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Behavioral Response to Conspecific Sounds in Loricariid Catfishes (Pterygoplichtys Pardalis and Otocinclus Vittatus)

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BEHAVIORAL RESPONSE TO CONSPECIFIC SOUNDS IN LORICARIID CATFISHES (*PTERYGOPLICHTYS PARDALIS* AND *OTOCINCLUS VITTATUS*)

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ABSTRACT

BEHAVIORAL RESPONSE TO CONSPECIFIC SOUNDS IN LORICARIID CATFISHES (*PTERYGOPLICHTYS PARDALIS* AND *OTOCINCLUS VITTATUS*)

Sound production is a means of communication among many fish species. In fishes, sound is produced through various mechanisms, but in the family Loricariidae, known as armored suckermouth catfishes, sound is produced primarily through pectoral fin spine stridulation. Previous experiments have described the sounds produced and shown the mechanism of sound production in two species of loricariid catfishes, *Pterygoplichthys gibbiceps* and *Otocinclus affinis*, but the functional significance of loricariid sounds is still unknown. To address this question, I examined the behavioral responses of *Pterygoplichthys pardalis* and *Otocinclus vittatus* to conspecific calls. Individual fish (N=10 for *P. pardalis*) or groups of 20 fish (N=4 groups for *O. vittatus*) were acclimated to an aquarium for at least 24 h. Individual *P. pardalis* were video recorded for 2 minutes with no sound, plus another 2 minutes with a playback of either a 500 Hz tone control or conspecific call through an underwater speaker. In contrast, *O. vittatus* was video recorded for 5 minutes with no sound, with an additional 5 minutes of either conspecific call or 500 Hz tone stimuli. This procedure was repeated for each individual or group using either a 500 Hz tone and conspecific call for playback so that each individual or group received both stimuli. I hypothesized that *P. pardalis* would avoid, while *O. vittatus* would be attracted to, the conspecific sound-emitting speaker, respectively. The rationale for this hypothesis is that *P. pardalis* produces calls when it is under duress, so it may be an alarm call, while *O. vittatus* produces calls spontaneously in large groups of fish, suggesting it may be a cohesion call or involved in other intraspecific interactions. *P. pardalis* showed an increased activity level to conspecific sound compared to the 500 Hz tone, although movement in general was minimal, while *O. vittatus* exhibited a short-lived response to conspecific calls by moving toward the speaker with the sound source. Since both species showed a minimal behavioral change to sound playbacks, more research is needed to better understand the function of sound production in loricariid catfishes.

Keywords: fish, communication, acoustic, loricariid, catfishes

I dedicate this thesis to my parents and siblings for their support despite the miles between us.

ACKNOWLEDGMENTS

I would like to thank Dr. Michael Smith for his patience and kindness toward me since the start of my program. He has been of tremendous help in my transition to this new environment two years ago. I would also like to thank my other thesis committee members, Dr. Steve Huskey and Dr. Philip Lienesch, for all their insight, recommendations, and guidance since the proposal stage of this project and for giving me great advice as my thesis progressed. Furthermore, I am grateful to the WKU Biology Department for their financial support through a graduate assistantship that funded my program and to the WKU Graduate School for the Graduate Research Grant that funded this project.

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CHAPTER 1 - INTRODUCTION

Sound production in fishes

Animals communicate with each other using various signals such as pheromones, sound, visual, and tactile cues (Leonardo 2016). Most terrestrial animals depend heavily on vision and smell as means of communication, but these are restricted in water by light absorption and the steady physical movement of water. Due to this, many aquatic species, including fishes, evolved the use of sound and hearing as their basic means of communication and environmental awareness (Frankel 2009).

A wide range of fish species are known to produce sounds. Over 800 species from 109 families of fishes are vocal (i.e., sound producing; Rountree et al. 2002, Amorim 2006). The first mention of sound production in fishes was by Aristotle (Gohlke 1957) in the fourth century BC, while the fundamental mechanisms of sound production were extensively defined by Müller (1857) and Duffose (1874) in the nineteenth century (Ladich and Fine 2006). Fish can produce sounds by several mechanisms, including swim bladder compression, stridulation of the pectoral or dorsal spine, and grinding of teeth (Ladich and Fine 2003). Fishes, like other animal taxa, produce sounds for a variety of purposes, including spatial orientation (Tavolga 1977), predator defense, mating, alarm calls (Ladich and Fine 2003), as well as a response to stress (Ladich and Bass 1998; Ladich 2000).

Some catfish species produce sounds through pectoral spine stridulation or swim bladder compression using sonic muscles, while others can produce sounds through both mechanisms (Ladich and Fine 2006). Swim bladder compression as a medium of sound production in teleost fishes has been thoroughly studied (Demski et al. 1973). The swim bladder is swiftly contracted and protracted by specialized sonic muscles (intrinsic and extrinsic muscles), triggering its radiant surface to vibrate. The two sides of the intrinsic muscles are directly attached to the swim bladder, while the extrinsic muscles have an insertion point on a close-by bony structure such as the skull or vertebrae (Ladich and Fine 2003).

Stridulation sounds are produced via the rubbing together of skeletal elements such as teeth, skull bones, jaw apparatus, branchial apparatus, fin rays, and vertebrae. Specialized stridulation sounds (i.e., the rubbing of body parts in a regular pattern) are generated by the fish families Haemulidae, Centrarachidae, Cichlidae, Theraponidae, and many others (Schneider 1961; Mahajan 1963; Lanzing 1974; Ballantyne and Colgan 1978; Ladich and Bass 2003). Almost all fish species generate unspecialized stridulation sounds (i.e., the rubbing of body parts in an irregular pattern; Ladich and Bass 2003). Stridulatory sounds have been most thoroughly studied in catfishes and damselfishes (Fine and Parmentier 2015). In catfishes that produce stridulatory sounds, the sounds are produced during the abduction and/or adduction of pectoral fin spines (Ladich 1997; Heyd and Pfeiffer 2000) but in *Sisor rhabdophorus* stridulation is done via the dorsal fin (Mahajan 1963).

Loricariid catfish

The family *Loricariidae* is the largest catfish taxa, with up to 900 valid species (Fricke et al. 2023). Commonly known as suckermouth catfishes, they are native to Central and South America and have a round, ventral, oral opening that forms a sucker disc and are covered with bony dermal plates (Evers and Seidel 2005).

Little is known about sound production in loricariid catfishes, and a majority of what is known was discovered by previous students in the Smith Lab at Western Kentucky University (Webb 2011; Stewart 2012). In these previous studies by Webb (2011) and Stewart (2012), it is suspected that the species used, referred to as *Macrotocinclus affinis* (*Macrotocinlus* is an invalid genus now), could have been *O. vittatus* because many *Otocinclus* species are commonly incorrectly labeled as *Macrotocinclus affinis* in the aquarium trade and *O. vittatus* is the most common species imported.

In the current study, I examined *P. pardalis* (Figure 1), a loricariid catfish similar in size and ecology to *P. gibbiceps*, and *O. vittatus* (Figure 2). These two species differ considerably in size (*P. pardalis* grows up to 50 cm; *O. vittatus* up to 5 cm) and behavior (*P. pardalis* can be solitary, while *O. vittatus* are found in large schools; Evers and Seidel 2005). The natural habitat of *P. pardalis* is the inland waters of South America, mostly in the middle stretch and the upstream section of the Orinoco and Amazon rivers that flow through Venezuela, Brazil, and Peru (Armbruster and Page 1996; Weber 2003). Its main food source is algae. *Otocinclus vittatus* is indigenous to southeastern Brazil. It usually forages in river bottoms and spends most of its time eating algae on demersal objects (Zhang et al. 2021). Loricariid catfishes can produce sounds through pectoral spine stridulation, similar to other catfish species like the channel catfish, *Ictalurus punctatus* (Webb 2011; Fine 1996). The stridulation sounds are produced when the ridges on the dorsolateral surface of the dorsal process of the base of the pectoral spine are rubbed against the pectoral girdle. Larger loricariid individuals and species have greater distances between the ridges on the dorsal process of the pectoral spine, and this greater size is inversely correlated with dominant frequency of their sound production (Webb 2011).

P. gibbiceps produces sound through alternating abduction and adduction of the pectoral spine with a longer duration and lower frequency, in contrast to the sound produced by *O. affinis* through adduction, only producing higher frequency click-like sounds (Webb 2011). In contrast, Ladich (1997) and Heyd and Pfeiffer (2000) stated that loricariids only stridulate during the abduction of the pectoral spine, unlike bagrids, mochokids, doradids, and aspredinids which stridulate during both adduction and abduction.

Rationale/Hypotheses

Previous research performed on sound production in catfishes focused on the mechanisms- i.e., stridulation and swim bladder drumming/compression. These studies have not been able to ascertain if some of these sounds produced are spontaneous or more specific to certain behaviors (i.e., have a functional meaning).

The purpose of this project was to begin to understand why two loricariid catfish species, *P. pardalis* and *O. vittatus,* produce sound. These two species were selected because previous studies showed that similar species can produce sounds, and because these species represent the extremes in the size spectrum of loricariid catfishes. I hypothesized that *P. pardalis* would exhibit antipredator behavior (i.e., swim faster and more erratically in response to a conspecific call and avoid the underwater speaker emitting the call). The rationale for this hypothesis is that *P. gibbiceps*, a closely-related *Plecostomus* catfish, produces its calls when being handled by a human experimenter (Webb 2011). Thus, the stridulation sounds they produce may be alarm calls. In contrast, I predicted that *O. vittatus* movement would be towards the speaker emitting a conspecific call. Preliminary experiments revealed that they were attracted to such calls (Stewart 2012). *Otocinclus spp.* possesses a small sized bi-lobed swim bladder located on each side of the head adjacent to the inner ear and a single Weberian ossicle (with connected tripus and scaphium bones) that transmits vibrations from each swim bladder lobe directly to the inner ear. In addition, they exhibit large fenestrae in their pterotic + supracleithrum capsule-like bone that covers each swim bladder lobe and is adjacent to the inner ear (Weitzman 2005, Botta 2009). All of these, together, may account for why *Otocinclus spp.* can localize sound well (Stewart

2012). *Otocinclus spp.* can be found in large schools (Evers and Seidel 2005), and it is possible that their calls are used to maintain cohesion within the school or in other intraspecific interactions.

CHAPTER 2 -
BEHAVIORAL RESPONSE OF *P. PARDALIS* TO CONSPECIFIC SOUNDS

Experimental fish

Eighteen *P. pardalis* were purchased from local suppliers for use in this behavioral study. Fish with a mean total length and mass of 14.61 cm (± 0.43) and 22.89 g (± 1.39), respectively, were maintained in two 210 L aquaria in the Animal Facility in the Engineering and Biological Science Building at WKU. The aquaria had gravel bottoms, heaters, and filters to maintain water quality and were kept on a 12h:12h light:dark cycle at 26-27 ºC. Fish were fed algae wafers daily.

Sound stimuli

Two treatment sounds were used in this experiment. The first was a previously recorded *P. pardalis* stridulatory sound with a peak frequency of 106 Hz (Figure 3). The sound was amplified, and background noise filtered with Raven Pro 1.6 and Audacity 3.2.2. The second treatment was a tone with a peak frequency of 500 Hz created with Audacity, which acted as a control to test whether behavioral changes were specific to conspecific sounds or simply sound in general. (Figure 4).

Calibration

A GRAS Pistonphone Type 42AC, GRAS RA00043 hydrophone coupler was used to calibrate the sound pressure levels of our playback recordings. The hydrophone (GRAS Type 10CT) was placed 2 cm directly above the center of the underwater speaker (University Sound UW-30) and connected to an amplifier (Kistler Type 5010B charge-voltage amplifier). Treatment sounds were amplified (AudioSource Amp 5.3 monoblock power amplifier) and played through the underwater speaker (University Sound UW-30), and the Peak-to-Peak and

Cyclic RMS voltages were recorded using an oscilloscope (Tektronix TDS 2012). During experimental trials, both sounds were played back (separately) at 171 dB re 1 µPa.

Experimental Set-Up

In a sound dampening room (SE 2000 Sound Isolation Enclosure, Whisper Room, Inc.), a glass 210 L aquarium was set up with gravel, a filter, heater, and an underwater speaker (University Sound UW-30) 3 cm away from the right side of the tank (Figure 5). A hydrophone (GRAS Type 10CT) was inserted into the center of the tank to detect acoustic signals produced by the playback signal coming from the underwater speaker, with this acoustic stimulus going through a National Instruments Hi-Speed NI-9162 USB and a Kistler Type 5010B charge-voltage amplifier. A Humbug (Quest Scientific, Canada) electrical conditioner was used to filter electrical noise that might interfere with the recording of the conspecific and tone sounds. Video was recorded through a Logitech HD 1080p webcam. Both audio and video data were recorded using custom data acquisition software built in LABVIEW on a laptop outside of the sound dampening room.

Experimental Procedure

Treatments were randomized to determine which sound an individual fish was exposed to first (500 Hz versus *P. pardalis* conspecific sound). An individual fish was randomly selected from the maintenance tank and put in the experimental tank and allowed to acclimate for approximately 24 hours. All experiments started at approximately noon on the experimental day. First, their control behavior (no sound stimulus) was video recorded for 2 minutes. Then, the pre-recorded conspecific sound or the 500 Hz tone file (depending on which treatment was randomly selected to be used first) was played back to the fish for an additional 2 minutes. Both stimuli consisted of three pulses of the sound, (0.75 secs each for the conspecific call, and 0.5 secs for the tone) within 5 seconds, followed by silence for 25 seconds, looped for a total of 2 minutes. This experimental procedure was repeated with all experimental fishes. The filter and heater were turned off during the experimental period to eliminate potential background electrical and/or acoustical noise.

Data Collection

Data collected during the behavioral trials included mass (g), total length (cm), fork length (cm), standard length (cm), water temperature (°C), NSS (No Sound Start Time), NSE (No Sound End Time), SS (Sound Start Time), SE (Sound End Time), and time of first movement following sound onset. Following behavioral trials, fish behavior was quantified by movement both before and after the onset of the playback of the conspecific sound or tone. As fish movement was minimal for all experiments, behavioral data was coded as binary: 1 signified a movement response to sound at any point during the 2-minute control period or sound playback period, while 0 represented no movement.

Statistical Analysis

A non-parametric Friedman's Multiple Comparisons Test was used to test for differences in response by using sound treatment as a grouping variable and fish ID as a blocking variable.

Results

P. pardalis moved during only 22% of the experimental trials in response to the 500 Hz tone stimulus (4 out of 18) and 44% of trials in response to the conspecific sound stimulus (6 out of 18; Figure 6). In general, the fish did not move at the onset of either sound, as the earliest response to the sound was 13 secs after the onset of the sound stridulatory call stimulus. Thus, a startle response to the either acoustic stimulus was not evident. *P. pardalis* increased movement following the conspecific stridulatory call compared to the no-sound control period ($P < 0.01$), while the response to the 500 Hz tone was not significantly different from the control period (Figure 7).

Figure 1: Loricariid catfish species used in the current experiment described in Chapter 2: *Pterygoplichthys pardalis*.

Figure 2: Loricariid catfish species used in the current experiment described in Chapter 3: *Otocinclus vittatus*.

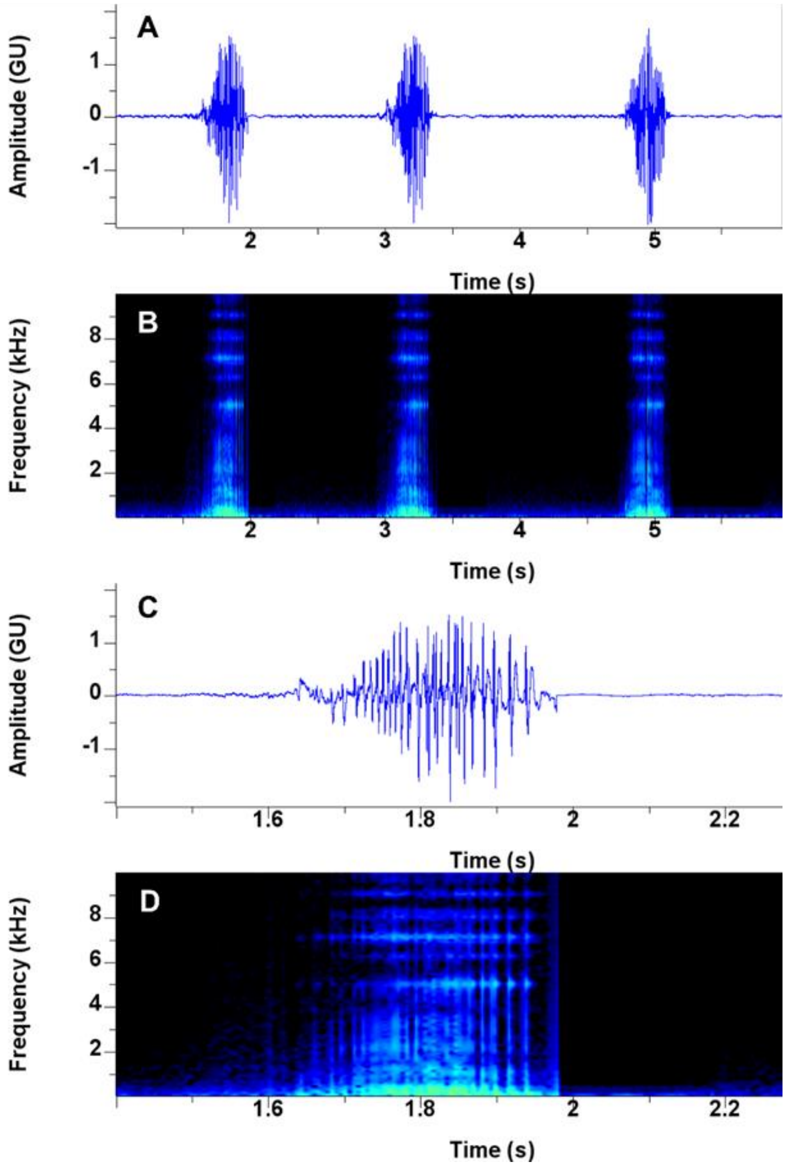


Figure 3: Oscillograms (A and C) and spectrograms (B and D) of a *P. pardalis* call with three repetitions (A and B) and an enlarged image of one of the calls (C and D).

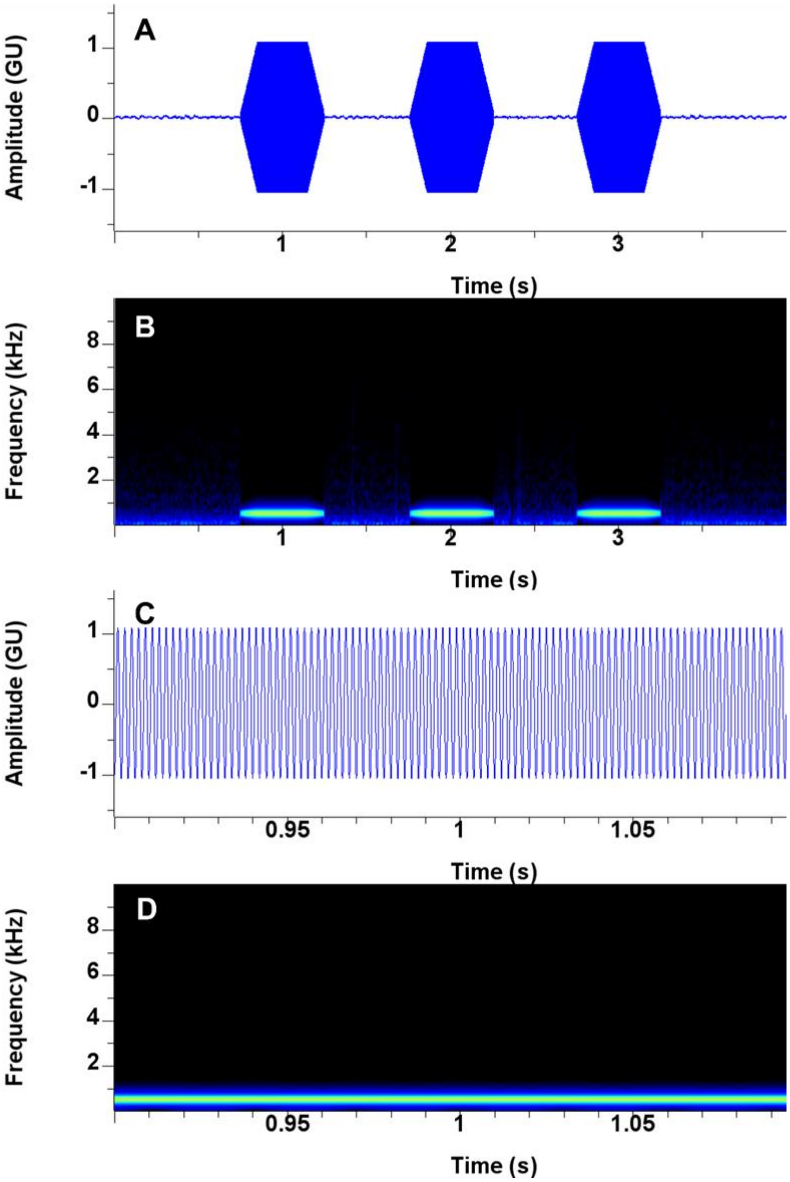


Figure 4: Oscillograms (A and C) and spectrograms (B and D) of the 500 Hz tone with three repetitions (A and B) and an enlarged image of one of the tones (C and D).

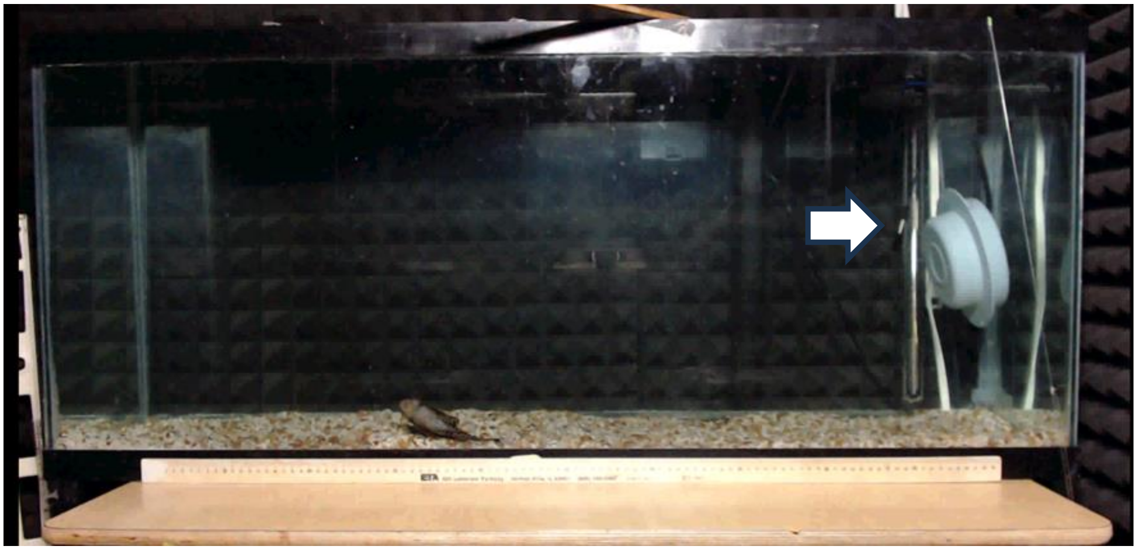


Figure 5: Experimental set up showing the experimental tank, an individual *P. pardalis*, a University Sound UW-30 underwater speaker on the right, hydrophone (white arrow), heater, and aquarium filter.

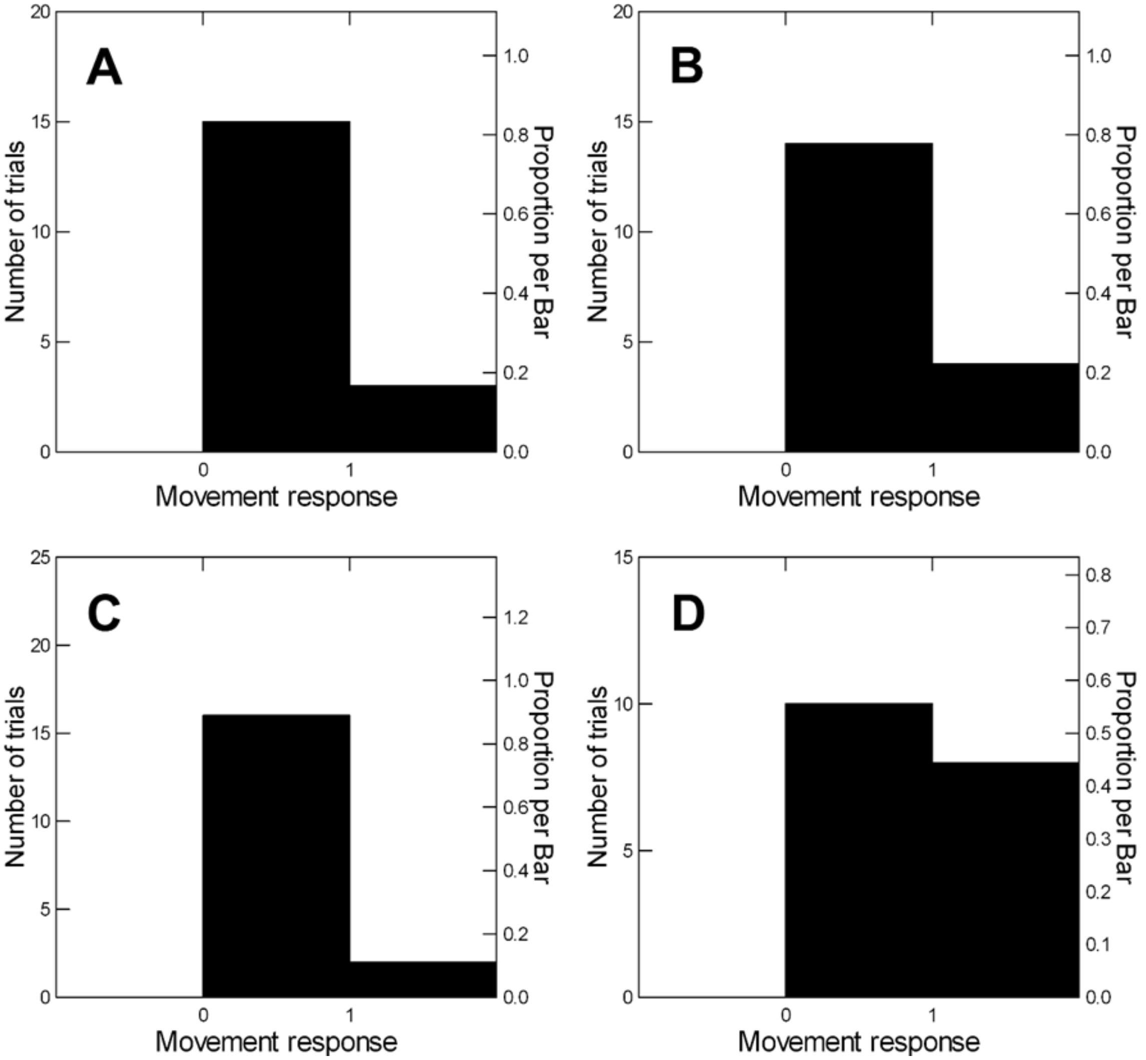


Figure 6: Histograms showing numbers of experimental trials in which *P. pardalis* moved before (A and C) or after (B and D) exposure to sound playback of the 500 Hz tone (A and B) and *P. pardalis* stridulatory call (C and D).

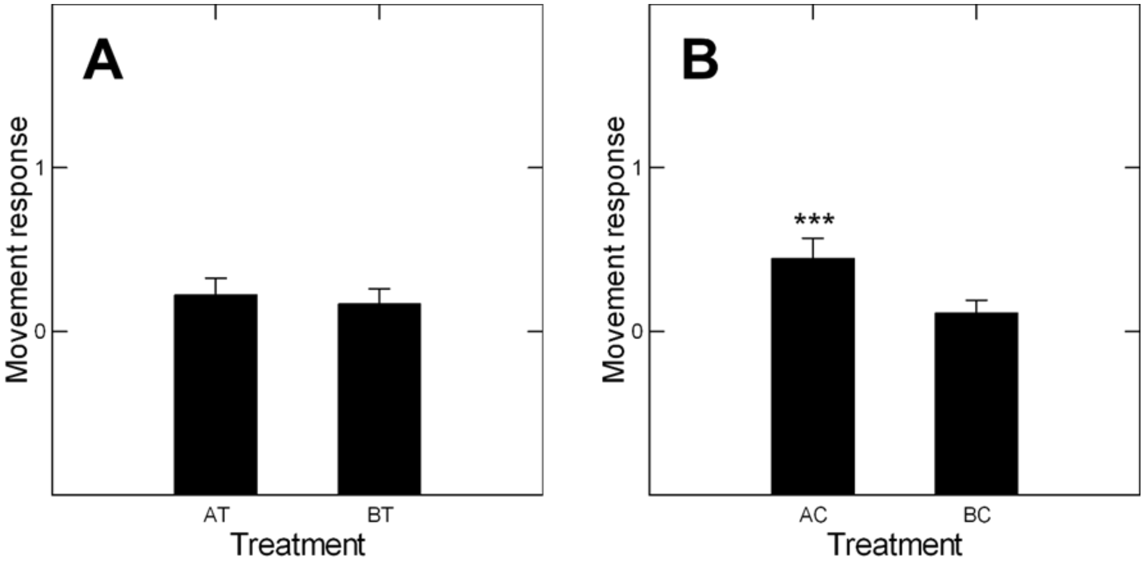


Figure 7: Mean (± S.E.) response of *P. pardalis* to either 500 Hz tones (A) or conspecific calls (B). *** P < 0.001. (AT = After tone, BT = Before tone, AC = After call and, BC = Before call).

CHAPTER 3 -
BEHAVIORAL RESPONSE OF *O. VITTATUS* TO CONSPECIFIC SOUNDS

Experimental fish

Eighty *O. vittatus* purchased from local suppliers were used in this behavioral study. Fish had a mean total length and mass of 3.37 cm (± 0.20) and 0.42 g (±0.02), respectively. They were maintained in aquaria in the Animal Facility in the Engineering and Biological Science Building at WKU. Aquaria had gravel bottoms, heaters, and filters to maintain water quality and were kept on a 12 h:12 h light:dark cycle at 26-27 ºC. Fish were fed algae wafers daily.

Sound stimuli

Two treatment sounds were used in this experiment. The first was a previously recorded conspecific stridulatory call of *O. vittatus* with a maximum peak of 407 Hz and secondary peak of 3,579 Hz (Figure 8). This call was 0.25 secs in duration and was a broadband click. The second was a tone with a frequency of 500 Hz and a duration of 0.5 secs which was created using Audacity (Version 3.2.2) (Figure 4). The recorded stridulatory sound was amplified, and background noise was filtered using Audacity (Version 3.2.2). Both stimuli consisted of three pulses of the sound, within 5 sec, followed by silence for 10 sec.

Calibration

A G.R.A.S Pistonphone Type 42AC G.R.A.S RA00043 hydrophone coupler was used to calibrate the sound pressure levels of the 500 Hz tone. The hydrophone (GRAS 10CT 3505028) was consecutively placed 3 cm from two underwater speakers (University Sound UW-30) in the experimental tank, connected to two separate amplifiers (AudioSource Amp 5.3A). The tone was played, and the Peak-Peak and Cycle RMS voltage were recorded from the oscilloscope (Tektronix TDS 2012). During experimental trials, both sounds were played back (separately) at approximately 155 dB re 1 µPa as measured 2 cm from the underwater speaker.

Experimental Set-Up

In a sound-dampening room (SE 2000 Sound Isolation Enclosure, Whisper Room, Inc.), a glass 210 L aquarium was set up with gravel, two filters, a heater, and two underwater speakers (University Sound UW-30), one hung on each side of the tank with fishing line such that the speakers did not touch the glass of the aquarium and were hanging 9 cm above the gravel bottom of the tank (Figure 9). Each speaker was connected to an independent amplifier (AudioSource Amp 5.3A). A hydrophone was placed centrally in the experimental tank to detect acoustic signals produced by the playback signal coming from the underwater speaker. The hydrophone was attached to a Kistler Type 5010B charge-voltage amplifier which was then attached to National Instruments Hi-Speed NI-9162 USB and fed into a laptop computer outside of the sound dampening room. Custom-built LABVIEW data acquisition software was used to simultaneously record both video and audio data through a Logitech HD 1080p webcam. A Humbug (Quest Scientific, Canada) electrical conditioner was used to filter electrical noise from the audio signal that might interfere with the recording.

Experimental Procedure

The order of speaker use (left or right) and treatment sounds (control, conspecific click or 500 Hz tone) was pseudo-randomized for experimental trials. The schedule was determined before the experiment started to ensure that the random order was never replicated between weeks (Table 1). All eighty fish were divided into four groups of 20 fish. The 20 fish were haphazardly selected from the maintenance tank to be put into a specific trial group, put in the experimental tank, and then allowed to acclimate for 72 hours. All experiments took place at approximately noon. First, their control behavior (no sound stimulus) was video recorded for 5 minutes, after which the conspecific sound tone or the 500 Hz tone (depending on which treatment was randomly selected to be used first) was played back on a continuous loop for an additional 5 minutes. The filter and heater were turned off during the experimental period to minimize background noise. Fish in the first group remained in the experimental tank for all five treatments, after which they were removed to maintenance tanks and replaced with a new group of 20 fish (Table 1).

Data Collection

White tape was used to make a 6 X 3 grid with each square measuring 21.5 x 15.5 cm on the tank's front glass (Figure 9). The experiments were analyzed by stopping the video at each minute (1, 2, 3….10 minutes) following either the start of the experiment (for the control period) or the start of the sound stimulus (for the treatment period). The total number of fish in each grid square on the right and the left side of the aquarium were counted at each minute.

Statistical Analysis

Analysis of variance was used to examine the effect of location (rows and columns of the tank grid) on fish density. The percentage of total fish on the right and left sides of the tank was calculated for the conspecific click, tone, and control treatments. A Kruskal-Wallis test was used to test significant differences in percentage of fish on both sides of the tank between the 5th and 6th minutes for all treatments. The start of the 5th minute was the moment the sound playback started and where a response was most expected. In addition, to test for potential effects of previous exposure to the sound stimuli, analysis of variance (ANOVA) was used to test for the differences between percent fish on the sound speaker tank side between the first and second exposures to treatment sounds. SYSTAT (Version 13) was used for all statistical analyses.

Results

Fish preferred the glass sides of the aquarium closest to both speakers for all treatments (click, tone, and control) from the 1st to the 10th minute (i.e., throughout each experiment) and when they were located in the middle of the tank they were usually at the bottom of the tank (row 3; Figure 10). The same trend was observed in the 5th and 6th minute of the experimental period for all treatments (Figure 11). Similar distributions of fish were exhibited in the before and after playbacks of the conspecific click and tone treatments for all time periods of the experiments (Figure 12). In general, even though there was an overall row and column effect ($P < 0.001$) there was no treatment effect ($P > 0.05$). In addition, there was no timing effect on fish distribution when before (1-5 min) and after (6-10 min) sound playback were compared.

Significant “side” effects occurred in sound-treated experiments. In tone treatments trials, fish were most often on the side of the speaker producing the tone. In the click treatment trials, fish were most often on the opposite side of the aquarium from the sound source if it was the left speaker, but not the right speaker. There was a significantly greater percentage of fish on the right side of the tank with the right speaker as the sound source in tone treatment trials ($P < 0.001$), but a similar side effect was not evident for the control and click treatment trials (Figure 13). When the left speaker was the sound source, the percent of fish on the right side of the tank was significantly greater than the left side for the conspecific click treatment ($P < 0.001$) and significantly greater on the left side for the tone treatment but not the control (Figure 14). For conspecific click treatment trials, the percent of fish on the right side of the tank was significantly greater than on the left side when the sound was coming from the left speaker at 5 min (Figure 15A), and the percentage of fish on the right was significantly greater than on the left side when the sound was coming from the right speaker at 6 min (Figure 15B). For tone trials, the percentage of fish did not differ by side when the sound was coming from the left speaker at either 5 or 6 min (Figure 16A), and the percentage fish on the right was significantly greater than on the left side when the sound was coming from the right speaker at both 5 and 6 min (Figure 16B). For the control treatment trial, percentage of fish on the right side of the tank was significantly greater at 5 min but not at 6 min (Figure 17). Thus, between the 5th and 6th minute, fish moved towards the speaker producing conspecific clicks (Figure 15; $P < 0.01$) but not tones (Figure 16; $P > 0.05$) and no directional movement was evident in controls (Figure 17; $P > 0.05$).

Differences between percent fish on the sound speaker tank side between the first and second exposures (one being from the left speaker and the other from the right speaker but in a random order) to treatment sounds (conspecific clicks or 500 Hz tone) were tested separately. The difference was significant for the click treatment ($P < 0.001$) but not the tone treatment ($P > 0.05$; Figure 18). Fish were more attracted to the sound speaker tank side in the first trial exposing them to the conspecific click compared to the second trial exposing them to the same stimulus.

Table 1. Schedule of the four-week experimentation period. LS = left side speaker, RS = right side speaker.

Day	Fri	Sat	Sun	Mon	Tues	Wed	Thurs	Friday
Week 1	Acclimation period			LS Click	RS Tone	RS Click	LS Tone	Control
Week 2	Acclimation period			RS Click	Control	RS Tone	LS Tone	LS Click
Week 3	Acclimation period			LS Tone	RS Tone	Control	RS Click	LS Click
Week 4	Acclimation period			RS Tone	RS Click	LS Click	Control	LS Click

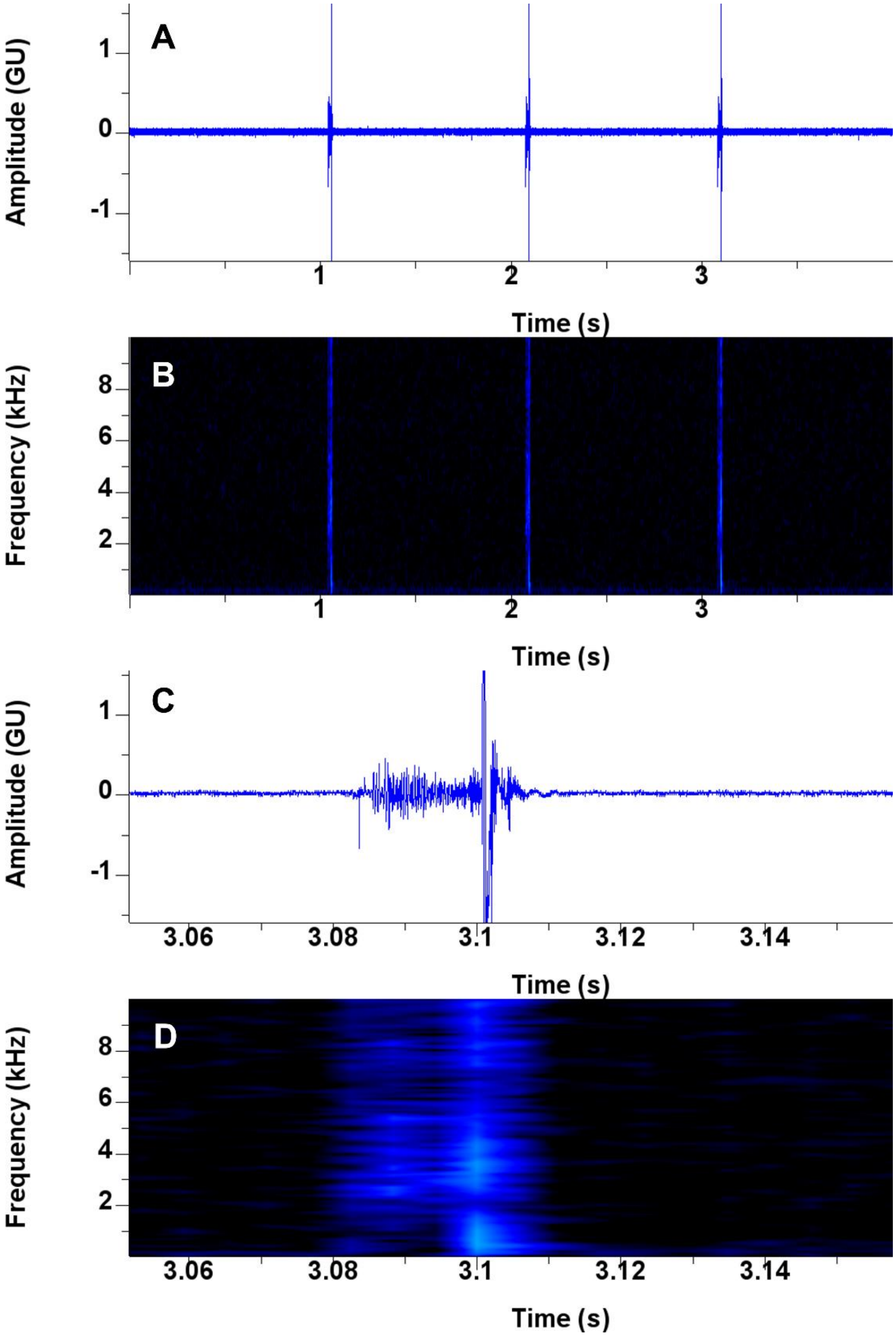


Figure 8: Oscillograms (A and C) and spectrograms (B and D) of the *O. vittatus* call with three repetitions (A and B) and an enlarged image of one of the calls (C and D).

Figure 9: Experimental set-up showing experimental tank, left and right University Sound UW-30 speakers, hydrophone, two filters, and a heater with a group of 20 fish.

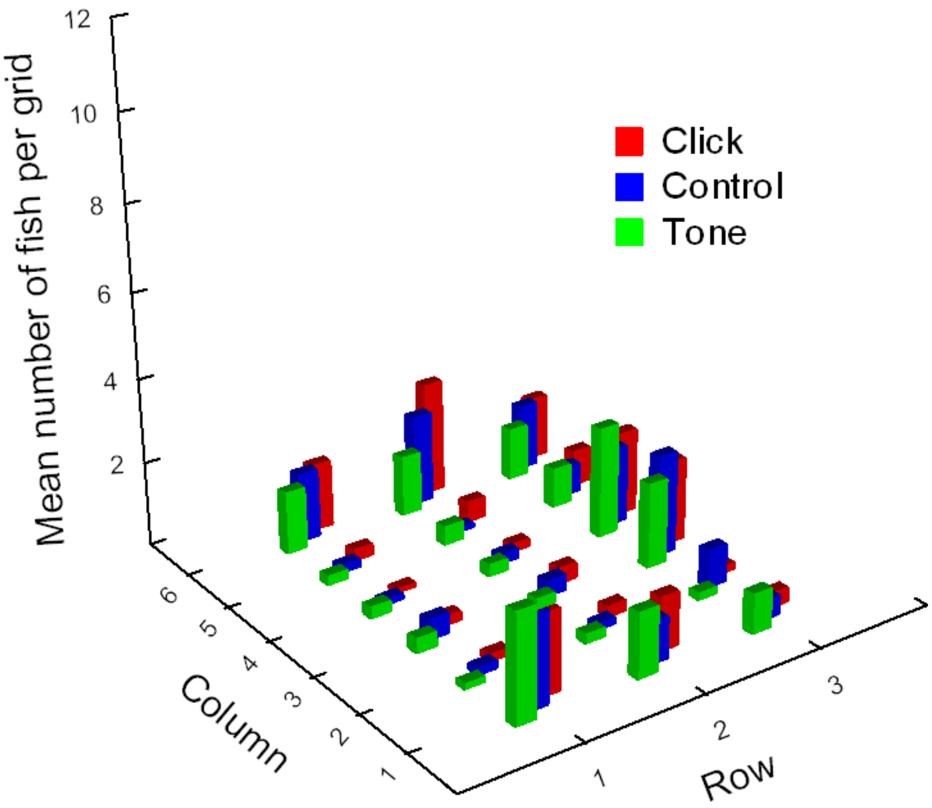


Figure 10: Fish location on the grid for all treatments during the experimental period from the 1st to the 10th minute.

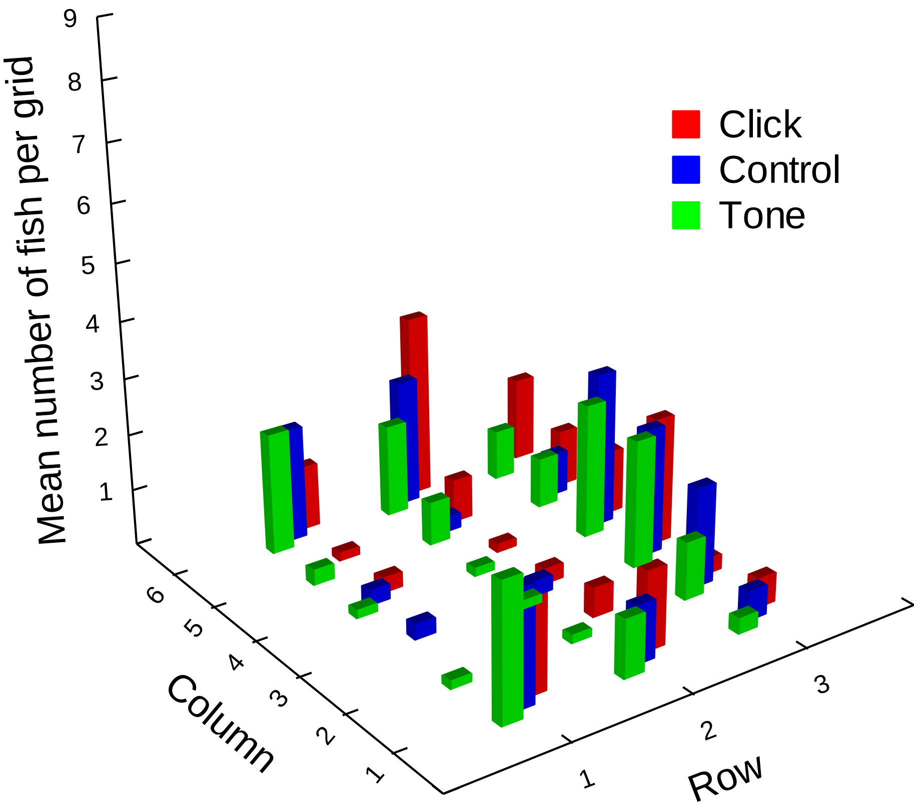
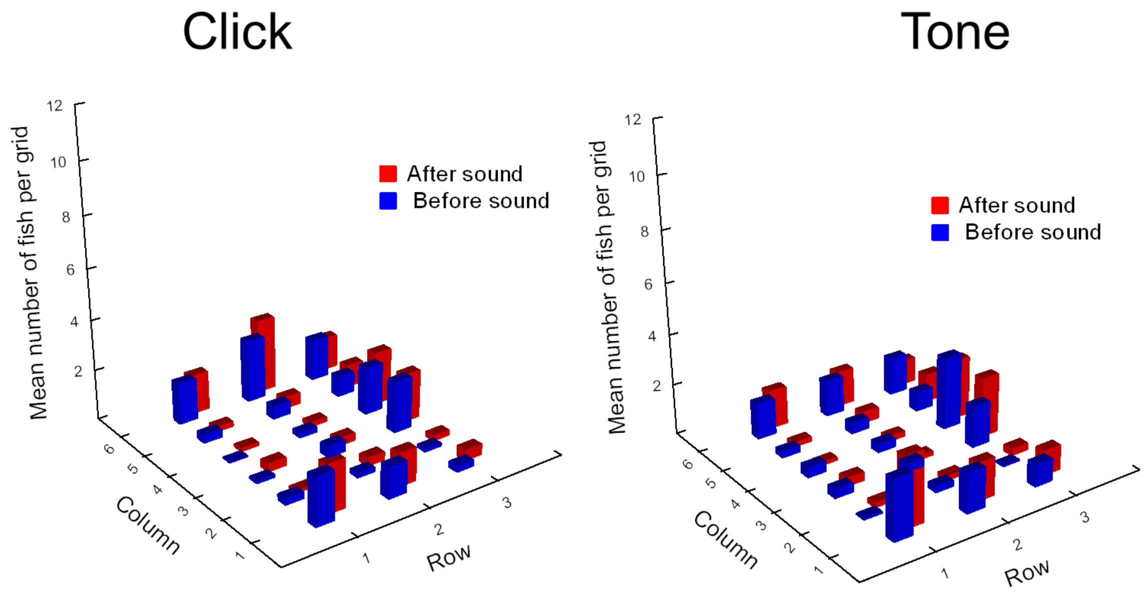


Figure 11: Average fish location on the grid for all treatments at the 5th and 6th minute of the experimental period.



B = Before sound stimulus
A = After sound stimulus

Figure 12: Experimental data showing fish positioning for conspecific click (left) and tone (right) throughout the experimental period.

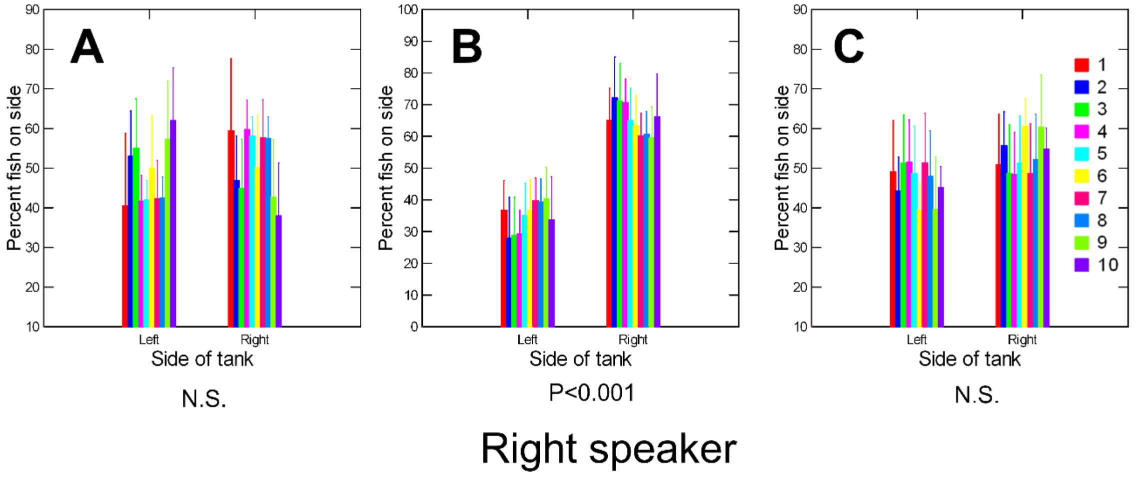
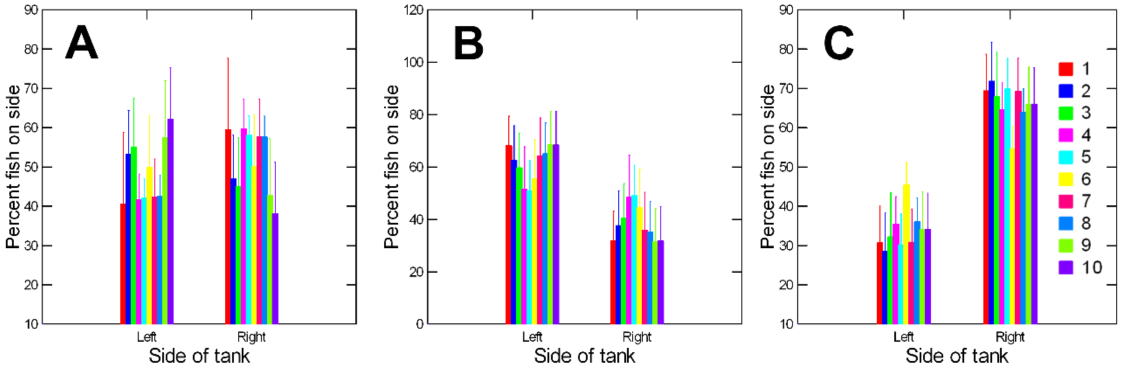


Figure 13: Mean (±SE) percentage of fish on both sides of the tank with the right speaker as the sound source. A, B, and C represent control, tone, and click, respectively.



N.S.

P<0.001

P<0.001

Left speaker

Figure 14: Mean (±SE) percentage of fish on both sides of the tank with the left speaker as the sound source. A, B, and C represent control, tone, and click, respectively.

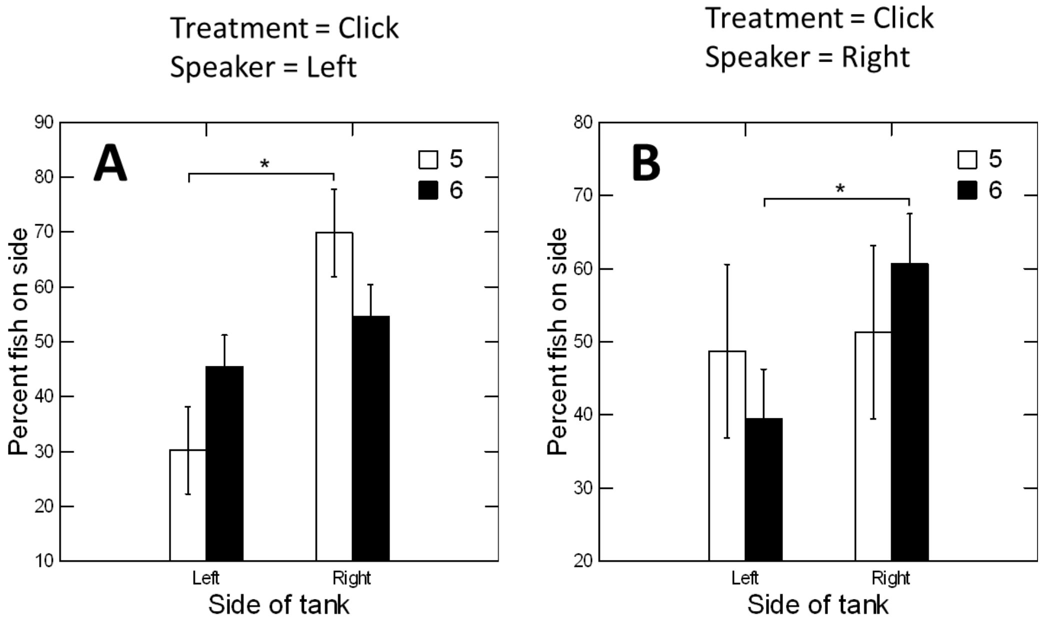


Figure 15: Mean (±SE) percentage of fish on either the left or right side of the tank at 5th and 6th min of the experiments in which conspecific sound clicks came from the left speaker (A) or right speaker (B).

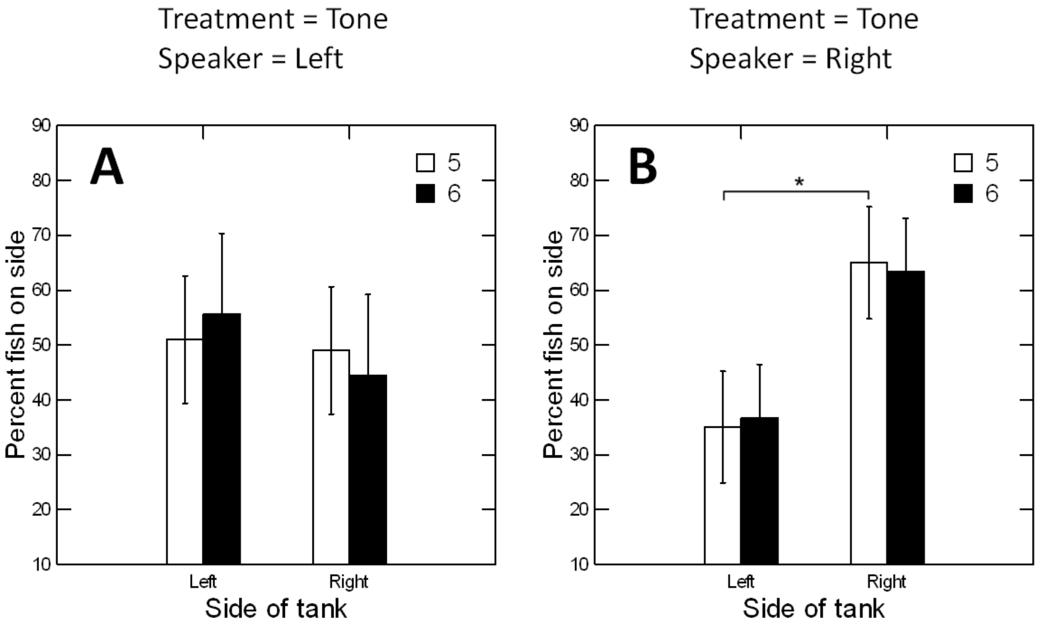


Figure 16: Mean (±SE) percentage of fish on either the left or right side of the tank at 5th and 6th min of the experiments in which the tone sound came from the left speaker (A) or right speaker (B).

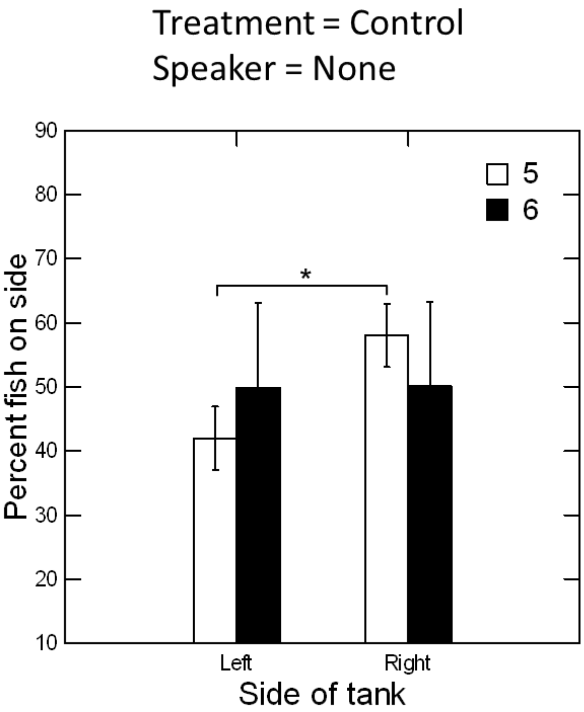


Figure 17: Mean (±SE) percentage of fish on either the left or right side of the tank at 5th and 6th min with the control experiment.

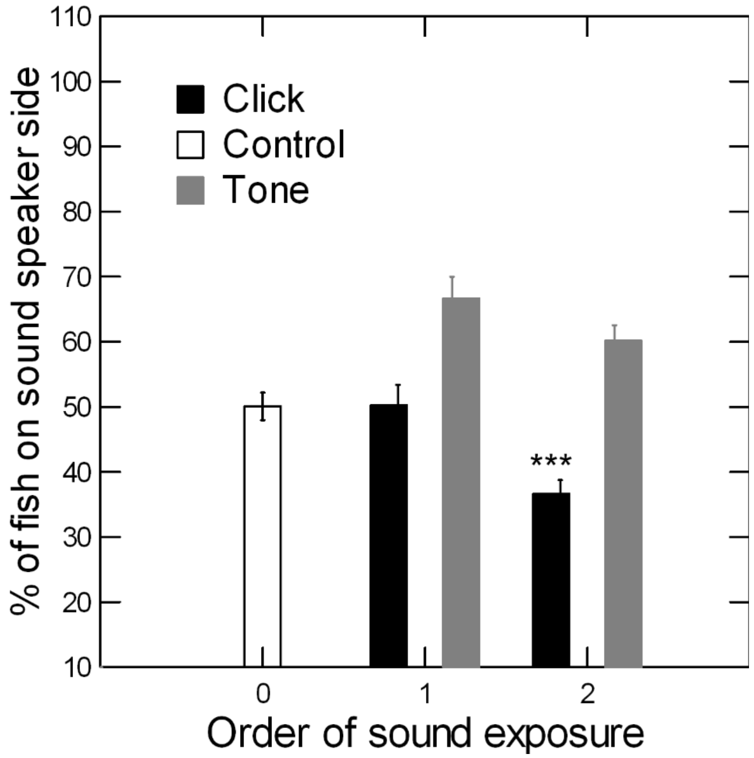


Figure 18: Mean (±SE) percentage of fish on the side of the tank with the sound-producing speaker as a function of the order of exposure to treatment sounds (first or second exposure). The control, which did not have a sound-producing speaker, was coded as 0 and shown for reference. Separate ANOVAs were used to test for significant differences between percent fish on the sound speaker side between first and second exposures for the two sound treatments (click or tone). *** P < 0.001.

CHAPTER 4 – DISCUSSION

The current study is the first to examine behavioral responses of loricariid catfishes to conspecific acoustic stimuli. In the first experiment, I found that even though *P. pardalis* showed a significant response to conspecific sound, for much of the experimental period, individual fish were static most of the time. Loricariid catfishes have evolved several antipredator morphological strategies, including flexible, bony plates and strong pectoral fin spines (Covain and Fisch-Muller 2007). In addition, they are mostly benthic species, utilizing their suckermouths to adhere to objects in their habitat while scraping algae from their surfaces (Weber 2003). Most loricariids exhibit a darkly colored dorsal surface, usually with cryptic patterning that helps them camouflage against the darker bottoms of most river environments. Thus, simply remaining motionless may be an effective anti-predator response for *P. pardalis*.

P. pardalis sometimes moved in response to conspecific sounds, which could also be an anti-predatory response, while movement in response to the 500 Hz tone rarely occurred. If the conspecific sound stimulus represented an alarm call from another individual, movement in response to the sound could be the fish seeking shelter, although in our experimental tank there were no structures provided to allow the fish to hide. In contrast to the current study, a previous study found that *P. pardalis* displayed no significant change in behavior to conspecific stridulation sounds (Slusher 2018) even though they should have been able to detect such low-frequency sounds since they are otophysans with sensitive hearing (Popper et al. 2022). Slusher (2018) hypothesized that the stridulation sound's function could be to keep predators away rather than trigger a startle response. This may be why *P. pardalis* moved minimally during the playback of the sounds in the current study. Playback of stridulation sounds of channel catfish (*Ictalurus punctatus*) had no effect on predation of them by largemouth bass, which does not support this antipredator function, but this could mean that the stridulation sound produced could be for distinct predators and not a broad range of predator species (Bosher et al. 2006). Catfish are known to stridulate more when out of water, which is usually due to high-stress levels, which results in greater sound production and behavioral response in air as compared to under water (Kaatz 1999).

Stridulation sounds in *P. pardalis* could be a warning signal to other conspecifics. Such acoustic warning signals are common in other animals. For example, prairie dogs use alarm calls that are primarily auditory cries which have been used to detect vocabulary, cognitive recognition, and intercolony dialect (Frederiksen and Slobodchikoff 2007; Kiriazis and Slobodchikoff 2006; Slobodchikoff et al. 2009; Smith et al. 1977). Meerkats mostly produce alarm calls when the risk of running into a predator is greater (Townsend 2012). A fish example is the Western striped grunter (*Pelates octolineatus*) producing alarm calls before and during attacks from predators. These calls cause behavioral changes in conspecifics as they seek safety and avoid predators by moving away from conspecific calls (Ladich 2022).

While I hypothesized that *P. pardalis* sound production is an alarm call, I hypothesized that stridulation sounds in *O. vittatus* are contact calls. In the present study, *O. vittatus* revealed that individual members of each group showed a significant response to conspecific clicks. In general, the fish were not distributed randomly within the tank. They were mostly attached to the right or left side of the glass near the speakers, or centrally at the bottom of the tank. This is not unexpected as this species likes to hide and attach vertically to surfaces to scrap algae off these surfaces (Britto et al. 2002). The hanging speakers on either side provided a structure for them to hide behind. They rarely swam in the middle of the water column of the tank since they were mostly oriented on surfaces (either the glass sides or the gravel bottom). *O. vittatus* showed a short-lived response to conspecific clicks by moving toward the speaker between the 5th and 6th minute of the experiment (this minute immediately after the onset of the conspecific sound had the most evident change in response during the experimental period). During conspecific click trials, only one to three of the fish moved out of the 20 within a group in response to the sound. *O. vittatus* may have moved toward the sound source of the conspecific clicks to find other individuals and it is possible they use sound to maintain cohesion within a school of individuals. *Otocinclus spp.* occur in large schools of up to 10,000 individuals (Evers and Seidel 2005). A potential reason that relatively few numbers of *O. vittatus* moved in response to conspecific sound may have been because they were already in a school (i.e., the experimental tank is a relatively small space compared to what they would be used to in the wild) and remaining in that space with other individuals could be their best form of protection rather than moving toward the sound source.

Another schooling fish example of acoustic communication is seen in Pacific herring (*Clupea pallasii*). They produce high-frequency calls by releasing gas from their anuses (Wilson et al. 2004) and have been shown to be able to detect sounds at 1 kHz and higher (Mann et al. 2001). Thus, it is hypothesized that they use these sounds for intraspecific communication. The herring sounds could be for other purposes, like mating and alarm calls, but contact calls seem more plausible. Herring occur in large schools like *O. vittatus,* and may use sound to maintain cohesion (Wilson et al. 2004). Other examples of contact calls are found in migrating warblers like the Canada warbler (*Cardelina canadensis*). They use contact calls to keep in touch with each other while foraging for food. Such calls are also used to communicate location to conspecifics, especially in dense vegetation where visual contact is easily obscured (Bradbury and Vehrencamp 2011). In addition, ring-tailed lemurs use meow calls to maintain group movement and avoid separation (Oda 1996). As *O. vittatus* travel in large groups and live in South American ecosystems where the water is often very turbid (Evers and Seidel 2005), contact calls might be useful to maintain school cohesion.

In the current study, it is unclear how realistic the sound stimuli used for the *P. pardalis* and *O. vittatus* experiments were because recordings of these two species have never been performed in the wild. Thus, stridulation sounds were induced from these species by holding them by their pectoral fin spines (Webb 2011, Stewart 2012). It is unknown how these human-induced sounds compare to sounds produced naturally by these two species, so I had no basis of comparison in terms of number of calls at a given time, intensity, frequency, spacing of the pulses, and repetition. Stewart (2012) found that *Otocinclus affinis* (which was likely *Otocinclus vittatus*) were attracted to conspecific clicks after food conditioning when clicks were played once every 3 seconds for 15 minutes. The stimulus used in the current study was similar but differed in that three conspecific clicks were presented in a row, followed by either 25 s (in the *P. pardalis* experiment) or 10 s (in the *O. vittatus* experiment) of silence. In the current study, *O. vittatus* actively avoided the conspecific click sound during the second trial in which they were exposed to it (Fig. 18). It is possible that the first exposure to the conspecific sound initially induced curiosity and investigative behaviors to find a potential source of the sound (e.g., another fish), but when no such sound-producing individual was found, the conspecific clicks may have induced avoidance upon secondary exposure to it.

Without sound recordings from these two species in the wild, or at least in the lab during normal fish interactions, it will be impossible to know which aspects of sound production may be most relevant for behavioral responses. During spawning, the male oyster toadfish (*Opsanus tau*) produces a continuous boat whistle to attract females (Mensinger 2014). Recordings of this boat whistle sound have been recorded in the wild, making it possible to playback the sound for experimental purposes and study which characteristics are most attractive to females (Mensinger

2014). Such recordings of loricariid sounds will be needed to improve future behavioral studies on the bioacoustics of sound production in these catfishes.

The sound stimuli used for the two experiments in this study (171 dB re 1 µPa for *P. pardalis* and 155 dB re 1 µPa for *O. vittatus*) were sufficiently loud enough for loricariid catfish species to detect. Botta (2009) showed that *M. affinis* were able to detect sound as low as 80 dB re 1 µPa with frequencies between 600 Hz and 1800 Hz, while *Corydoras sodalis* catfish are able to detect sound between 117 and 121 dB at 4 and 5 kHz (Lechner and Ladich 2008). The sound stimuli intensity for both current experiments was considerably above the hearing threshold for catfishes. Loud sounds significantly above hearing thresholds can produce hearing loss in fishes (Smith et al. 2006, Smith et al. 2011, Smith 2012), but it is unlikely that *O. vittatus* and *P. pardalis* experienced any hearing loss. First, the sound stimuli were short pulses of less than a half a second each and total exposure duration was for only 2 or 5 min. Most studies of hearing loss in fishes exposed fish for 24-48 hours of continuous sound, at sound pressure levels that were greater than those used in this study. For example, hearing loss and sensory hair cell loss was induced in goldfish (*Carassius auratus*) exposed to tones of 176 dB re 1 µPa for 48 h (Smith et al. 2011). In addition, the sound pressure levels of the current study were measured 2 cm away from the underwater speaker. Since sound pressure attenuates rapidly with the distance from the sound source, sound pressure levels were considerably reduced throughout much of the tank compared to the maximal levels presented.

Although I have hypothesized that *P. pardalis* produce stridulation sounds with an antipredator function and *O. vittatus* with a cohesion function, fishes produce sounds under other contexts as well. Many fishes produce sound for mating purposes (Ladich 2022). For example, male *Prochilodus spp.* produce calls during the spawning season to attract females. Only males possess sonic muscles on their swim bladder used for sound production (Smith et al. 2018). No sexual dimorphism has been reported in the dorsolateral surface of the dorsal process of the base of the pectoral fin spine that is used to produce sounds (i.e., the ridges of the pectoral fin spine) in the species used in the current study, although males can have more developed spines with hypertrophied odontodes on the spine in some loricariid catfishes (Pereira et al. 2014). Thus, we predict that both sexes should be able to produce similar sounds via stridulation in both *P. pardalis* and *O. vittatus* and that males likely do not produce calls to attract females as is found in other sound producing fish species.

Fish often produce sounds at frequencies where their hearing is the most sensitive (Ladich and Yan 1998). A match between sound and hearing frequencies was found in midshipman, damselfish, and piranha (Cohen and Winn 1967; Myrberg and Spires1980; Stabentheiner 1988; McKibben and Bass 1996), but a slight mismatch was found in toadfish (Fine 1981). *O. affinis* clicks have a hearing frequency range between 1,200 Hz to 6,600 Hz (Webb 2011), while their hearing is most sensitive between 600 Hz and 1,500 Hz (Botta 2009). The *O. vittatus* clicks have a peak frequency of 407 Hz and a secondary peak of 3,579 Hz, which is well within the range of hearing sensitivity of *O. affinis*. *O. vittatus* hearing sensitivity should be similar to that of *O. affinis,* whose best hearing sensitivity matches fairly well with the frequencies of *O. vittatus* click sounds.

In conclusion, *P. pardalis* and *O. vittatus* respond to their individual conspecific ssounds. *P. pardalis* moved in response to conspecific sounds but not tones, while the *O. vittatus* moved towards the speaker producing conspecific clicks but not tones. The result of this research is novel in loricariid behavioral studies and provides background work for future studies. Behavioral studies could be performed in other loricariid species to evaluate their responses to conspecific sounds to compare to the responses presented here for *P. pardalis* and *O. vittatus*. For a better replication of this study, future studies should record stridulatory sounds in the wild or in natural tank environments to assess how these fish would respond to potentially more realistic sound stimuli.

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