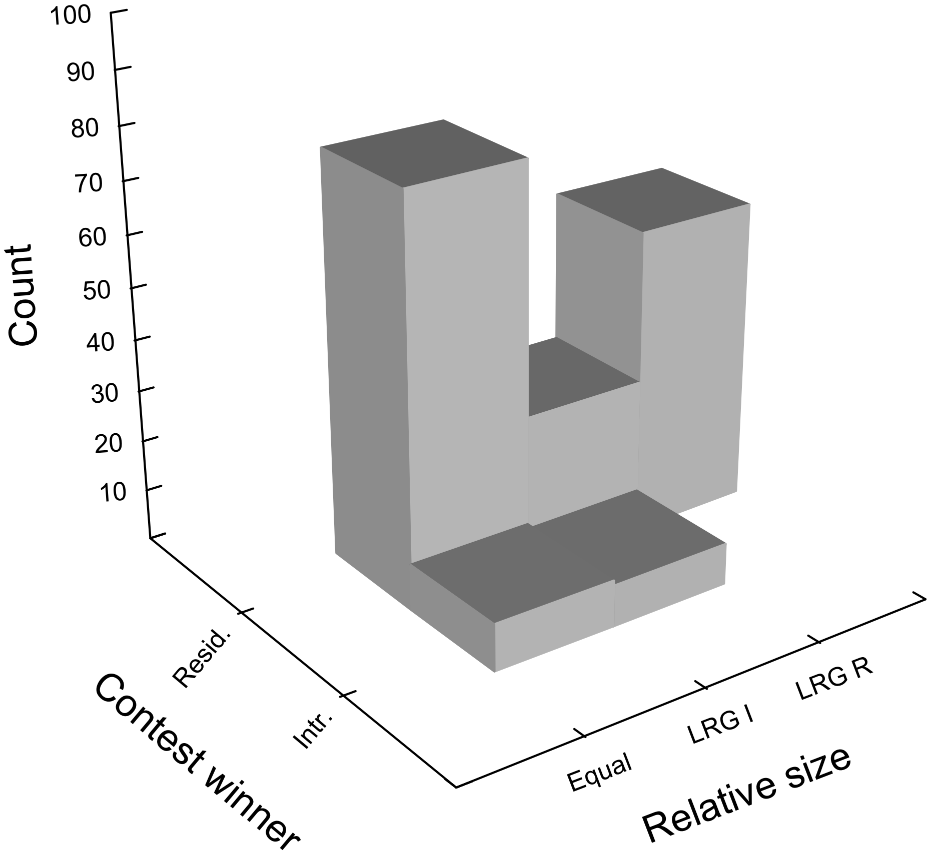


Figure 3. Histogram of the total number of calls as a function of contest winner and relative size. Winners were always either resident or they were intruders with greater mass. Calls were produced in much greater numbers by residents than intruders, and only by intruders of equal or greater size than the resident.

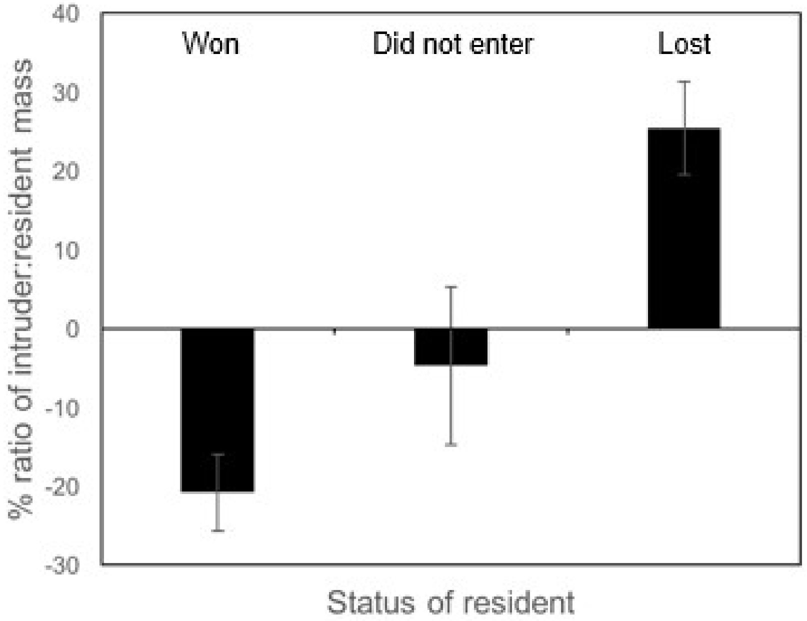


Figure 4. Mean (±SE) intruder: resident mass ratio as a function of status of the resident (won contest, did not enter contest area, lost contest). Intruding fish of smaller relative mass lost contests, while relatively larger intruders won contests. Negative values indicate a larger resident while positive values indicate a larger intruder.

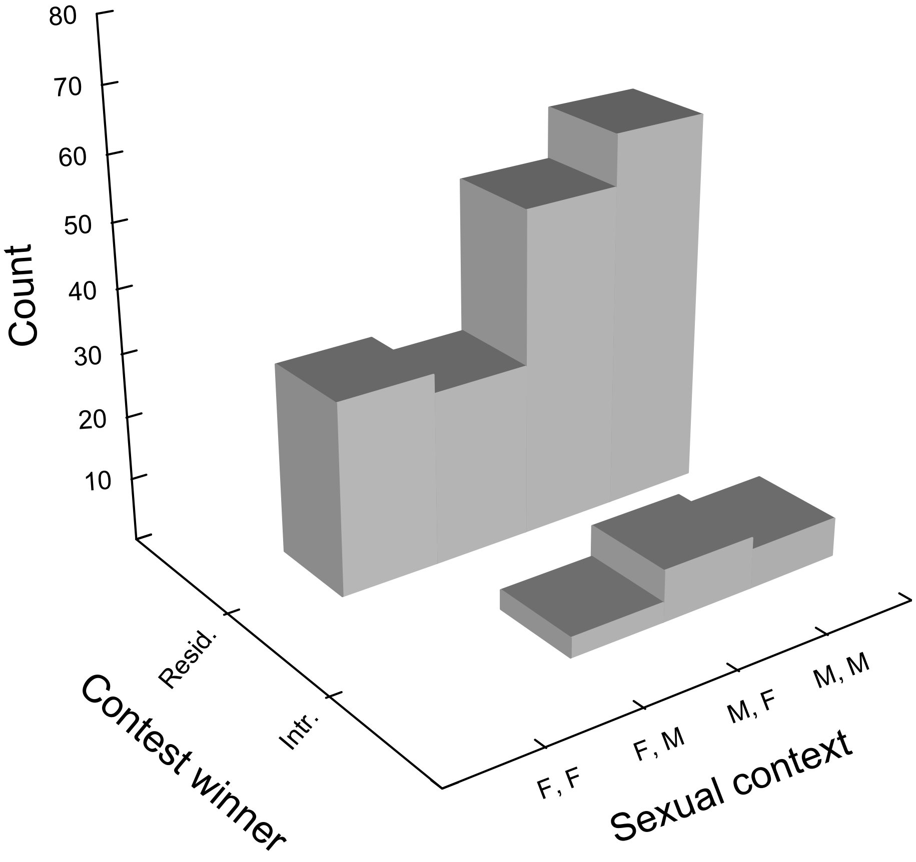


Figure 5. Histogram of total number of calls as a function of contest winner and sexual context. Calls were produced in much greater numbers by residents than intruders, with male residents producing greater numbers of calls than female residents. (Note: An ANOVA with total calls as the dependent variable and sexual context and winner as dependent variables did not reveal significant differences, suggesting that the pattern in total calls was largely caused by a relatively small number of experimental trials).

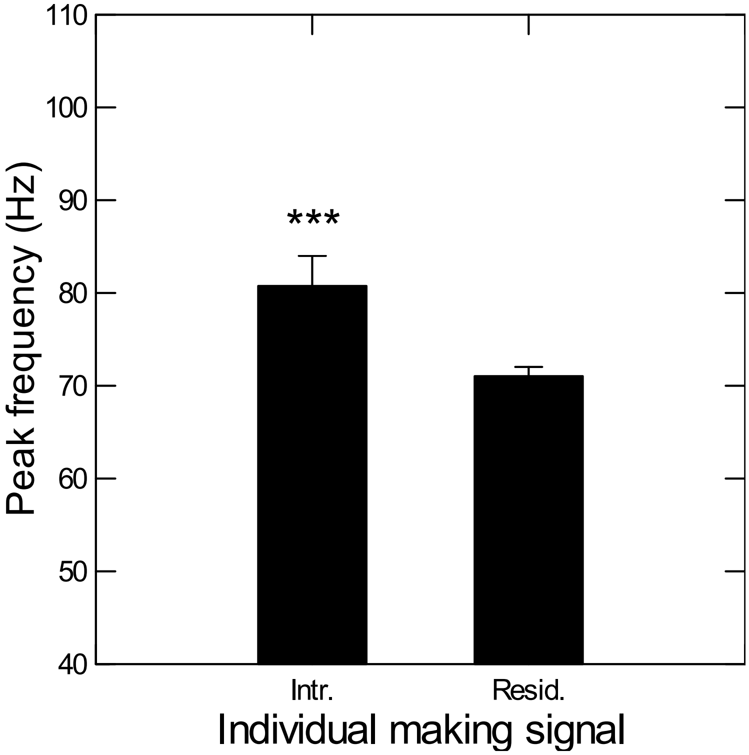


Figure 6. Mean (±SE) peak frequency as a function of caller (intruder versus resident). Peak frequencies were higher for intruders compared to residents ($P<0.001$).

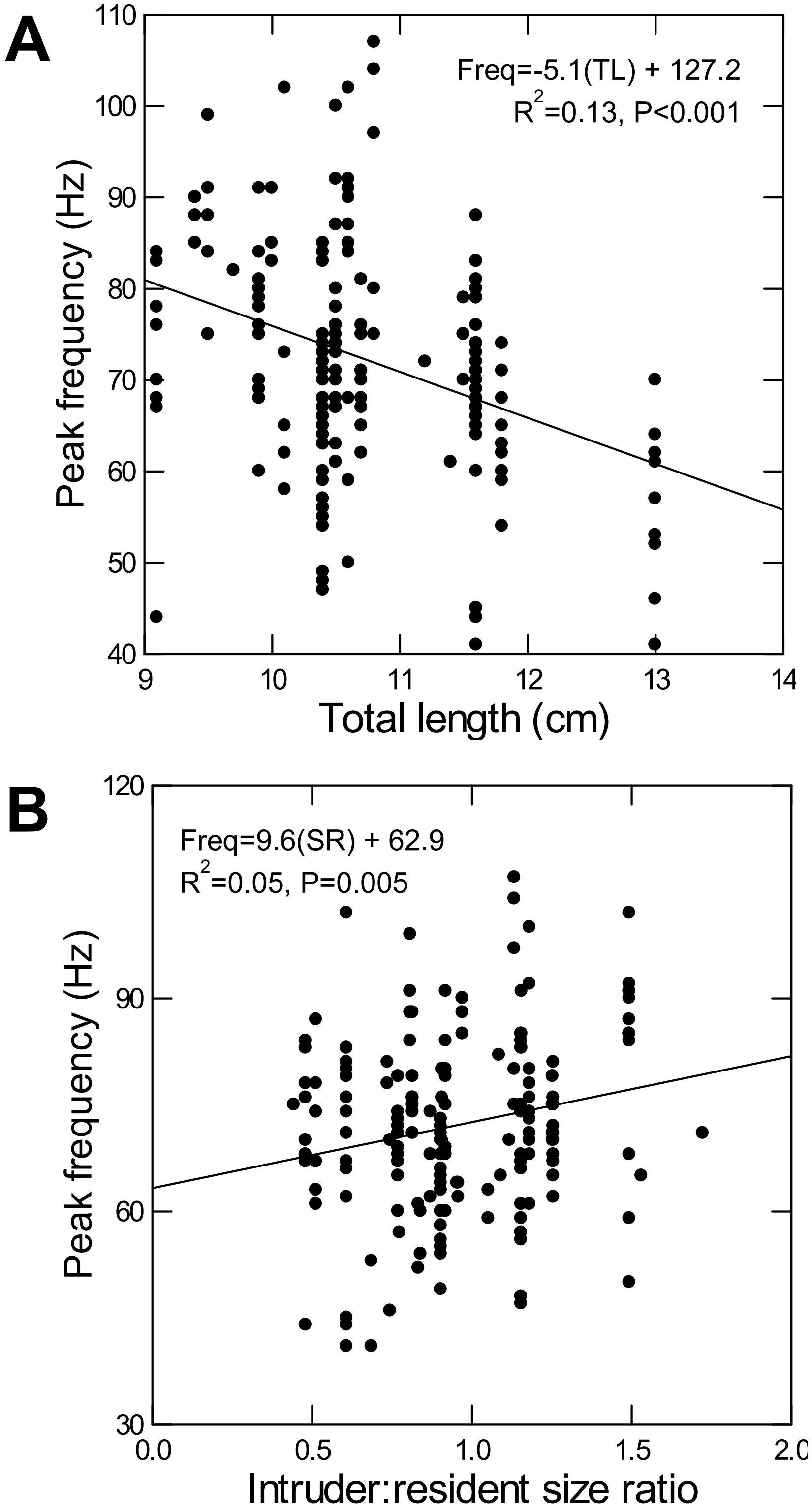


Figure 7. The linear regression relationships between A) fish total length and call peak frequency and B) intruder:resident mass ratio and call peak frequency. A higher ratio indicates an intruder increasing in size relative to the resident.

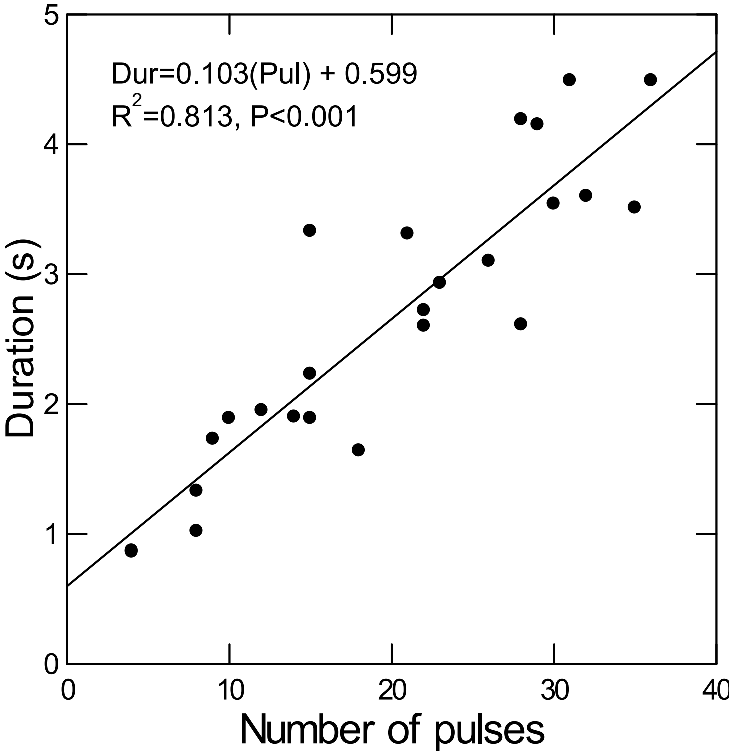


Figure 8. Linear regression relationship between pulse train duration and number of pulses.

DISCUSSION

This is the first study showing that *P. barbarus* emits acoustic signals that can be measured as vibrations in the mud. The only other mudskipper species previously examined acoustically, *Pn. septemradiatus* [6], also produced tonal and pulsatile vibrations that could be measured through the mud substrate during aggressive dyadic contests. However, the two mudskippers exhibit some species-specific characteristics. Tonal signals emitted by these two species lasted for <1.0 s (*Pn. septemradiatus* ~0.43 s, *P. barbarus* ~0.26 s), and pulse-trains lasted multiple seconds (0.86–4.49 s in both species). The mean peak frequency of *Pn. septemradiatus* pulses was just below 100 Hz, while some tonal segments reached 168 Hz [6]. In contrast, peak frequency ranged from 44 to 104 Hz in *P. barbarus*, with a mean of ~72 Hz.

Behavioral observations suggest that mudskippers communicate acoustically or vibrationally, while out of the water. However, typically aquatic gobies (e.g., *Padogobius martensii*) communicate acoustically in water [18]. Mudskippers are gobioids (Teleostei, Gobioidei or Gobiiformes), being phylogenetically related to other groups of soniferous aquatic gobies, including the *Pomatoschistus* lineage [17], [19]. Consistently, both studied mudskipper species produce tonal and pulsatile signals that are structurally and temporally similar to typical aquatic gobioid sounds. A rapid contraction of the *levator pectoralis* muscle is currently hypothesized as the sound-production mechanism in *Gobius paganellus* [20], [21]. Additionally, tonal sounds with slightly higher frequency (~120 Hz) in the aquatic *Perccottus glenii* (Gobiiformes, Odontobutidae) have been observed to be produced by a similar sonic mechanism [22]. These findings in aquatic gobies currently offer the best hypothesis for the sound production mechanism in the closely related intertidal mudskippers.

Call structure and variation in substrate-borne vibrations observed in my study of *P. barbarus* are mostly consistent with what has been observed in the aquatic *P. martensii* (both tonal and pulsatile) with a slightly lower general frequency range in *P. barbarus* (*P. martensii*: 80-200 Hz, *P. barbarus*: 30-160 Hz) [18]. Another aquatic gobioid, *Odontobutis obscura,* produces its calls at a peak frequency just below 1 kHz [23]. Despite significantly higher frequencies, these calls are composed of short pulses that are structurally similar to the vibrations recorded in *P. barbarus*. It is possible that *P. barbarus'* morphological adaptations for an amphibious lifestyle, such as a more robust pectoral girdle and fins, account for the differences in call frequency between *O. obscura* and *P. barbarus* despite similarities in general call structure.

Several teleost species use a gas bladder to amplify the vibrations produced by sonic muscles [13]. However, migration onto land in amphibious gobioid species such as *Pn. septemradiatus* resulted in the loss of a gas bladder [6]. While mechanisms of call production are still unknown in *P. barbarus* and are not the current focus of our studies of communication during these agonistic interactions, our results fit the hypothesis that amphibious gobies such as *P. barbarus* and *Pn. septemradiatus* produce calls that are similar to those produced from strictly aquatic species. They likely use a similar sound production mechanism, differing only in transmission medium and acoustic filtering through the mud substrate instead of water. In other words, *Pn. septemradiatus* and *P. barbarus* likely retained sound production mechanisms observed in aquatic gobies and now effectively utilize them to send vibratory signals through the mud surface, most likely through their pectoral fins [6].

During my observed dyadic interactions in *P. barbarus*, vibrations were produced both before and after territorial contests. Ritualistic acoustic communication in gobies is commonly associated with courtship and territorial behaviors as seen in *Pomatoschistus pictus* and *Pomatoschistus minutus* [24-26], suggesting that territorial acoustic communication may be a common characteristic of gobiiforms [6]. My analysis of the variables of size, sex, and role (Resident: established owner of territory; Intruder: trespassing fish) established patterns of predictable contest outcomes. Resident individuals never lost to intruders unless the intruder was at equal or greater size than the resident. However, a larger or equally large intruder still rarely won and was rather found to be the exception to the tendency for residents to win. This indicates that residency status is a greater influence on contest outcomes than size. However, the effects of size in territorial matters within gobies are still present and are shared among many species. For example, mudflat wall construction and territorial ownership of mud walls are known to be exhibited in the species *Boleophthalmus boddarti* and illustrates a similar effect of size and residency status on territorial relationships [27]. Territories are often built and abandoned by their owners to soon be replaced by other individuals. Studies indicate that replacement fish were often smaller than the original owners of the territory suggesting that contests for newer and more profitable territories are often unattainable to the smaller individuals based primarily on the competition of larger individuals for those new territories [27]. Our studies illustrate the same disadvantage smaller *P. barbarus* intruding individuals have.

Unlike size, sex as a variable of the observed dyadic trials resulted in no significant indication of contest outcome or pattern of vocalization. Instances of residents of both sexes won dyadic contests and produced similar calls with no statistical difference in call type, duration, or frequency. Absence of differences between the sexes in visual and audible behaviors and vibrations suggests a fundamentally shared mechanism (potentially the *levator pectoralis*) and context (territory protection/ acquisition) for vibration production in both sexes of *P. barbarus*. Many species of teleost fish share this feature of non-discriminatory call production between sexes such as the pectoral stridulation and swim bladder drumming in the neotropical catfish *Iheringichthys labrosus* [28]. On the other hand, many fish species also exhibit sex exclusive sound production utilized in courtship behaviors such as the sciaenid *Macrodon atricauda*, in which only males exhibit bilateral sonic muscles used in advertisement calls for female attraction [29]. Additionally, sexually dimorphic visual actions are also prevalent in related fish species. A behavioral study demonstrated differences in visual courtship behavior in *Scartelaos histophorus*, with a tail standing action that is present in the males of the blue mudskipper but not present in the females [30]. No distinct visual or audible mannerisms were noted by either sex in *P. barbarus* during video analysis other than the dorsal fin display which was shared by both male and female fish during contest bouts. Therefore, no sex-specific behaviors were observed in *P. barbarus* at the level of analysis done in this study.

During territorial disputes, residents were much more “vocal” than intruders (residents produced 152 of the total 173 calls) indicating the function of vibrational communication is more often used as a defensive tactic by the resident during territory protection rather than as an offensive strategy by the intruder to overcome the resident. Vibrational communication could also be produced to influence the motivation to fight in favor of the caller. In other words, prominent vibration production by the resident can suggest a risky cost-benefit payoff to the intruder when choosing to engage in a contest. Abundant vibration production paired with relatively low call peak frequency would suggest a highly competitive individual on the basis of size of the resident (following the inverse relationship between size and peak frequency; Figure 7A). Most of the recorded vibrations were produced by the winner of the contest (resident or intruder). The observed negative relationship between size and frequency also suggests that sound frequency may convey information about the emitter size (hence competitive ability), during dyadic contests. Therefore, active acoustic production at relatively low peak frequency could convey the message of a large and competitive emitter. In particular, pulse trains were only emitted by the contest winner and were twice as likely to occur after the contest outcome. Studies observing acoustic signals in the damselfish, *Parmecentrus partitus*, show a similar dependence on acoustic communication to convey physical traits of the caller. Acoustical recognition of members within a colony of bicolor damselfish is achieved by recurrent exposure to the sounds and correlated actions of neighbors [31].

Interestingly, as intruder: resident size ratio increased (i.e., as the intruder became larger relative to the resident), frequency of the vibrations in both the resident and intruder decreased. This is a reverse trend of what was anticipated. I hypothesize that this pattern may be the result of submission by non-dominant fishes as has been found in other species. For example, non-dominant members of the social fish *Neolamprologus* *pulcher* will attempt to appease dominant group members using various visual submissive signals to avoid assumptions of hostile intent [32]. Although additional data would need to be collected, it is possible that, as intruders increase in mass and size, resident vibrations would increase in frequency to suggest a smaller size or submissive posture like what is seen in *N. pulcher* (due to the inverse relationship between size and frequency; Figure 7) and avoid conflict. This assumes that the fish has the anatomical and physiological ability to purposefully shift characteristics of its call, but the mechanism of vibration production in mudskippers is currently unknown.

Sound production mechanisms remain a topic for further research in intertidal gobioids, but mudskipper sounds do resemble sound characteristics of strictly aquatic fish species. Some teleost fish species produce acoustic signals that may be transmitted through the substrate like *P. barbarus*. The aquatic, but benthic mottled sculpin *Cottus bairdii* illustrates the morphological utilization of substrate as a medium to receive signals. After pharmacological reduction of the lateral line sensory reception in *C. bairdii*, acoustic signals were perceived by placing the fish's mandible on the substrate [33]. After visual analysis of vibrational communication during territorial interactions, it is reasonable to hypothesize that a similar morphological behavior is observed in the use of the pectoral fins and girdle as the first chain in vibration reception in *Pn. septemradiatus* and *P. barbarus*. An example of such substrate-mediated reception of vibration is observed in the opercularis system of the bullfrog *Rana catesbeiana*. The opercularis muscle connects the shoulder girdle skeleton to the operculum allowing vibrations to be carried to the inner ear after initial reception at the substrate level [34]. I hypothesize that a similar mechanism could be present in *P. barbarus* allowing conspecific signals on land to be communicated through substrate in the absence of water as a medium.

As this is the first bioacoustic study of *P. barbarus*, and only the second such study for mudskippers, additional acoustic communication analysis in both behavioral contexts and the mechanisms of production and detection of these vibrations are still needed. Transitional fossil vertebrate species such as *Tiktaalik roseae* are important to our understanding of the evolution of the transition of some aquatic taxa to terrestrial ones. It is believed that *Tiktaalik* ventured onto land just as present-day mudskippers do, propping up on their fins [35]. Tiktaalik exhibited the development of terrestrial-like middle ear structures and pectoral girdle modifications suggesting that it might have been able to detect vibrations through a muddy substrate in a similar way that I hypothesize mudskippers do- through their pectoral fins. Studies comparing the evolution of the oval window from earlier anapsid amphibians and turtles to diapsids and eventually synapsids also provide insight on the proliferation of auditory sensory systems between aquatic and terrestrial life [36]. Although scientists can speculate as to the function of fossil pectoral and skull/ear bones and their role in sound and vibration detection, examining living aquatic-terrestrial transition species like mudskippers may be a good way to test their hypotheses. It is likely that continued examination of amphibious mudskippers will provide information that will fill in missing gaps in our understanding about the evolutionary transition from aquatic to terrestrial life in vertebrates, with *P. barbarus* as a critical component in the discovery.

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