Sound Production And The Effect Of Noise On Communication In The Blacktail Shiner (*Cyprinella venusta*)

by

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A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
May 7, 2012

Keywords: acoustic communication, Cyprinella, environmental noise,
fish, hearing, propagation

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Abstract

Animal communication is important in the success and survival of species. In order to find a potential mate, individuals must be able to communicate effectively with each other. This communication may involve identification of the proper species, individuals which are in the proper physiological and motivational states for reproduction, and possibly the coordination of gamete release. Communication is also necessary for many aspects of an animal's life that relate indirectly to reproduction. The interaction and competition between males for resources and mates, for example, is often dependent upon communication. Although most people are not aware of it, the underwater world of freshwater streams is alive with sound, and many fishes in these environments communicate acoustically. The ability to communicate effectively using acoustics can be highly affected by levels of ambient noise. In a natural setting, this noise may be a selective pressure in the evolution of acoustic signals. However, in recent years, the breadth and level of anthropogenic noise has increased drastically, and little attention has been given to the effect it may have on freshwater fishes, especially those inhabiting small to moderately sized streams. Freshwater fishes in the genus *Cyprinella* have been shown to communicate acoustically during the reproductive season. Several species have been described in detail, but *Cyprinella venusta*, an extremely common species in the southeast United States, has not. This dissertation provides the first detailed description of acoustic signals and associated behaviors in *C.*

venusta and makes the first comparison of acoustic signals and behaviors recorded in the field and laboratory in the genus *Cyprinella*. A description is also made of the natural soundscape, anthropogenic noise sources, hearing abilities, and propagation of signals and noise sources in the natural environment in order to determine how far acoustic signals are utilized, and how anthropogenic noises may impact the ability of *C. venusta* to communicate. Finally, I investigate the ability of *C. venusta* to adapt to elevated noise levels in the laboratory. The results of the current study advance our understanding of sound production in the genus *Cyprinella*, and also our knowledge of how noise may impact vocal fishes in freshwater systems.

Acknowledgments

Partial funding was provided by: the Auburn University Graduate Research Award, the Department of Fisheries and Allied Aquaculture, and the Sigma Xi Grants-in-Aid of Research award.

I would first like to give my most sincere thanks to my advisor, Dr. Carol Johnston, for all the support, encouragement, and insightful advice she has provided me during the portion of my life at Auburn. I would also like to thank the many colleagues who provided valuable input and instruction, especially Drs. David Mann, Dennis Higgs, and Frank Jordan.

I would also like to thank my parents, brother and sister for their unwavering support and love. They have been a great source of strength in my life, and have always been there when the going gets tough, a common occurrence during the course of a dissertation.

To Ivey. From late nights at buffalos, making check-lists on how to graduate on time, and spending an hour under a little covered bridge, to the moment we said “I do” under our Arbor, the time I’ve been blessed with you has been the greatest of my life. I love you!

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Chapter 1

Sound Production And Associated Behaviors In *Cyprinella venusta*; A Comparison Between The Laboratory And Field.

Although it has been known that *C. venusta* produced acoustic signals during reproductive interactions, the only previous description was basic and provided very limited information describing both behaviors and physical characteristics of vocalizations. Since the initial description of sounds in *C. venusta*, the acoustic signals and associated behaviors of a number of other fishes in the genus *Cyprinella* have been investigated and described in detail. A detailed description of sounds produced by *C. venusta* is a necessary first step in the quest to further understand how this species lives in close association with other closely related, acoustically active species and its environment. In the current study, I described the sounds produced by *C. venusta*, along with associated behaviors in both a laboratory, and a field setting. I found that males were the only sex to vocalize and did so during reproductively associated behaviors such as courtship, aggression, and spawning. Sounds were similar in gross structure to a few other species of *Cyprinella* in that they were composed of bursts and knocks, but seemed to differ in a number of signal parameters including frequency, pulse duration, pulse interval, and pulse rate. One of the more striking findings was that the frequency distributions of both growls and knocks in *C. venusta* were bi-modal, a characteristic not mentioned for any other species in the genus *Cyprinella*. I also observed several differences between field and laboratory recordings with the laboratory generally showing a smaller subset of behaviors that were observed in the field. Also, sounds recorded in the laboratory were found to be significantly different from those recorded in the field.

Introduction

In all sexual animals, successful reproduction is not possible without some form of communication (Bradbury and Vehrencamp 1998). The use of acoustics as a mode of communication has been documented in a wide variety of terrestrial taxa including birds (Slater 2003), reptiles (Dooling et al. 2000), frogs (Narins et al. 2007), mammals (McComb and Reby 2005), and insects (Römer 1998). Acoustic communication has also been documented in aquatic organisms including marine mammals (Janik 2005), aquatic insects (Aiken 1985), and fishes (Ladich 2004). Despite the fact that fishes account for approximately 51% of all recognized vertebrate biodiversity (Nelson 2006), and inhabit a wide variety of habitats ranging from shallow, freshwater streams, to the deepest reaches of the oceans, only a small percentage of fish species have had their acoustic repertoires thoroughly described. A more complete understanding of the commonalities between the acoustic signals of different fish species may provide additional insight into the evolutionary history of acoustic signaling in fishes, as well as provide a better framework on which further bioacoustic studies may be based.

Acoustic communication is likely important for many fishes due to its association with important tasks that directly influence fitness such as territory defense (Lugli 1997; Myrberg 1997), aggressive interactions (Ladich and Myrberg 2006), and courtship and reproduction (Winn and Stout 1960; Stout 1975; Lugli et al. 1995; Phillips and Johnston 2008). Acoustic signals likely act as indicators of male quality, which can be advantageous to both males during agonistic interactions, and females during courtship interactions. When competing for a resource, a male will fare better if it is able to assess the fighting ability of a competitor without escalating directly to a physical contest, which typically risks injury or death. Ladich (1998) found that body size was negatively correlated with dominant frequency, and positively correlated with the level of acoustic signals produced by the male croaking gourami (*Trichopsis vittata*), and that larger males typically won agonistic contests. Ladich also showed that traits not correlated with male body size (such as number of sounds produced) did not predict winning. A thorough description of acoustic signals and the relationships between signal parameters and potential indicators of fitness such as body size are necessary before further studies investigating more complex issues such as functional significance can be conducted.

Descriptions of sound production for many fishes have often been conducted in small aquaria under laboratory conditions (Drewry 1962; Stout 1963; Stout 1975; Vilinski and Rigley 1981; Colson et al. 1998; Ladich and Yan 1998; Pruzsinszky and Ladich 1998; Lugli and Torricelli 1999; Johnston and Johnson 2000a, b). Numerous complications may arise when recording sounds in an aquarium due to the effects of reverberation and resonance in the tank. Although empirical solutions have been determined to help minimize these effects (Akamatsu et al. 2002), direct comparisons should also be used to verify that signal parameters measured in the lab are consistent with sounds produced under natural conditions. A few studies have provided evidence that sounds and behaviors observed in the laboratory are representative of those under natural conditions (Lugli et al. 1995; Crawford et al. 1986; Kaatz and Lobel 2001), however, the majority of studies have not attempted to make this comparison.

In recent years, the acoustic signals of several new species in the genus *Cyprinella* have been described in great detail (*C. galactura,* Phillips and Johnston 2008; *C. gibbsi*, *C. trichroistia, C. callisema*, Phillips and Johnston 2009; *C. lepida*, Phillips et al. 2010). However, all of these descriptions, as well as earlier, general descriptions of *C. lutrensis*, *C. venusta* (Delco 1960), and *C. analostana* (Winn and Stout 1960) were based on signals obtained under laboratory conditions. Despite the growing number of studies, no study to date has attempted to compare the acoustic signals recorded under natural field conditions to those recorded in laboratory conditions for fishes in the genus *Cyprinella*.

Although sound production has been documented in *C. venusta* by Delco (1960), the detail with which the signals were described was minimal, reporting only the duration and dominant frequency of the acoustic signals. Furthermore, Delco only reports one sound type from *C. venusta*, which is suspicious considering most other descriptions of sounds from fishes in the genus *Cyprinella* report two or more sound types. The current study provides a detailed description of the acoustic signal structure of *C. venusta*, and compares acoustic signals recorded under laboratory conditions to those recorded under natural conditions in the field. Acoustic signals produced during agonistic and reproductive contexts, as well as specific behaviors are compared in terms of signal structure. Correlations between fish size and signal parameters are discussed, and agonistic and reproductive behavioral repertoires and corresponding sounds are briefly described.

Methods

Laboratory Recordings

For the laboratory experiments, fish were captured by seine from tributaries to the Chatahoochee River in Lee County, East Alabama, specifically Little Uchee Creek (32.508579° N, -85.184215° W), Halawakee Creek (32.697579° N, -85.266951° W, and Wacoochee Creek (32.622799° N, -85.132685° W). Fish were transported back to the lab and stored in 76 l aquariums with gravel substrates. Hanging, external filters were used on all tanks. Fish were kept at approximately 26°C, given a 12L:12D light cycle, and fed a diet of commercial flake food. Males that had not been recorded were kept isolated from other males, and females were stored with other females and males that were not to be, or had already been used. A 76 l experimental tank was setup in an acoustic chamber to conduct each experiment (Figure 1-1). A tile propped up on a brick forming a 4 mm crevice served as an artificial nest, and was placed on the wall in the center of the tank. The tank also contained an underwater speaker (University Sound UW-30, Oklahoma City, OK) suspended behind a thin mesh barrier located on one end of the tank. This speaker was used to test the potential effect of elevated noise on acoustic communication, the results of which will be included in a forthcoming paper.

To determine the distance between fishes during trials, a white piece of plexiglass with gridlines at 5 cm intervals was placed on the bottom of the tank, and an identical grid was placed on the back of the tank. A 60 W blub was hung approximately 1.5 m above the tank, and a camera was placed in front of the tank in such a way that both the bottom and rear grids were visible. The light bulb above the tank cast shadows of fish in the tank on the bottom grid, allowing the position of the fish along the X and Z axes of the tank to be determined from the video camera. The grid on the back of the tank allowed the position of the fish along the Y axis to be determined from the video camera.

Trials were conducted between 2 June – 6 August 2009, and 6 June – 12 August 2010. During each trial, behavioral data was gathered from male-male agonistic interactions, and male-female courtship interactions. Typically for each trial, 1 male and 1-4 females were placed in the experimental tank and allowed to acclimate. Acclimation periods varied by trial, and fish were considered to be acclimated when normal behaviors resumed (swimming freely), and the male appeared to be defending the artificial nest. After the fish had acclimated, a second male was placed into the tank in an attempt to stimulate aggressive behavior. During a typical successful trial, males would perform agonistic behaviors toward each other to establish dominance. After dominance had been established, the victorious male would begin courting the females while the losing male tried to avoid interaction with the dominant male. If dominance had obviously been established, and agonistic behaviors persisted, the losing male was removed from the tank and recording continued on the dominant male. Each trial was video recorded on a Sony handycam digital HD video recorder (HDR-SR11) and sounds were recorded onto Raven 1.3 (Cornell University, Ithaca, NY, U.S.A) using a Brüel and Kjaer 8103 hydrophone and a Brüel and Kjaer 2635 charge amplifier. Recording began at the onset of agonistic or courtship behaviors and ended when the behaviors ceased, or after 2 hours had passed.

The percent occurrence of sound production was determined for behaviors from all the trials. Percent occurrence of sound for each behavior was calculated as the number of times the behavior was observed with sound accompaniment divided by the total number of times the behavior was observed. For laboratory trials, video of each trial was watched, starting from the beginning. Behaviors that were clearly observed and well within acoustic range of the hydrophone (about 15 cm) were documented, along with whether or not they were accompanied by sound production, and if so, what type of sound was produced (knock or growl). Because in the laboratory, many trials contained too many behaviors to reasonably analyze, a subset was selected by analyzing each trial from beginning to end, or until 200 individual behaviors had been observed, whichever came first. In the field, because there were many more fishes present, it was often difficult to assign a sound to a particular fish performing a behavior. For this reason, field trials were always analyzed from beginning to end, and only behaviors that could be clearly distinguished as being accompanied by sound or not were included in the analysis.

Field Recordings

Field behavior recordings were conducted between 18 May – 28 July 2010, and again on 7 - 8 June, 2011 at Moffits Mill (Little Uchee creek, Lee Co., AL, 32.508579° N, -85.184215° W). Recordings were also attempted at other sites including Beans Mill (Halawakee Creek, Lee Co., AL, 32.697658° N, -85.266788° W) and Meadows Mill (Little Uchee Creek, Lee Co., AL, 32.528591° N, -85.254299° W), but a number of factors (such as a lack of abundant reproductive aggregations and highly turbid water) hampered my efforts at these sites, and useful data was unable to be collected. On each day of sampling, I would begin by walking up and down the study site, looking for spawning aggregations of *C. venusta*. Care had to be taken not to startle the aggregations, and polarized sunglasses were used to help detect them at farther distances. Reproductive males were readily identified by the white tips on their fins, and were typically found at the nesting site. Females and smaller males could be found at the nesting site and in surrounding areas, but the majority of them were almost always downstream of the active nest. When an aggregation was found, I carefully placed an underwater camera (Aqua-Vu ZT 60, Nature Vision, Inc., Brainerd, MN, U.S.A) and hydrophone (Hi-Tech HTI-96-MIN, sensitivity -164.4 re 1V/µPa) as close to the active nest as possible. Video and audio were recorded separately onto an AquaVu Digital Video Recorder, and a Marantz PMD 661 digital audio recorder (sampling rate 44100, PLM). A click was made in front of the camera to synchronize the audio and video for later combination.

When the aggregation was disrupted by placement or adjustment of the recording equipment, the fish would swim away. In some circumstances, the fish would not return after disruption, and could not be found afterwards. Other times, the fish would begin spawning on another nearby nest after disruption. In these cases, it was typically impossible to film the fish because they would switch nests any time the recording equipment was placed near them. In the rarely successful cases, the fish would return to the nesting site containing the recording equipment and resume reproductive activity.

Because of the nature of the field, I was unable to keep a count of behaviors for individual males. Multiple males that were indistinguishable on video would continuously swim in and out of the field of view, making it impossible to keep a count on any one specific male. Because of this, the data could not be classified into distinct samples, which made it impossible to statistically compare the occurrence of behaviors, and the presence of sound with behaviors between the laboratory and the field.

Signal Analysis

Cyprinella venusta acoustic signals were composed of two main components, knocks and growls (Figure 1-2), and after some experience looking at and listening to the sounds, it was fairly easy to identify an uncontaminated sound from the waveform and spectrogram. Knocks were always produced as a single pulse. Growls, on the other hand, were composed of two smaller subunits: bursts, and pulses. Pulses were the most basic component of growl call types and occurred at relatively consistent intervals within bursts. Bursts were composed of groups of pulses that occurred in succession without a break that deviated much from the typical interpulse interval. Bursts were variable in duration and in length, and growls typically consisted of multiple bursts made in succession with variable inter-bust intervals.

Video of each laboratory trial was watched while simultaneously reviewing the audio in Raven. Any time a behavior occurred in the video that was accompanied by a sound that was suitable for analysis (not contaminated by the fish hitting something, or mostly buried under the ambient noise), it was labeled in Raven. A total of 78 males were included in the laboratory trials, but only 20 males produced sounds. Of the 20 sound producing males, a varying number produced sounds associated with each behavior that were of high enough quality for analysis. A male, for example, may have performed a wide variety of behaviors accompanied by sound, but only a small subset of those behaviors may have been performed close enough to the hydrophone to obtain recordings of a quality that was adequate for analysis. Because of this, the ability to statistically compare signals between agonistic and courtship contexts, and between specific behaviors in the laboratory was limited to only the most commonly performed behaviors.

Methods for describing sounds produced in the field were similar to those for the laboratory. Recording was attempted at a total of 22 sites. All of the sites that produced data (14 sites) were located at Moffits Mill. Some behaviors (male-female chase, male-female circling, and male-female zig zag) whose signals were unable to be compared in the laboratory due to low sample sizes were recorded more frequently in the field, and were able to have their associated sounds compared statistically.

Temporal parameters of acoustic signals were measured using the waveform view in Raven (Table 1-1). Pulse duration was measured as the time difference between the first deviation from random background noise in the waveform to the point at which the pulse pattern in the waveform could no longer be detected. Pulse interval was measured as the time difference between identical peaks in adjacent pulses. Pulse rate was defined as the number of pulses within a single burst divided by the duration of that burst. Burst duration was measured as the time difference between the first deviation of the first pulse within the burst, and the point at which equilibrium was regained after the last pulse of the burst (the first and last pulses of a burst were subjectively determined by the same researcher throughout the study). Burst interval was measured as the time difference between the end of one burst and the beginning of the next. Burst rate was measured as the number of bursts within a call divided by the duration of the call (the number of bursts within a call was subjectively determined by the researcher, and depended on how long the corresponding behavior lasted). Knock duration was measured in a manner similar to pulse duration.

Spectral parameters were measured from the power spectrum function of Raven (Table 1-1). Both bursts and knocks showed two peaks in their spectral composition. For bursts, the peak at the lower frequency was referred to as the burst fundamental frequency, and the peak at the higher frequency was referred to as the burst harmonic frequency. Knock fundamental frequency and knock harmonic frequency also refer to the distinct low and high spectral peaks in knocks. The sound pressure level for each of the frequencies was referred to as the level for that frequency.

Due to the fact that in the lab, fish signals were recorded in a small glass tank, only signals that occurred within the calculated attenuation distance from the hydrophone for frequencies below 1 kHz (15 cm) were included in any type of analysis (Akamatsu et al. 2002). Because I was not certain of the distances fish were from the hydrophone in the field, only sounds that were clearly audible and clearly visible on the waveform were included in analyses. All statistical analyses were conducted in PASW software package (IBM SPSS Corporation, Chicago, Illinois, U.S.A.). To obtain the most accurate frequency information from the power density spectrum, sample sizes of FFT's were maximized for each knock or burst so that each point on the power density spectrum represented the smallest frequency increment possible. Decibel levels of the fundamental frequency and first harmonic of knocks and bursts were also measured from the pressure density spectrum, and were adjusted to represent actual levels by taking into consideration the gain imposed by each unit in the recording chain. Decibel levels were also corrected to represent spectrum level (1Hz bands; dB re 1µPa^2/Hz) by subtracting 10log(bin resolution) of each measured signal.

Prior to all statistical analyses, an average value for each signal parameter (pulse duration, burst duration, dominant frequency, etc) was calculated for each individual male. For sounds recorded in the laboratory, Pearson correlation coefficients were calculated using these averaged values to determine if any relationship existed between body size measured as standard length and signal parameters. If a correlation did exist, standard length was included as a covariate for that parameter during any analysis that was not a repeated measures or paired design. Correlations were also performed on sounds recorded in the laboratory (prior to averaging all data for each individual) to determine if there was a relationship between level and distance from the hydrophone. If levels were correlated with distance from the hydrophone, levels were corrected by multiplying the distance at which each sound was produced from the hydrophone by the slope of the linear best fit line, and adding this value to the original level of the knock. This eliminated the effect of distnace without affecting variability around the best fit line. Values that had been corrected for distance from the hydrophone were used in further analysis. Correlations between sound parameters and fish standard length and distance from the hydrophone were not performed on sounds recorded in the field because there was no consistently reliable reference for determining fish size or distance from the hydrophone.

Repeated measures ANOVAs were used, independently for laboratory and field conditions, to determine whether knock, burst, and pulse call components differed significantly from each other in terms of duration. Paired T tests, also independent for laboratory and field conditions, were used to determine whether knocks and bursts differed in fundamental frequency, first harmonic frequency, or level. Pulses were not included in frequency or dB comparisons because those signal parameters were not measured for pulses. Comparison of knock, burst, and pulse call components was limited to approach behaviors for both laboratory and field conditions because the approach behavior was the only behavior that was consistently accompanied by both knock and growl call types, and I believed it was more appropriate to compare knocks, growls, and pulses within a single behavior than to compare them among multiple behaviors.

To determine whether a difference existed between growls or knocks produced during agonistic and courtship contexts, behaviors that were accompanied by either knocks or growls under both contexts had to be identified. A challenge to this was that behaviors that were definitely part of the courtship or agonistic sequences were never shared. I therefore had to settle for behaviors that were somewhat ambiguous in context, and that were preformed towards both males and females. In the laboratory, knocks and growls were produced for approach and lateral display behaviors during both male-male and male-female interactions. Multivariate analysis of variance (MANOVA) with standard length as a covariate was used to determine whether signal parameters (dependent variables) differed between agonistic and courtship contexts (fixed factors) under laboratory conditions. This resulted in 4 separate MANOVA's; growls during approaches, knocks during approaches, growls during lateral displays, and knocks during lateral displays. In the field, knocks were not associated with courtship contexts and so only growls associated with approaches and chases were compared between male-male and male-female interactions. For sounds produced in the field, two separate MANOVA's were conducted; growls during approaches, and growls during chases. No covariate was used in the field because body sizes were not known. If the MANOVA showed a significant main effect of context for either laboratory or field conditions, then univariate ANOVA's or ANCOVA's were run on the specific signal parameters with fish standard length applied as a covariate where appropriate.

Bonferroni corrections were applied to the alpha values by multiplying 0.05 by the number of ANOVA's being run.

Methods used to determine whether signal parameters differed between specific behaviors (eg. approach vs lateral display behaviors) were similar to those used to test for differences in context, but in this case, I used doubly multivariate analysis of variance (with body size as a covariate in the laboratory; MANCOVA) with signal parameters as dependent variables and specific behaviors as the fixed factors. If behaviors were shared between courtship and agonistic contexts, and signals had been shown not to be different between contexts within a behavior, then the contexts were combined (eg. if signals produced during male-male approach and male-female approach behaviors were not different, they were combined into simply approach behaviors). A wider variety of behaviors were able to be included in the analysis because I was not confined by the requirement of the behavior being present in both agonistic and courtship contexts. In the laboratory, I was able to compare growls produced during approach, lateral display, and male (m) crevice inspection behaviors. In the field, I was able to compare sounds associated with m circling/guarding nest, m crevice inspection, approach, chase, mf circling, mf hover, mf spawning, and mf zig zag behaviors for growls, and approach, mm circle swim, and mm lateral display behaviors for knocks. If a doubly multivariate MANOVA showed a significant main effect of behavior type, then univariate ANOVAs with Bonferroni corrected post hoc tests on each dependent variable were conducted separately to see which behaviors differed, and in what signal parameters they differed.

Comparisons were made to determine whether sounds recorded in the laboratory differed from sounds recorded in the field for given behaviors. Behaviors that were recorded in both the lab and field with sufficient sample sizes for this comparison included m crevice inspection and approaches for growl call types, and approaches and lateral displays for knock call types. Four MANOVA's were performed, one for each behavior associated with growls, and one for each behavior associated with knocks. Signal parameters were used as the dependent variables, and the location in which they were recorded (laboratory or field) as the fixed factors. Pulse interval, burst first harmonic frequency, and levels were not included as dependent variables. Pulse interval and burst first harmonic frequency were not included because they were significantly correlated with fish standard length in the laboratory, and I could not test for this correlation in the field. I did not include any levels because they could not be tested for correlations with distance from the hydrophone in the field. If a MANOVA showed a significant main effect of laboratory or field locations, then univariate ANOVA's with Bonferroni correction were run on the specific signal parameters.

Results

Behavioral description

I observed a number of behaviors from *C. venusta* in the field and in the laboratory. Males were always the initiators of behaviors. Agonistic behaviors were typically directed at other males and courtship behaviors were typically directed at females. Sounds directed towards females in the laboratory included in order of increasing escalation; male-female (mf) approach, mf chase, mf lateral display, mf zig zag, mf circling, and mf hover. Behaviors performed towards other males in the laboratory included in order of increasing escalation; male-male (mm) approach, mm chase, and mm lateral display. I also observed three other behaviors that were not directed at another fish. The first, I called male circling/guarding nest behavior, which did not fall under either agonistic or courtship contexts due to the fact that I did not know if the male was guarding the nest, or advertising it to potential females. The other behaviors I called male crevice inspection and male crevice pass.

A wider variety of behaviors were observed in the field than in the laboratory. Field behaviors directed at females included in order of increasing escalation; mf approach, mf chase, mf zig zag, mf circling, mf hover, and mf spawning. Field behaviors directed at males included in order of increasing escalation; mm approach, mm chase, mm lateral display, mm circle swim, mm fight, mm lip locking, and mm bite. Other behaviors that were not directed at another fish included male circling/guarding nest, m crevice inspection, and m crevice pass, which were the same as under the laboratory setting.

Approach, lateral display, and chase behaviors were similar when directed towards either males or females. During approaches, the sound producing male would slowly approach the other fish, from any direction, and vocalize. Lateral display behaviors were rather variable, and consisted of the sound producing male displaying next to another fish with its fins erect. If the other fish was a male, it would show no response, swim away, or respond back with a lateral display. If the other fish was a female, it would never respond with another lateral display, and would not respond at all, or swim away. Chases consisted of a male quickly pursuing another fish for a distance greater than approximately 10 cm. During courtship, mf zig zag consisted of the male swimming very rapidly between the nest and the female being courted. The zig zag behavior seemed to function as a way of drawing the female closer to the nest, and only occurred with females that were already relatively close to the nest. Male-female circling behavior would occur closer to the nest than mf zig zag behaviors, and consisted of the male rapidly orbiting the female numerous times in rapid succession. The next behavior that typically took place was the mf hover, which took place in very close proximity to the nest. During mf hovers, the male would hover just above or beside the female, directly in front of the nest. The next behavior was typically mf spawning, during which time the male and female would swim together along the nest crevice, vibrating their bodies and presumably releasing gametes.

More escalated agonistic behaviors included mm circle swim, which involved two males chasing each other's tails in a circular pattern. Male-male fights and mm lip locking were the two most advanced agonistic behaviors. Male-male fights consisted of the two males circling each other while simultaneously hitting the sides of their bodies together, or hitting the each other with their heads. Male-male fights occurred extremely quickly and required slowing the video down in order to decipher what was happening. Male-male lip locking has been described previously for one species (*C. galactura* ; Phillips and Johnston 2008), and consisted of the males locking at the mouth for a short period of time. When mm lip locking did occur in *C. venusta*, it always occurred just prior to a mm fight. Male-male fights, however, did not have to be preceded by a mm lip lock.

Variation was observed in the type of sound that accompanied behaviors, as well as the degree to which behaviors were accompanied by sound in both the laboratory (Table 2), and field (Table 3). In the laboratory, mm approaches were accompanied by knocks 29.11% (n=158, total number of mm approaches) of the time, growls 59.49% of the time (n=158), and no sound 10.76% (n=158) of the time. Male-male chases were accompanied by knocks 17.17% (n=99) of the time, growls 62.63% (n=99) of the time, and no sound 16.16% (n=99) of the time. Male-male lateral displays were accompanied by knocks 42.5% (n=240) of the time, growls 51.25% (n=240) of the time, and no sound 6.25% (n=240) of the time.

In the laboratory, mf approaches were accompanied by knocks 19.34% (n=181) of the time, growls 64.64% (n=181) of the time, and no sound 16.02% (n=181) of the time. Male-female lateral display behaviors were accompanied by knocks 11.41% (n=184) of the time, growls 72.83% (n=184) of the time, and no sound 15.22% (n=184) of the time. Male-female chase behaviors were accompanied by knocks 25.00% (n=44) of the time, growls 63.64% (n=44) of the time, and no sound 11.36% (n=44) of the time. Male-female zig zag behaviors were accompanied by knocks 0% of the time (n=42), growls 92.9% (n=42) of the time, and no sound 7.14% (n=42) of the time. Male-female circling behavior was accompanied by knocks 5.1% (n=118) of the time, growls 84.7% (n=118) of the time, and no sound 10.2% (n=118) of the time. Male-female hovers were accompanied by knocks 1.9% (n=53) of the time, growls 92.4% (n=53) of the time, and no sound 5.7% (n=53) of the time. Male guarding/circling nest was accompanied by knocks 7.1% (n=14) of the time, growls 92.9% (n=14) of the time, and no sound 0% (n=14) of the time. Male crevice inspections were accompanied by knocks 1.7% (n=121) of the time, growls 67.8% (n=121) of the time, and no sound 29.8% (n=121) of the time. Male crevice passes were accompanied by knocks 0% (n=51) of the time, growls 25.5% (n=51) of the time, and no sound 72.5% (n=51) of the time.

In the field, mm approaches were accompanied by knocks 46.7% (n=107) of the time, growls 36.4% (n=107) of the time, and no sound 16.8% (n=107) of the time. Male-male chases were accompanied by knocks 30% (n=40) of the time, growls 55% (n=40) of the time, and no sound 15% (n=40) of the time. Male-male lateral displays were accompanied by knocks 50.4% (n=141) of the time, growls 26.2% (n=141) of the time, and no sound 23.4% (n=141) of the time. Male-male circle swims were accompanied by knocks 85.4% (n=48) of the time, growls 0% (n=48) of the time, and no sound 14.6% (n=48) of the time. Male-male fighting was accompanied by knocks 89.4% (n=47) of the time, growls 0% (n=47) of the time, and no sound 10.6% (n=47) of the time. Male-male lip locking was accompanied by knocks 60% (n=10) of the time, growls 0% (n=10) of the time, and no sound 40% (n=10) of the time. Male-male biting was never accompanied by sound (n=10).

In the field, mf approaches were accompanied by knocks 0% (n=110) of the time, growls 95.5% (n=110) of the time, and no sound 4.5% (n=110) of the time. Male-female chases were accompanied by knocks 0% (n=58) of the time, growls 96.6% (n=58) of the time, and no sound 3.4% (n=58) of the time. Male-female zig zag (n=78), mf circling (n=112), mf hover (n=83), and mf spawning (n=33) behaviors were all accompanied by growls 100% of the time. Male circling/guarding behaviors were accompanied by knocks 0% (n=133) of the time, growls 86.5% (n=133) of the time, and no sound 13.5% (n=133) of the time. Male crevice inspections were accompanied by knocks 0% (n=167) of the time, growls 72.5% (n=167) of the time, and no sound 27.5% (n=167) of the time. Male crevice passes were accompanied by knocks 0% (n=71) of the time, growls 4.2% (n=71) of the time, and no sound 95.8% (n=71) of the time.

Acoustics

No correlation was found between distance from the hydrophone and level for either the fundamental frequency (Pearson correlation = 0.002, P = 0.931, N = 1397; Figure 1-3a) or the first harmonic frequency (Pearson correlation = -0.021, P = 0.438, N = 1397; Figure 1-3b) of bursts recorded in the lab. A significant negative correlation was found, however, between distance from the hydrophone and level for both the fundamental frequency (Pearson correlation = -0.384, P < 0.001, N = 143) and level of the first harmonic frequency (Pearson correlation = -0.378, P < 0.001, N = 143) of knocks recorded in the lab (Figure 1-4a). After correction, the correlation was eliminated for knocks (Figure 1-4b).

In the laboratory, pulse interval showed a statistically significant positive correlation with fish standard length for both approach (Pearson correlation = 0.564, P = 0.015, N = 18), and lateral display behaviors (Pearson correlation = 0.638, P = 0.019, N = 13; Figure 1-5b,d). Burst harmonic frequency showed a statistically significant negative correlation with fish standard length for both approach (Pearson correlation = -0.665, P = 0.002, N = 19), and lateral display behaviors (Pearson correlation = -0.660, P = 0.007, N = 15; Figure 1-5a,c). No other signal parameters showed a significant correlation with body size in the laboratory. No correlations between signal parameters and body size could be performed in the field.

An overall difference was found in duration between the three call types in both the laboratory (repeated measures ANOVA: $F_{2,9} = 26.37$, $P < 0.001$) and field (repeated measures ANOVA: $F_{2,6} = 62.10$, $P < 0.001$; Table 1-4). Under laboratory conditions, burst duration (mean ± SD; 0.08928 ± 0.03689 sec, N = 11) was significantly longer than both knock duration (0.01576 ± 0.005279 sec, N=11; Bonferroni corrected post hoc: P < 0.001) and pulse duration (0.007576 ± 0.001006 sec, N=11; Bonferroni corrected post hoc: P < 0.001). Under field conditions, burst duration (0.04527 ± 0.01555 sec, N=8) was also significantly longer than both knock duration (0.009314 ± 0.001720 sec, N=8; Bonferroni corrected post hoc: P = 0.001) and pulse duration (0.005901 ± 0.000972 sec, N=8; Bonferroni corrected post hoc: P < 0.001). Knocks were significantly longer in duration than pulses under both laboratory (Bonferroni corrected post hoc: P = 0.001), and field (Bonferroni corrected post hoc: P = 0.010) conditions.

Paired-samples T tests (Table 1-4) found that, in the laboratory, the fundamental frequency of knocks (165.07 ± 58.64 Hz, N=12) was significantly higher than the fundamental frequency of bursts (80.10 ± 3.93 Hz, N=12; paired t test: $t_{11}=4.905$, P < 0.001), and that the first harmonic frequency of knocks (587.68 ± 101.43 Hz, N=12) was also significantly higher than the first harmonic of bursts (301.58 ± 45.11 Hz, N=12;

paired t test: t_{11}=8.806, $P < 0.001$). The level of the fundamental frequency of knocks (99.32 ± 5.75 dB, N=12) was significantly greater than the level of the fundamental frequency of bursts (76.79 ± 5.50 dB, N=12; paired t test: t_{11}=13.544, $P < 0.001$), and the level of the first harmonic of knocks (102.73 ± 7.00 dB, N=12) was significantly greater than the level of the first harmonic of bursts (70.70 ± 5.72 dB, N=12; paired t test: t_{11}=17.080, $P < 0.001$).

Paired-samples T tests (Table 1-4) also found that, in the field, the fundamental frequency of knocks (297.03 ± 45.77 Hz, N=8) was significantly higher than the fundamental frequency of bursts (114.15 ± 5.77 Hz, N=8; paired t test: t_7=10.870, $P < 0.001$), and that the first harmonic frequency of knocks (615.11 ± 40.19 Hz, N=8) was significantly higher than the first harmonic of bursts (391.89 ± 45.79 Hz, N=8; paired t test: t_7=14.718, $P < 0.001$). The level of the fundamental frequency of knocks (89.84 ± 11.81 dB, N=8) was significantly greater than the level of the fundamental frequency of bursts (68.40 ± 9.42 dB, N=8; paired t test: t_7=7.360, $P < 0.001$), and the level of the first harmonic frequency of knocks (95.08 ± 9.04 dB, N=8) was also significantly greater than the level of the first harmonic of bursts (73.03 ± 9.37 dB, N=8; paired t test: t_7=9.468, $P < 0.001$).

Signal parameters did not differ between contexts (male-male versus male-female interactions) for approach behaviors (growls; MANCOVA: $F_{10,10}$=0.867, P=0.587; knocks; MANCOVA: $F_{5,8}$=0.903, P=0.523; Table 1-5) or lateral display behaviors (growls; MANCOVA: $F_{10,4}$=1.743, P=0.312; knocks; MANCOVA: $F_{5,10}$=1.166, P=0.389; Table 1-6) in the laboratory. There was also no significant difference between contexts for approach behaviors (growls; MANOVA: $F_{10,4}$=0.647, P=0.738; Table 1-7) or chase behaviors (growls; MANOVA: $F_{10,2}$=0.570, P=0.777; Table 1-8) in the field.

Growl signal parameters did not differ between specific behaviors in the laboratory (MANCOVA: $F_{20,48}$=0.709, P=0.797; Table 1-9), or field (MANOVA: $F_{60,204}$=0.933, P=0.615; Table 1-10). Knocks were also not significantly different between specific behaviors in the laboratory (MANCOVA: $F_{5,17}$=0.449, P=0.808; Table 9) or in the field (MANOVA: $F_{5,8}$=2.407, P=0.129; Table 1-11).

An overall main effect of location (laboratory vs field) was found for growls and knocks produced during approach behaviors (growls; MANOVA: $F_{6,21}$=15.072, $P < 0.001$; knocks; MANOVA: $F_{3,17}$=12.680, $P < 0.001$). Individual univariate ANOVA's with Bonferroni correction found that all signal parameters included in the MANOVA's for growls and knocks were significantly different between the laboratory and field (Table 1-12). An overall main effect of location was also found for knocks produced during lateral display behaviors (MANOVA: $F_{3,17}$=9.897, P = 0.001). Univariate ANOVA's with Bonferroni correction found that knock fundamental frequency and harmonic frequency differed between the laboratory and field (fundamental frequency; F=23.957, $P < 0.001$, harmonic frequency; F = 9.043, P = 0.007), while knock duration did not (F=3.527, P=0.076; Table 1-13). An overall main effect of location was also found for growls produced during crevice inspection behaviors (MANOVA: $F_{6,7}$=4.541, P=0.034; Table 11). Univariate ANOVA's with Bonferroni correction found that pulse duration (ANOVA: F = 11.354, P = 0.006), burst interval (ANOVA: F = 15.050, P = 0.002), and burst fundamental frequency (ANOVA: F = 20.446, P = 0.001) were significantly different between the laboratory and the field for growls produced during crevice inspection behaviors (Table 1-14).

Discussion

This descriptive study provides new information that advances our understanding of sound in the reproductive behaviors of *C. venusta*. An appreciable amount of variability exists in the sound production of different species within the genus *Cyprinella* (Delco 1960; Winn and Stout 1960; Stout 1975; Phillips and Johnston 2008; Phillips and Johnston 2009; Phillips et al. 2010), and my results indicate that sounds used by *C. venusta* are equally distinct. Phillips and Johnston (2009) found that although the tricolor shiner (*Cyprinella trichroistia*) and the Tallapoosa shiner (*Cyprinella gibbsi*) produced growls similar to that of *C. venusta* during courtship behaviors, they rarely produce sounds during male-male aggressive interactions, and when they did, they were growls, not knocks. Phillips et al. (2010) also found that the Edwards plateau shiner (*Cyprinella lepida*) produces only growls and single pulses during agonistic interactions. *Cyprinella trichroistia* and *C. gibbsi* also produce chirps and rattles, two types of sounds that are not produced by *C. venusta*. *Cyprinella galactura* and *Cyprinella callisema* both produce knocks and growls under similar contexts as *C. venusta* and do not produce chirps or rattles. Differences between *C. venusta* and other species existed in dominant frequency of knocks, which varied between 79 - 92 Hz in other species of *Cyprinella*, and was approximately 165 Hz (293 Hz in field) in *C. venusta*. Pulse duration was much shorter in *C. venusta* (laboratory 7.5 ms; field 5.9 ms) than in other species of *Cyprinella*, which varied between 15.8 - 34.3 ms. Pulse interval was also shorter in *C. venusta* (laboratory 13.3 ms) than in other species, which ranged from 17.3 to 27.3 ms. Due to shorter pulses and pulse intervals, the pulse rate of *C. venusta* (approximately 81 pulses/sec) was considerably higher than other species (24.3 – 32.8 pulses/sec).

Although I was unable to statistically compare the acoustic signals of *C. venusta* to other species, the relatively drastic differences in signal parameters such as pulse duration and pulse interval suggests that acoustic signals are likely different, and could act as an interspecific isolating mechanism (Wells and Henry 1992). This is especially intriguing due to the fact that *C. venusta*'s range overlaps with so many other sister species.

The behavioral repertoire observed in the laboratory differed slightly from that observed in the field for male-female interactions. Spawning events, for example, were only observed in the field. This is not entirely surprising considering that spawning is the end product of courtship behaviors, which have been shown to be negatively affected by stressors in the Atlantic cod (*Gadus morhua*; Morgan et al. 1999). As with all laboratory studies, a large number of differences beyond my control existed between the physical conditions in the laboratory and the field, any number of which could have played a role in the absence of spawning behaviors in the laboratory. The absence of flow, which was present at all field nest sites, and which is thought to be an important factor in nest selection in *C. venusta* (Baker et al. 1994), I believe, may be the reason that spawning was not observed in the laboratory. Because of the energetic investment associated eggs, females should be more selective about a nest than males when it comes to spawning. A nest associated with no flow is likely less desirable because there is less oxygen availability. Females were probably more responsible for the lack of spawning in the laboratory, a suggestion that is supported by the fact that courtship behaviors preceding spawning (mf zig zag, mf circling, and mf hover) were seen in both the laboratory and field, suggesting that males were prepared to spawn, but females were not.

Male-female lateral display was the only other behavior (besides spawning) that occurred between males and females that was not observed under both laboratory and field conditions. I do not have a clear explanation as to why this discrepancy was observed. One possibility is that the inability of the female to exit the nesting arena, combined with the lack of motivation to cooperate with the courting male, provided a mixed signal to the male that is probably not normally encountered in the field. This may result in a type of displacement activity (Tinbergen and Vaniersel 1948) by the male, in this case, a lateral display. Lateral displays are typically one of the lowest levels of the agonistic behavioral sequence performed between two males challenging each other. The reason I believe this may be a displacement activity is that the male does not proceed along the normal escalation of agonistic behaviors with the female, but continues to perform courtship behaviors and nest guarding behaviors. If a mf lateral display were part of a normal aggressive interaction between the male and female, some normal progression of aggression should have been observed. However, such a progression was never observed in the laboratory, and a mf lateral display was never observed at all under field conditions.

Observation of only the first three agonistic behaviors between males (mm approach, mm chase, and mm lateral display) in the laboratory was somewhat of a surprise to us. I originally believed that the constraints of the tank in the lab would force males into highly escalated states of aggression. I believe that the most likely explanation for the lack of escalated agonistic behaviors between males in the laboratory is the same as for the lack of spawning between males and females. Just as spawning is very costly for females, escalating to advanced agonistic behaviors is more energetically costly for males, and poses a greater risk of injury. If the nest is considered substandard, and only a limited number of females are present, a male may not consider it beneficial to escalate to a higher aggressive state.

Based on the accompaniment of growls and knocks with certain behaviors, an indication of the function of the sounds may be inferred. In both the laboratory and field, behaviors that are undoubtedly part of courtship such as mf zig zag, mf circling, mf hover, and mf spawning (mf spawning observed in the field only) are almost always exclusively associated with growls sounds. Likewise, behaviors that were exclusively performed during male-male agonistic interactions such as mm circle swim, mm fight, and mm lip lock (all of which were only observed in the field) were associated exclusively with knock sounds. From these associations, it is clear that knocks carry a more aggressive meaning, while growls are used more to communicate courtship.

There are, however, a number of behaviors that are observed during both male-male and male-female interactions. These behaviors include approaches, chases, and lateral displays. The association of knocks and growls with these behaviors is not as clear as with behaviors that are unique to courtship and agonistic contexts. During male-male and male-female approaches, chases, and lateral displays produced in the laboratory, growls and knocks both make up a substantial percentage of the accompanying sounds. Male-male approaches, chases, and lateral displays in the field also show accompaniment by a mixture of knocks and growls. However, mf approaches and mf chases in the field are unique in that when they are accompanied by sound (approximately 95% of the time), they are always accompanied by growls, never by knocks. This is an interesting finding as it shows a significant discrepancy between laboratory and field findings. Because field conditions are the more natural setting, the lack of knocks during mf approach and mf chase behaviors should be considered the normal condition. The association of knocks with mf approach and mf chase behaviors in the laboratory may be explained through the hypothesis that under laboratory conditions, the inability of a reproductively unmotivated female to leave the nesting area could prompt males to increase the level of aggression towards that female, whereas under field conditions, an unmotivated female would not likely persist around the nesting area.

My results also indicated that during highly aggressive behaviors between males, the association of sound production with behaviors decreased (as with mm lip lock), or ceased altogether (as with mm bite). These results corroborate, to some extent, those found for *C. galactura* (Phillips and Johnston, 2008). Phillips and Johnston (2008) found that during highly aggressive behaviors (mm circle swim and mm lip lock), *C. galactura* did not produce any sounds. They attributed this to a possible tradeoff between the energetic costs of calling and the benefits acquired from the calls. If sound production has not resolved an aggressive encounter at lower levels of aggression, it is unlikely to help once behaviors have escalated to physical contests. I agree with this logic, but found that *C. venusta* differs in the level at which sound production is abandoned. *Cyprinella venusta* continues to produce sounds at a high rate during mm circle swims (85.5%) and mm lip locks (60.0%), and only ceases to accompany aggressive behaviors with sounds during bite behaviors. I am unsure whether this relates to differences in the physiology of sound production, or whether the payoff of sound production at higher levels of aggression differs between *C. galactura* and *C. venusta*. To answer these questions, more elaborate studies will be needed comparing the energetic costs of sound production, and the benefit of sounds during agonistic behaviors in the two species. Both of these studies will probably require knowledge of the sound producing mechanism in these species for purposes of muting or stimulating sound production.

I also found that *C. venusta* continued to produce sounds throughout the entire courtship and spawning process, with sounds being produced during 100% of the observed spawns. Sound production during the act of spawning has been found to be inconsistent across several closely related species of the family Cyprinidae. Stout (1975) reported that no sounds were made during the act of spawning in the satinfin shiner (*Cyprinella analostanus*). Phillips and Johnston (2008) reported that although sounds were observed during the act of spawning in *C. galactura*, they were produced less frequently relative to other courtship behaviors. Johnston and Vives (2003) reported that, like *C. venusta*, the ornate minnow (*Codoma ornata*) produced sounds during 100% of observed spawning acts. The reason for the inconsistency in sound production during spawning is unknown, and especially interesting considering the importance of effective communication at the moment of gamete release.

The correlations between burst harmonic frequency and pulse interval with fish standard length may be used by females to evaluate the fitness of a male. In the field, I observed that the dominant male guards the nest from potential predators such as sunfish and other smaller fishes who frequently attempt to eat eggs from the crevice nest. Large sunfishes were not observed attempting to eat from the nest, possibly because of difficulties reaching eggs deposited in the small crevice. On a few occasions, the dominant male was observed chasing a sunfish away from a nest at which he had frequently spawned. Since the defending male protects the nest from egg predators such as sunfishes or other fishes, a female may prefer to spawn with a larger male that is better suited to protect the nest.

An interesting side note is that the male *C. venusta* sometimes produced knocks during the sunfish chase, indicating that knocks are not reserved specifically for interspecific communication, but also function as a general sign of aggression. The use of agonistic acoustic signals is typically found in intraspecific interactions. However, there are records in the literature indicating agonistic acoustic signals being used interspecifically (Brawn 1961; Gray and Winn 1961; Winn et al. 1964; Salmon 1967; Ballantyne and Colgan 1978; Rigley and Muir 1979)

I found in the laboratory and field that both knocks and growls were bimodal in their frequency distribution, a finding that has not yet been mentioned in the sound production literature for fishes in the genus *Cyprinella*. The dominant frequency for *C. venusta* was typically the lower (fundamental) frequency for both knocks and growls, and closely resembles the dominant frequencies of growls and knocks of other closely related species in the genus *Cyprinella* (Delco 1960; Phillips and Johnston 2008, 2009; Phillips et al. 2010). I am not yet certain of the significance of this bimodal frequency distribution, but (D.E. Holt and C.E. Johnston, unpublished data) showed that the harmonic frequency of *C. venusta* growls corresponds nicely to a quiet window in their natural spawning habitat. This, along with the fact that body size is correlated with the harmonic frequency and not the fundamental frequency suggests that the harmonic frequency may carry a greater biological significance than the dominant, fundamental frequency.

Although knocks were more typically associated with aggressive contexts, and growls were more associated with courtship contexts, the lack of a difference in growl signal parameters between male-male and male-female interactions for approach, lateral display, and chase behaviors was surprising. Other studies with closely related fishes have found differences in the characteristics of sounds produced under different contexts. Phillips and Johnston (2008), for example, found that in the whitetail shiner (*C. galactura*), growls produced during lateral display behaviors differed in numerous signal parameters between male-male (agonistic) and male-female (courtship) interactions including dominant frequency, pulse duration, pulse interval, pulse rate, and burst duration. The lack of this specialization in *C. venusta* was further seen by the absence of a difference in growl or knock signal parameters between not just agonistic and courtship contexts, but between any behaviors able to be compared for *C. venusta*. These results portray a more general function of acoustic communication by *C. venusta* during courtship and agonistic behaviors than I had expected based on other studies of closely related species. According to my results, a growl is a growl regardless of what type of behavior it is associated with, and a knock is a knock.

I am unsure why growls showed differences between the laboratory and the field. These differences were highly significant for approach behaviors, with sounds recorded in the laboratory generally being sluggish, and more drawn out with longer pulse durations, burst durations, and burst intervals, and slower pulse and burst rates. These same trends were seen for growls produced during crevice inspection behaviors, although only pulse duration, burst interval, and burst rate were statistically significant. Growl harmonic frequency was also significantly lower under laboratory conditions for both approach and crevice inspection behaviors. A possible explanation for the differences in growls between the laboratory and field could be a lack of motivation. If males are less motivated to court females, they may expend less energy in sound production. If muscle contraction on the swim bladder is the source of sound production in *C. venusta*, a slower rate of muscle contraction will result in slower pulse repetition rate (pulse rate), which is directly tied to the fundamental frequency of the growl. Exposure to stressors associated with capture, transport, and movement through an unfamiliar laboratory environment could also decrease motivation and energy availability (Bonga 1997), which could have a similar effect on growl fundamental frequency, pulse duration, and pulse rate.

The slower burst rate under laboratory conditions is naturally explained by the longer burst durations and intervals. However, I do not have an explanation as to why, under laboratory conditions, burst duration and interval are longer than under field conditions. There are many parameters that were unable to be controlled between the laboratory and the field that may have led to differences in signal parameters. For example, Penteriani (2003) found that the Eurasian Eagle Owl (*Bubo bubo*) decreased call duration in response to a more sparse conspecific density. Call duration is an honest signal of male quality in *B. bubo*, and when competition is scarce, males do not put as much energy into calling. Conspecific density in the current study was very different between laboratory and field settings, and could have played a role in burst duration and interval. To answer this question, further studies will have to be performed that control for density.

I am also unsure why knock fundamental and harmonic frequencies are significantly higher under field conditions than in the laboratory. This cannot be explained through pulse repetition rate because knocks are composed of a single unit. The only other explanation is that the difference in knock duration between laboratory and field conditions is actually real, even though it is not statistically significant in the current study. A shorter knock duration would result in a higher acoustic frequency for knocks (Connaughton et al. 2002). Although knock duration between the laboratory and the field for *C. venutsta* was not significant for lateral display behaviors, it was significant for approach behaviors, and showed the same trend of being shorter in the field for both behaviors. I believe that knock duration is the most likely explanation for the difference in frequency between laboratory and field conditions.

Although it is not the first to describe sound production by *C. venusta*, the current study is the first to provide a detailed description of sounds and associated behaviors in *C. venusta*, and a comparison of sounds and behaviors performed in the laboratory to those in the field for the genus *Cyprinella*. This is also the first study within the genus *Cyprinella* that provides data concerning the decibel levels of vocalizations. Knowing decibel levels of acoustic signals is crucial in understanding how fish communicate in their environment. Such information, in conjunction with environmental information such as propagation distances and the environmental soundscape of the habitat can be used to answer important questions such as the active area of vocalizations and the potential effect of natural and anthropogenic noises. Finally, the current study illustrates the importance of making field comparisons to substantiate findings in the laboratory.

A solid groundwork of detailed acoustic descriptions for the genus *Cyprinella* has been laid by Phillips and Johnston (2008; 2009; 2010), to which the current study adds another species. Future studies concerning the genus *Cyprinella* should attempt to include field comparisons in order to verify laboratory findings, and to see if the differences found between the laboratory and the field in *C. venusta* are consistent across other species. The incorporation of decibel levels into acoustic descriptions should also be considered.

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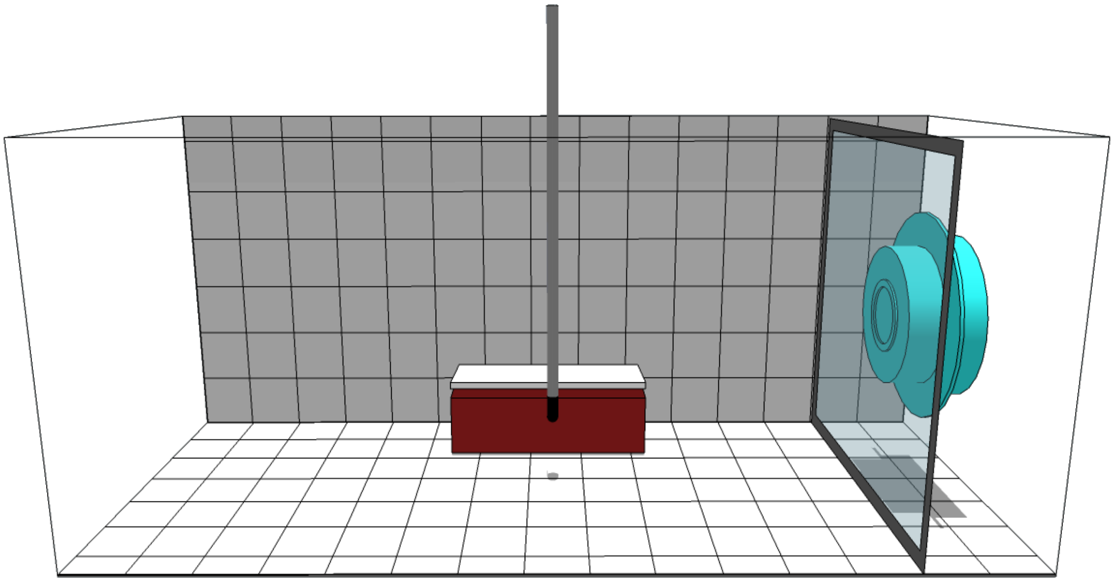

Figure 1-1 – Experimental tank setup. Underwater speaker is suspended behind a mesh barrier on the right hand side of the tank (according to the viewer's perspective). A hydrophone is suspended in front of an artificial nest (composed of a tile propped up on top of a brick), which is placed against the far wall of the tank. A light suspended above the tank cast shadow's onto the bottom grid, which together with the grid on the rear side of the tank allow the position of the fishes to be determined inside the tank.

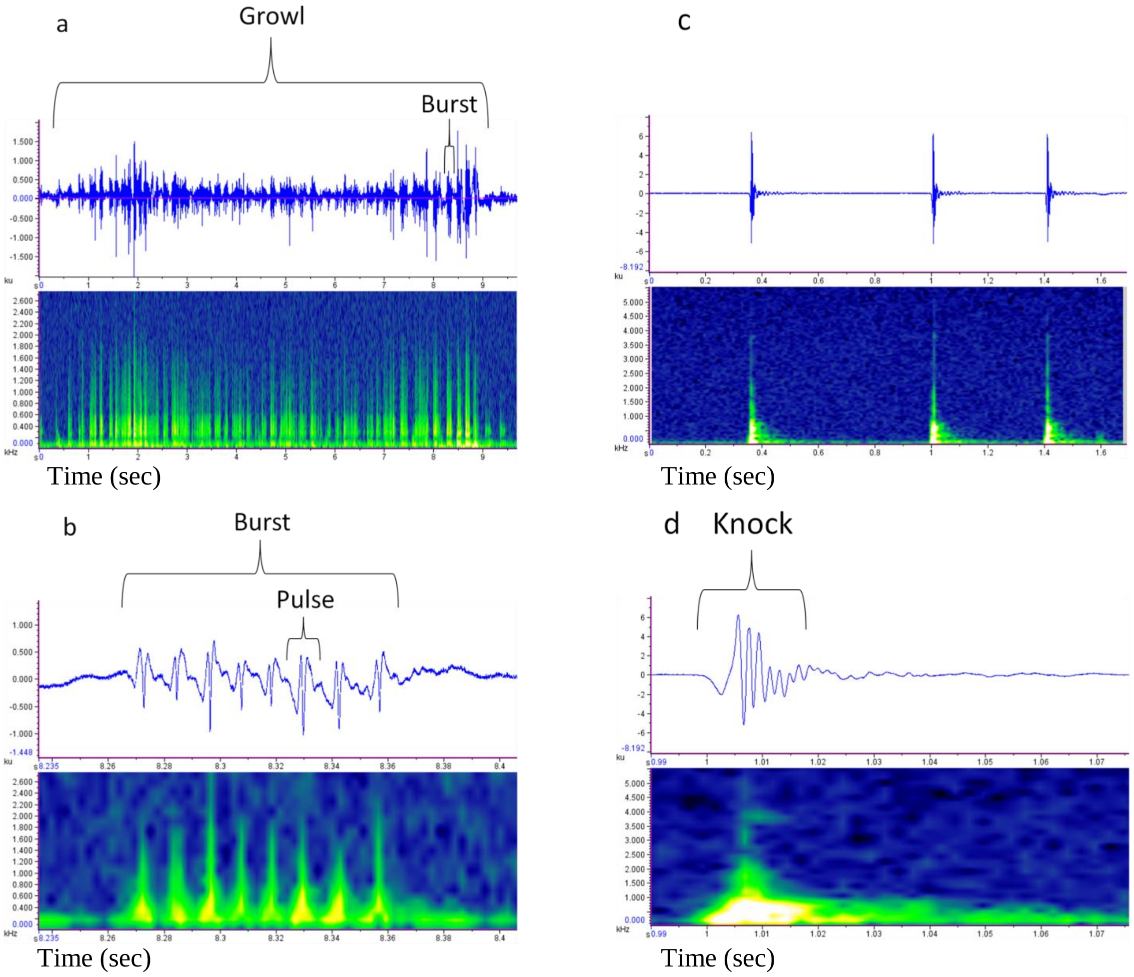

Figure 1-2 – Waveforms (blue lines with white background) and spectrograms (green signature with blue background) of *C. venusta* call repertoire. Both the (a) growl and (c) knock were recorded from the same individual under laboratory conditions. The growl is approximately 9 seconds long and represents an entire call. (b) A closer look at the burst bracketed in the growl. A total of 8 pulses making up the burst can be clearly seen, along with the relatively uniform pulse interval separating them. The empty space on either side of the burst in (b) represents the burst interval between this burst and adjacent bursts. Waveform amplitude values and units are arbitrary. The 3 knocks are produced in succession by the same male. Plate (d) is an expanded view of the middle knock of plate (c).

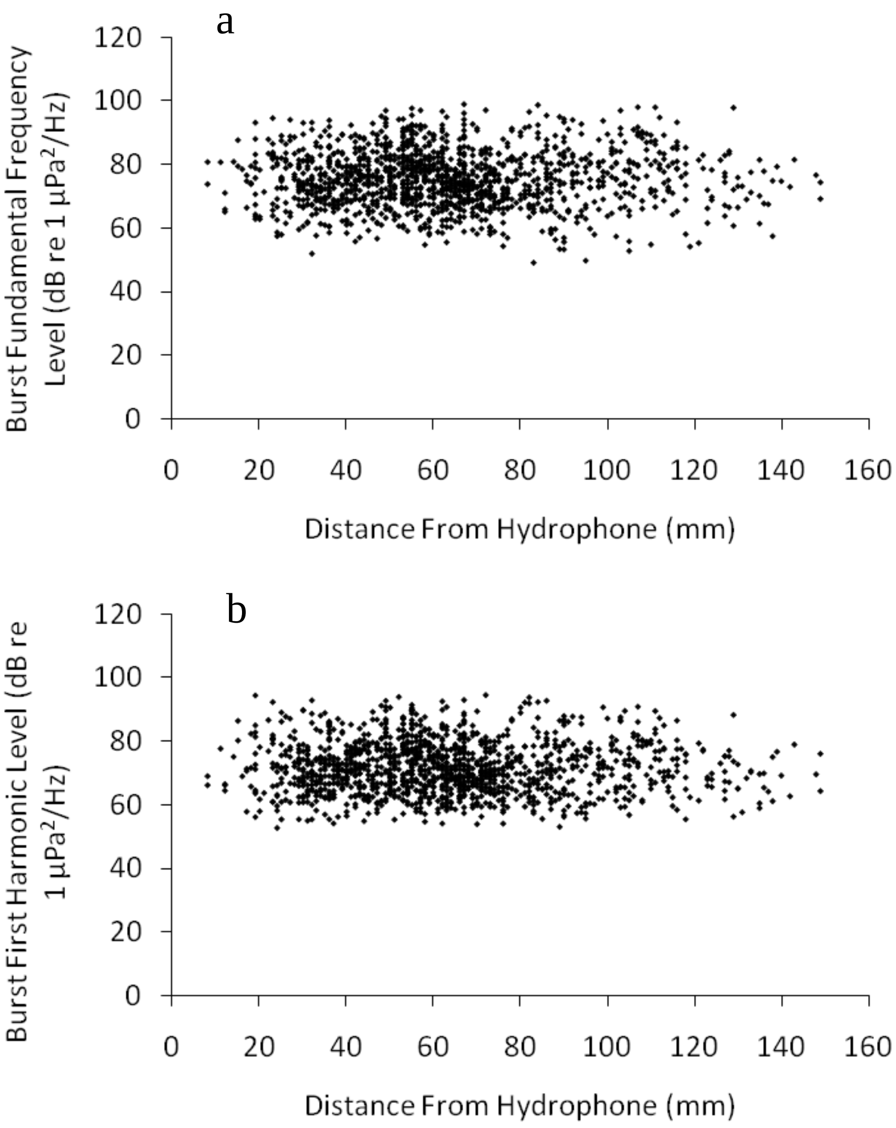

Figure 1-3 – Scatterplot of burst fundamental frequency level (a) and first harmonic level (b) with distance of sound producing fish from the hydrophone. Neither showed a significant correlation.

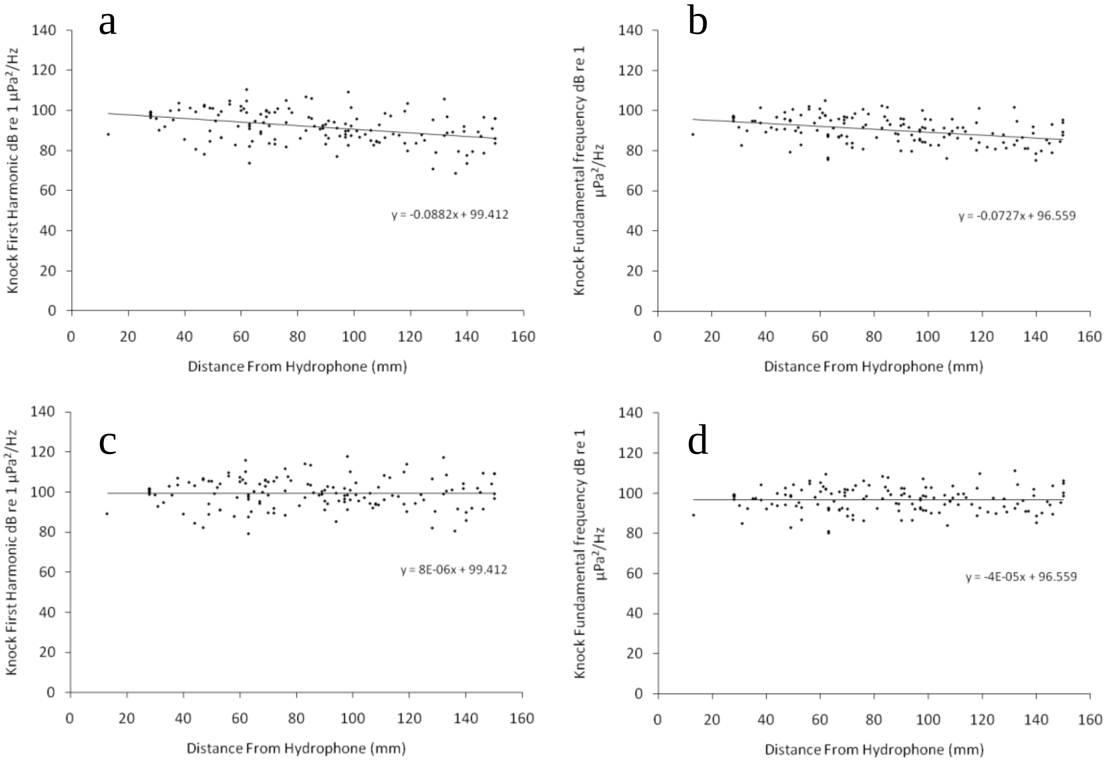

Figure 1-4 – Scatterplot with best fit line of fundamental frequency and first harmonic levels of knocks and distance of fish from hydrophone uncorrected (a,b), and corrected (c,d). First harmonic level of knocks and distance of fish from hydrophone (top left) uncorrected, and (bottom left) corrected. Values were corrected by multiplying the distance from the hydrophone of each point by the slope of the best fit line (0.0727), and adding the product to the original dB value.

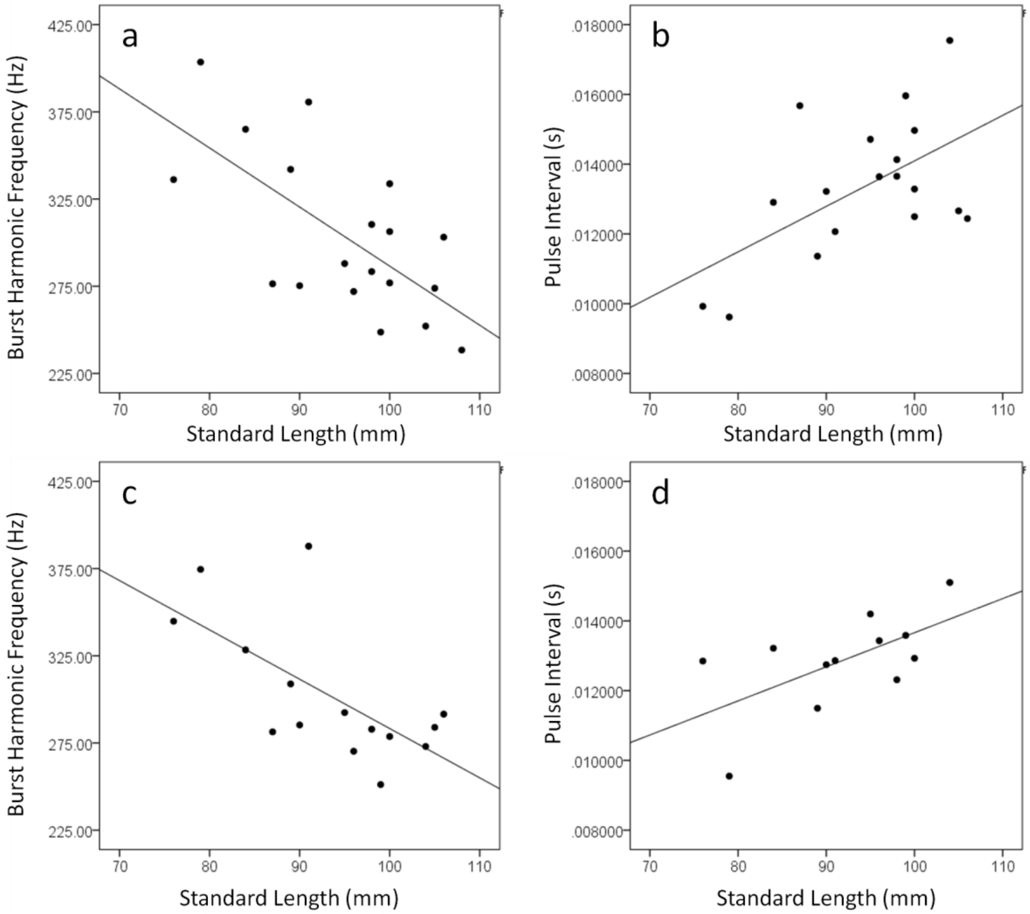

Figure 1-5 – Scatterplots showing correlations between fish standard length and burst harmonic frequency during approach behaviors (a), and lateral display behaviors (c). The correlations between standard length and pulse interval are also shown for approach behaviors (b) and lateral display behaviors (d). Data was combined for both agonistic and courtship contexts. Pearson correlation showed a significant negative correlation between SL and frequency of the first harmonic for both approach (Pearson correlation = -0.665, P = 0.002, N = 19), and lateral display behaviors (Pearson correlation = -0.660, P = 0.007, N = 15). Pulse interval also showed a significant positive correlation with SL for both approach (Pearson correlation = 0.564, P = 0.015, N = 18), and lateral display behaviors (Pearson correlation = 0.638, P = 0.019, N = 13).

Parameters	Description
Temporal:	
Pulse duration	Time from beginning to end of one pulse.
Pulse interval	Time between identical points on adjacent pulses.
Pulse rate	Number of pulses within burst/burst duration.
Burst duration	Time from beginning to end of burst.
Burst interval	Time between the last pulse and the first pulse of adjacent bursts.
Burst rate	Number of bursts within a call/call duration.
Knock duration	Time from beginning to end of one knock.
Spectral:	
Burst fundamental frequency	Lower frequency of the two frequency peaks measured from a burst.
Burst harmonic frequency	Higher frequency of the two frequency peaks measured from a burst.
Knock fundamental frequency	Lower frequency of the two frequency peaks measured from a knock.
Knock harmonic frequency	Higher frequency of the two frequency peaks measured from a knock.
Acoustic Intensity:	
Burst fundamental dB	Sound pressure level (in dB re 1µPa^2/Hz) of burst fundamental frequency.
Burst harmonic dB	Sound pressure level (in dB re 1µPa^2/Hz) of burst harmonic frequency.
Knock fundamental dB	Sound pressure level (in dB re 1µPa^2/Hz) of knock fundamental frequency.
Knock harmonic dB	Sound pressure level (in dB re 1µPa^2/Hz) of knock harmonic frequency.

Table 1-1 – Definitions and terminology of signal parameters measured for acoustic signals of *C. venusta*. All temporal parameters were measured from the waveform view and all spectral and acoustic intensity parameters were measured from the power spectrum view of Raven 1.3.

Laboratory	Knocks	Growls	No Sound
mm approach	29.2 (n=158)	60.0 (n=158)	10.8 (n=158)
mm chase	18.2 (n=99)	63.9 (n=99)	17.9 (n=99)
mm lateral display	42.5 (n=240)	51.2 (n=240)	6.3 (n=240)
mf approach	19.2 (n=181)	64.7 (n=181)	16.1 (n=181)
mf lateral display	11.5 (n=184)	72.9 (n=184)	15.6 (n=184)
mf chase	25.0 (n=44)	63.7 (n=44)	11.3 (n=44)
mf zig zag	0.0 (n=42)	92.9 (n=42)	7.1 (n=42)
mf circling	5.1 (n=118)	84.8 (n=118)	10.1 (n=118)
mf hover	1.9 (n=53)	92.5 (n=53)	5.6 (n=53)
m guarding/circling nest	7.1 (n=14)	92.9 (n=14)	0.0 (n=14)
m crevice inspection	1.7 (n=121)	68.3 (n=121)	30.0 (n=121)
m crevice pass	0.0 (n=51)	25.9 (n=51)	74.1 (n=51)

Table 1-2 – Percentage of behaviors associated with sound in the laboratory.

Field	Knocks	Growls	No Sound
mm approach	46.8 (n=107)	36.5 (n=107)	16.7 (n=107)
mm chase	30.0 (n=40)	55.0 (n=40)	15.0 (n=40)
mm lateral display	50.4 (n=141)	26.3 (n=141)	23.3 (n=141)
mm circle swim	85.5 (n=48)	0.0 (n=48)	14.5 (n=48)
mm fight	89.4 (n=47)	0.0 (n=47)	10.6 (n=47)
mm lip lock	60.0 (n=10)	0.0 (n=10)	40.0 (n=10)
mm bite	0.0 (n=10)	0.0 (n=10)	100.0 (n=10)
mf approach	0.0 (n=110)	95.5 (n=110)	4.5 (n=110)
mf chase	0.0 (n=58)	96.6 (n=58)	3.4 (n=58)
mf zig zag	0.0 (n=78)	100.0 (n=78)	0.0 (n=78)
mf circling	0.0 (n=112)	100.0 (n=112)	0.0 (n=112)
mf hover	0.0 (n=83)	100.0 (n=83)	0.0 (n=83)
mf spawning	0.0 (n=33)	100.0 (n=33)	0.0 (n=33)
m guarding/circling nest	0.0 (n=133)	86.5 (n=133)	13.5 (n=133)
m crevice inspection	0.0 (n=167)	72.5 (n=167)	27.5 (n=167)
m crevice pass	0.0 (n=71)	4.3 (n=71)	95.7 (n=71)

Table 1-3 – Percentage of behaviors associated with sound in the field.

Laboratory	Knock	Burst	Pulse	Statistic
Duration (s)	0.01576 ± 0.005279 (n=11)	0.08928 ± 0.03689 (n=11)	0.007576 ± 0.001006 (n=11)	ANOVA: F=26.37 P < 0.001
Fund. Freq. (Hz)	165.07 ± 58.64 (n=12)	80.10 ± 3.93 (n=12)	- - -	t=4.905 P < 0.001
Fund. Freq. (dB)	99.32 ± 1.66 (n=12)	76.79 ± 5.50 (n=12)	- - -	t=13.544 P < 0.001
First Harmonic (Hz)	587.69 ± 101.43 (n=12)	301.58 ± 45.12 (n=12)	- - -	t=8.806 P < 0.001
First Harmonic (dB)	102.73 ± 7.00 (n=12)	70.70 ± 5.72 (n=12)	- - -	t=17.080 P < 0.001

Field	Knock	Burst	Pulse	Statistic
Duration (s)	0.009314 ± 0.001720 (n=8)	0.04527 ± 0.01555 (n=8)	0.005901 ± 0.000972 (n=8)	ANOVA: F=62.10 P < 0.001
Fund. Freq (Hz)	297.03 ± 45.77 (n=8)	114.15 ± 5.76 (n=8)	- - -	t=10.870 P < 0.001
Fund. Freq. (dB)	89.84 ± 11.81 (n=8)	68.40 ± 9.42 (n=8)	- - -	t=7.360 P < 0.001
First Harmonic (Hz)	615.12 ± 40.19 (n=8)	391.89 ± 45.79 (n=8)	- - -	t=14.72 P < 0.001
First Harmonic (dB)	95.08 ± 9.04 (n=8)	73.03 ± 9.37 (n=8)	- - -	t=9.468 P < 0.001

Table 1-4 – Means and standard deviations of frequency and intensity properties of the three call types for approach behaviors produced in the lab and field. The far right column provides statistics (repeated measures ANOVA for duration, and paired-samples t tests for fundamental frequency, first harmonic, and levels of each).

Laboratory Approach behaviors	Agonistic (male-male interactions)	Courtship (male-female interactions)
Pulse duration	0.006860 ± 0.000976 (*n*=8)	0.007801 ± 0.000955 (*n*=14)
Pulse interval	0.012702 ± 0.002303 (*n*=8)	0.013625 ± 0.001936 (*n*=14)
Pulse rate	89.805 ± 15.913 (*n*=8)	77.758 ± 10.090 (*n*=14)
Burst duration	0.068195 ± 0.031114 (*n*=8)	0.090812 ± 0.034505 (*n*=14)
Burst interval	0.181828 ± 0.111263 (*n*=8)	0.144097 ± 0.073966 (*n*=14)
Burst rate	5.714 ± 2.666 (*n*=8)	5.161 ± 1.414 (*n*=14)
Burst fund. freq.	85.105 ± 12.553 (*n*=8)	78.704 ± 5.233 (*n*=14)
Burst fund. freq. dB	72.558 ± 5.517 (*n*=8)	77.520 ± 4.529 (*n*=14)
Burst harmonic freq.	321.017 ± 55.544 (*n*=8)	294.237 ± 34.218 (*n*=14)
Burst harmonic freq. dB	68.291 ± 3.380 (*n*=8)	71.999 ± 5.094 (*n*=14)
Knock duration	0.013799 ± 0.002953 (*n*=9)	0.017776 ± 0.006129 (*n*=6)
Knock fund. freq.	177.831 ± 68.453 (*n*=9)	147.117 ± 75.120 (*n*=6)
Knock fund. freq. dB	97.963 ± 5.975 (*n*=9)	100.365 ± 4.529 (*n*=6)
Knock harmonic freq.	589.381 ± 103.173 (*n*=9)	599.350 ± 105.841 (*n*=6)
Knock harmonic freq. dB	101.098 ± 6.754 (*n*=9)	103.635 ± 6.840 (*n*=6)

Table 1-5 – Means and standard deviations of growl and knock signal parameters produced during approach behaviors, for agonistic and courtship contexts. Multivariate analysis of co-variance with fish standard length as the covariate indicated no main effect of context on signal structure for either growls (MANCOVA: $F_{10,10}$=0.867, P=0.587), or knocks (MANCOVA: $F_{5,8}$=0.903, P=0.523).

Laboratory Lateral display behaviors	Agonistic (male-male interactions)	Courtship (male-female interactions)
Pulse duration	0.006739 ± 0.001223 (*n*=7)	0.007496 ± 0.000998 (*n*=9)
Pulse interval	0.012632 ± 0.001618 (*n*=7)	0.013382 ± 0.000957 (*n*=9)
Pulse rate	85.208 ± 14.987 (*n*=7)	81.210 ± 9.107 (*n*=9)
Burst duration	0.083522 ± 0.043875 (*n*=7)	0.081745 ± 0.032045 (*n*=9)
Burst interval	0.131530 ± 0.075161 (*n*=7)	0.113314 ± 0.048929 (*n*=9)
Burst rate	5.873 ± 2.42 (*n*=7)	5.908 ± 1.423 (*n*=9)
Burst fund. freq.	88.414 ± 14.133 (*n*=7)	78.246 ± 4.843 (*n*=9)
Burst fund. freq. dB	72.548 ± 3.936 (*n*=7)	75.756 ± 4.295 (*n*=9)
Burst harmonic freq.	314.893 ± 56.623 (*n*=7)	291.056 ± 27.516 (*n*=9)
Burst harmonic freq. dB	70.364 ± 4.192 (*n*=7)	71.001 ± 4.511 (*n*=9)
Knock duration	0.013616 ± 0.002958 (*n*=10)	0.01479 ± 0.004101 (*n*=7)
Knock fund. freq.	177.408 ± 55.438 (*n*=10)	181.678 ± 90.271 (*n*=7)
Knock fund. freq. dB	96.276 ± 4.725 (*n*=10)	96.342 ± 6.329 (*n*=7)
Knock harmonic freq.	585.369 ± 68.498 (*n*=10)	526.240 ± 113.825 (*n*=7)
Knock harmonic freq. dB	98.426 ± 5.764 (*n*=10)	99.032 ± 9.177 (*n*=7)

Table 1-6 – Means and standard deviations of growl and knock signal parameters produced during lateral display behaviors, for agonistic and courtship contexts. Multivariate analysis of co-variance with fish standard length as the covariate indicated no main effect of context on signal structure for either growls (MANCOVA: $F_{10,4}$=1.743, P=0.312), or knocks (MANCOVA: $F_{5,11}$=0.674, P=0.652).

Field **Approach behaviors**	Agonistic (male-male interactions)	Courtship (male-female interactions)
Pulse duration	0.006154 ± 0.001032 (*n*=6)	0.00584 ± 0.000872 (*n*=9)
Pulse interval	0.011071 ± 0.001415 (*n*=6)	0.01034 ± 0.000851 (*n*=9)
Pulse rate	93.191 ± 9.669 (*n*=6)	100.269 ± 10.852 (*n*=9)
Burst duration	0.050268 ± 0.020921 (*n*=6)	0.042519 ± 0.01406 (*n*=9)
Burst interval	0.090107 ± 0.067009 (*n*=6)	0.073431 ± 0.046326 (*n*=9)
Burst rate	6.134 ± 2.474 (*n*=6)	10.263 ± 3.254 (*n*=9)
Burst fund. freq.	116.729 ± 20.548 (*n*=6)	112.779 ± 5.563 (*n*=9)
Burst fund. freq. dB	67.242 ± 6.574 (*n*=6)	66.393 ± 8.432 (*n*=9)
Burst harmonic freq.	377.267 ± 54.236 (*n*=6)	388.427 ± 47.966 (*n*=9)
Burst harmonic freq. dB	72.659 ± 6.428 (*n*=6)	71.054 ± 8.287 (*n*=9)

Table 1-7 – Means and standard deviations of growl signal parameters produced during approach behaviors in the field, for agonistic and courtship contexts. Multivariate analysis of variance indicated no main effect of context on signal structure for growls (MANOVA: $F_{10,4}$=0.647, P=0.738).

Field **Chase behaviors**	Agonistic (male-male interactions)	Courtship (male-female interactions)
Pulse duration	0.005973 ± 0.001017 (*n*=5)	0.005324 ± 0.000582 (*n*=8)
Pulse interval	0.010261 ± 0.001354 (*n*=5)	0.010045 ± 0.001069 (*n*=8)
Pulse rate	95.648 ± 13.575 (*n*=5)	101.966 ± 8.130 (*n*=8)
Burst duration	0.041221 ± 0.010918 (*n*=5)	0.042722 ± 0.013421 (*n*=8)
Burst interval	0.117603 ± 0.11188 (*n*=5)	0.072041 ± 0.026946 (*n*=8)
Burst rate	8.329 ± 3.264 (*n*=5)	9.277 ± 1.923 (*n*=8)
Burst fund. freq.	119.413 ± 17.391 (*n*=5)	111.049 ± 9.448 (*n*=8)
Burst fund. freq. dB	72.962 ± 6.149 (*n*=5)	67.827 ± 4.655 (*n*=8)
Burst harmonic freq.	356.259 ± 48.456 (*n*=5)	401.912 ± 50.545 (*n*=8)
Burst harmonic freq. dB	77.956 ± 5.197 (*n*=5)	72.837 ± 6.920 (*n*=8)

Table 1-8 – Means and standard deviations of growl signal parameters produced during chase behaviors in the field, for agonistic and courtship contexts. Multivariate analysis of variance indicated no main effect of context on signal structure for growls (MANOVA: $F_{10,2}$=0.570, P=0.777).

Laboratory	Approach	Lateral Display	Crevice Inspection
Pulse duration	0.007481 ± 0.000965 (*n*=18)	0.007045 ± 0.000959 (*n*=12)	0.007212 ± 0.000697 (*n*=7)
Pulse interval	0.013348 ± 0.00201 (*n*=18)	0.012857 ± 0.00138 (*n*=12)	0.01314 ± 0.001881 (*n*=7)
Pulse rate	81.295 ± 12.417 (*n*=18)	84.282 ± 12.442 (*n*=12)	82.173 ± 14.583 (*n*=7)
Burst duration	0.082731 ± 0.036821 (*n*=18)	0.082752 ± 0.037888 (*n*=12)	0.06076 ± 0.026764 (*n*=7)
Burst interval	0.162006 ± 0.091154 (*n*=18)	0.12366 ± 0.051364 (*n*=12)	0.137435 ± 0.042685 (*n*=7)
Burst rate	5.445 ± 2.086 (*n*=18)	6.080 ± 2.117 (*n*=12)	5.905 ± 1.638 (*n*=7)
Burst fund. freq.	81.275 ± 9.577 (*n*=18)	82.243 ± 10.126 (*n*=12)	84.645 ± 9.139 (*n*=7)
Burst fund. freq. dB	75.819 ± 5.361 (*n*=18)	74.724 ± 4.710 (*n*=12)	73.945 ± 7.674 (*n*=7)
Burst harmonic freq.	307.090 ± 44.338 (*n*=18)	306.506 ± 43.349 (*n*=12)	313.556 ± 39.984 (*n*=7)
Burst harmonic freq. dB	70.887 ± 4.877 (*n*=18)	70.489 ± 4.395 (*n*=12)	70.720 ± 6.254 (*n*=7)
Knock duration	0.015812 ± 0.005037 (*n*=12)	0.014425 ± 0.003546 (*n*=12)	- -
Knock fund. freq.	165.066 ± 58.637 (*n*=12)	175.336 ± 52.481 (*n*=12)	- -
Knock fund. freq. dB	99.320 ± 5.752 (*n*=12)	96.951 ± 4.619 (*n*=12)	- -
Knock harmonic freq.	587.685 ± 101.434 (*n*=12)	571.439 ± 71.423 (*n*=12)	- -
Knock harmonic freq. dB	102.728 ± 7.002 (*n*=12)	99.613 ± 5.985 (*n*=12)	- -

Table 1-9 – Means and standard deviations of growl and knock signal parameters produced during approach, lateral display, and crevice inspection behaviors. Multivariate analysis of co-variance with fish standard length as the covariate indicated

Table 1-9 (Continued) - no main effect of behavior type on signal structure for either growls (MANCOVA: $F_{20,48}=0.709$, $P=0.797$), or knocks (MANCOVA: $F_{5,17}=0.449$, $P=0.808$).

Field	Circling/ guarding nest	Crevice inspection	Approach	Chase	mf circling	mf hover	mf spawning	mf zig zag
Pulse duration	0.005725 ± 0.000413 (*n*=7)	0.005975 ± 0.000678 (*n*=7)	0.005893 ± 0.000933 (*n*=10)	0.005672 ± 0.000799 (*n*=9)	0.005653 ± 0.000725 (*n*=7)	0.005936 ± 0.001033 (*n*=4)	0.00522 ± 0.00053 (*n*=3)	0.005612 ± 0.000599 (*n*=7)
Pulse interval	0.010715 ± 0.000637 (*n*=7)	0.01089 ± 0.000611 (*n*=7)	0.010488 ± 0.001149 (*n*=10)	0.01008 ± 0.000831 (*n*=9)	0.009646 ± 0.001091 (*n*=7)	0.009920 ± 0.001248 (*n*=4)	0.00968 ± 0.001177 (*n*=3)	0.009673 ± 0.001187 (*n*=7)
Pulse rate	94.643 ± 7.07 (*n*=7)	108.937 ± 39.167 (*n*=7)	98.889 ± 11.367 (*n*=10)	99.891 ± 6.095 (*n*=9)	103.966 ± 11.808 (*n*=7)	100.941 ± 13.926 (*n*=4)	101.469 ± 9.891 (*n*=3)	101.577 ± 11.889 (*n*=7)
Burst duration	0.050706 ± 0.018929 (*n*=7)	0.054071 ± 0.015526 (*n*=7)	0.044801 ± 0.014275 (*n*=10)	0.043427 ± 0.010944 (*n*=9)	0.059994 ± 0.032841 (*n*=7)	0.071781 ± 0.043438 (*n*=4)	0.046699 ± 0.017035 (*n*=3)	0.060793 ± 0.016351 (*n*=7)
Burst interval	0.091799 ± 0.020639 (*n*=7)	0.071837 ± 0.0134 (*n*=7)	0.090004 ± 0.055715 (*n*=10)	0.072042 ± 0.03241 (*n*=9)	0.068969 ± 0.049904 (*n*=7)	0.052074 ± 0.049117 (*n*=4)	0.057766 ± 0.022342 (*n*=3)	0.062494 ± 0.026878 (*n*=7)
Burst rate	8.151 ± 2.511 (*n*=7)	7.813 ± 1.798 (*n*=7)	8.744 ± 3.037 (*n*=10)	9.143 ± 2.231 (*n*=9)	9.127 ± 2.834 (*n*=7)	9.111 ± 4.677 (*n*=4)	8.134 ± 2.385 (*n*=3)	8.037 ± 2.114 (*n*=7)
Burst fund. freq.	112.102 ± 11.961	112.98 ± 13.833	113.368 ± 5.361	112.282 ± 10.595	110.384 ± 9.582	102.920 ± 4.899	114.415 ± 11.591	111.758 ± 9.347

	(*n*=7)	(*n*=7)	(*n*=10)	(*n*=9)	(*n*=7)	(*n*=4)	(*n*=3)	(*n*=7)
Burst fund. freq. dB	66.187 ± 5.598 (*n*=7)	66.609 ± 5.78 (*n*=7)	68.055 ± 8.351 (*n*=10)	70.033 ± 6.258 (*n*=9)	71.421 ± 10.829 (*n*=7)	74.125 ± 12.162 (*n*=4)	65.887 ± 14.279 (*n*=3)	71.331 ± 7.877 (*n*=7)
Burst harm. freq.	406.000 ± 36.004 (*n*=7)	396.801 ± 33.458 (*n*=7)	392.827 ± 40.876 (*n*=10)	384.431 ± 40.747 (*n*=9)	376.677 ± 44.58 (*n*=7)	350.546 ± 46.066 (*n*=4)	383.972 ± 73.319 (*n*=3)	391.579 ± 54.797 (*n*=7)
Burst harm. freq. dB	71.368 ± 5.493 (*n*=7)	72.574 ± 6.129 (*n*=7)	72.474 ± 8.417 (*n*=10)	74.803 ± 7.454 (*n*=9)	75.775 ± 10.619 (*n*=7)	79.456 ± 11.606 (*n*=4)	71.231 ± 14.651 (*n*=3)	76.043 ± 7.43 (*n*=7)

Table 1-10 (continued) – Means and standard deviations of growl signal parameters produced during 8 different behaviors in the field. Multivariate analysis of variance indicated no main effect of behavior on signal parameters for growls (MANOVA: $F_{60,204}$=0.933, P=0.615).

Field	Approach	mm circle swim	mm lateral display
Knock duration	0.00917 ± 0.001667 (*n*=9)	0.009052 ± 0.00134 (*n*=7)	0.010826 ± 0.005253 (*n*=9)
Knock fund. freq.	292.735 ± 44.711 (*n*=9)	286.421 ± 28.358 (*n*=7)	270.44 ± 28.707 (*n*=9)
Knock fund. freq. dB	90.321 ± 11.14 (*n*=9)	90.485 ± 8.162 (*n*=7)	91.704 ± 4.595 (*n*=9)
Knock harmonic freq.	628.112 ± 54.181 (*n*=9)	659.912 ± 61.393 (*n*=7)	665.256 ± 69.809 (*n*=9)
Knock harmonic freq. dB	95.487 ± 8.547 (*n*=9)	98.552 ± 8.356 (*n*=7)	98.878 ± 4.595 (*n*=9)

Table 1-11 – Means and standard deviations of knock signal parameters produced during approach, mm circle swim, and mm lateral display behaviors in the field. Multivariate analysis of variance indicated no main effect of behavior type on knock signal structure (MANOVA: $F_{5,8}$=2.407, P=0.129).

Approach behaviors	Laboratory	Field	F and P values
Pulse duration	0.007481 ± 0.000965 (*n*=18)	0.005893 ± 0.000933 (*n*=10)	F = 17.838, P < 0.001
Pulse rate	81.296 ± 12.418 (*n*=18)	98.889 ± 11.367 (*n*=10)	F = 13.670, P = 0.001
Burst duration	0.082731 ± 0.036821 (*n*=18)	0.044801 ± 0.014275 (*n*=10)	F = 9.665, P = 0.005
Burst interval	0.162006 ± 0.091154 (*n*=18)	0.090004 ± 0.055715 (*n*=10)	F = 5.122, P = 0.032
Burst rate	5.446 ± 2.087 (*n*=18)	8.744 ± 3.037 (*n*=10)	F = 11.581, P = 0.002
Burst fund. freq.	81.276 ± 9.578 (*n*=18)	113.368 ± 5.361 (*n*=10)	F = 94.690, P < 0.001
Knock duration	0.015811 ± 0.005037 (*n*=12)	0.009169 ± 0.001666 (*n*=9)	F = 14.309, P = 0.001
Knock fund. freq.	165.067 ± 58.638 (*n*=12)	292.735 ± 44.711 (*n*=9)	F = 29.595, P < 0.001
Knock harm. freq.	587.684 ± 101.435 (*n*=12)	628.112 ± 54.180 (*n*=9)	F = 1.169, P = 0.293

Table 1-12 – Means and standard deviations of growl and knock signal parameters produced during approach behaviors, under laboratory and field conditions. Multivariate analysis of variance indicated a significant main effect of location (lab and field) on signal structure for both growls (MANOVA: $F_{6,21}$=15.072, P < 0.001), and knocks (MANOVA: $F_{3,17}$=12.680, P < 0.001). Results from the Bonferroni corrected univariate ANOVA's are provided in the right column.

Lateral Display behaviors	Laboratory	Field	F and P values
Knock duration	0.014425 ± 0.003546 (*n*=12)	0.010825 ± 0.005252 (*n*=9)	F = 3.527, P = 0.076
Knock fund. freq.	175.337 ± 52.482 (*n*=12)	270.439 ± 28.707 (*n*=9)	F = 23.957, P < 0.001
Knock harm. freq.	571.44 ± 71.42 (*n*=12)	665.25 ± 69.81 (*n*=9)	F = 9.043, P = 0.007

Table 1-13 – Means and standard deviations of knock signal parameters produced during lateral display behaviors in both laboratory and field locations. Multivariate analysis of variance indicated a significant main effect of location (lab and field) on signal structure (MANOVA: $F_{3,17}$=9.897, P = 0.001). Results from the Bonferroni corrected univariate ANOVA's are provided in the right column.

Crevice Inspection behaviors	Laboratory	Field	F and P values
Pulse duration	0.007212 ± 0.000697 (*n*=7)	0.005975 ± 0.000678 (*n*=7)	F = 11.354, P = 0.006
Pulse rate	82.174 ± 14.584 (*n*=7)	108.937 ± 39.167 (*n*=7)	F = 2.870, P = 0.116
Burst duration	0.06076 ± 0.026764 (*n*=7)	0.054071 ± 0.015526 (*n*=7)	F = 0.327, P = 0.578
Burst interval	0.137435 ± 0.042685 (*n*=7)	0.071837 ± 0.0134 (*n*=7)	F = 15.050, P = 0.002
Burst rate	5.906 ± 1.639 (*n*=7)	7.813 ± 1.798 (*n*=7)	F = 4.301, P = 0.060
Burst fund. freq.	84.646 ± 9.14 (*n*=7)	112.98 ± 13.833 (*n*=7)	F = 20.446, P = 0.001

Table 1-14 – Means and standard deviations of growl signal parameters produced during crevice inspections, under laboratory and field conditions. Multivariate analysis of variance indicated that there was a significant main effect of location (lab and field) on signal structure (MANOVA: $F_{6,7}$=4.541, P = 0.034). Results from the Bonferroni corrected univariate ANOVA's are provided in the right column.

Chapter 2

The Effect Of Noise On Acoustic Communication In *Cyprinella venusta*.

Noise can be a problem for any acoustically communicating organism due to the masking effect it has on acoustic signals. Most naturally occurring noise is not problematic for organisms because they have had time to evolve mechanisms for eluding the adverse effects of the noise. However, rapid expansion of human populations, along with the noise that comes with industrialization and motorized transportation poses a threat for many acoustically communicating species. Aquatic organisms are no exception to this, and although much work has been devoted to marine organisms, relatively little has been directed at freshwater species, especially species inhabiting small stream and river systems. The aim of the current study was to determine the effect of elevated noise levels on acoustic signals, associated behaviors, and the interfish distance during sound production in the blacktail shiner, *Cyprinella venusta*. My results were not conclusive, but suggested it is unlikely that *C. venusta* is able to effectively compensate for elevated noise levels by increasing signal intensity, redundancy, or by decreasing interfish distance.

Introduction

The ability to communicate acoustically is a mode of communication that has been adopted by a wide variety of fishes (Ladich et al. 2006). Sound can be a useful tool for communication, especially in aquatic habitats where visual signals are often diminished due to high levels of turbidity, and complex substrates where line of sight is not available (Hawkins and Myrberg 1983). A wide variety of sound producing mechanisms and hearing adaptations have evolved in freshwater fishes (Fine et al. 1977), and sounds are often associated with important life history events such as reproduction (Myrberg and Lugli 2006), food localization (Holt and Johnston, 2011), territory defense (Lugli 1997), species recognition (Myrberg et al. 1978), and mate choice (Myrberg et al. 1986). Although experimental evidence is sparse, numerous studies have correlated acoustic signal production with behaviors critical for the reproductive success of numerous fish species, suggesting that acoustic signals play an important communicative role in the reproductive success of many fishes (Ladich et al. 1992; Hawkins and Amorim 2000; Kasumyan 2009).

Underwater communication through the use of sound does have its own set of unique challenges for fishes, especially those inhabiting shallow, freshwater systems. Shallow aquatic systems are subject to low cut-off frequencies, a phenomenon in which sounds below a certain frequency, defined mainly by water depth and substrate composition (Officier, 1958; Rogers and Cox 1988), attenuate very rapidly away from the source. Studies looking at fish signal propagation in shallow stream habitats have found that signals from both *C. venusta* (D.E. Holt and C.E. Johnston, unpublished data) and the gobies *Padogobius martensii* and *Gobius nigricans* (Lugli and Fine 2003) propagate only several decimeters away from their source, even under the best conditions.

Even in such challenging conditions, however, there are certain characteristics of the underwater environment that fishes are sometimes able to take advantage of in order to increase the efficiency of their acoustic communication. The few studies that have investigated the relationship between environmental noise and acoustic communication in fishes inhabiting small, freshwater streams have often found a quiet window in the natural environmental noise spectrum that matches the dominant frequencies utilized for communication by fishes in the habitat (Lugli et al. 2003; Speares et al. 2011; D.E. Holt and C.E. Johnston, unpublished data). This notch in the natural ambient noise allows hearing sensitivity within the window to remain relatively high and increases the signal-to-noise ratio of acoustic signals inside the window, both of which increase the active area and efficiency of acoustic signals. The use of quiet bands in the natural soundscape is not unique to fishes, and has been described in numerous other taxa including birds (Ryan and Brenowitz 1985), frogs (Parris et al. 2009), and primates (Waser and Waser 1977). It has been suggested that the correlation between the quiet window of the environmental natural noise and spectral composition of acoustic signals is an evolutionary strategy set up to increase the effectiveness of acoustic communication (Wiley and Richards 1982; Ryan and Brenowitz 1985; Waser and Brown 1986; Slabbekoorn and Smith 2002).

Elevations in environmental noise caused by anthropogenic sources can exacerbate the already limited active area of acoustic signals in shallow waters. The relatively recent development and rapid expansion of mass transportation, urbanization, and industrialization has contributed to the rise and spread of anthropogenic noise at unprecedented rates (Slabbekoorn et al. 2010). The effect of this noise on aquatic organisms is widely unknown, and what research has been conducted has focused mainly on marine organisms, especially marine mammals (Richardson et al. 1998; Southall et al. 2007). One area that has received significant attention in fishes is the effect of noise on hearing, and numerous studies on fishes have reported significant hearing threshold shifts after exposure to elevated noise (Scholik and Yan 2001; Scholik and Yan 2002a; Scholik and Yan 2002b; Amoser and Ladich 2003; Smith et al. 2004). Elevated noise levels can also hinder communication by decreasing the signal-to-noise ratio of acoustic signals, masking signals altogether, or altering the behavior of an organism. Sun and Narins (2005) found that anthropogenic noise from airplane flyby's and motorcycle playbacks caused a significant decline in call rate among a number of pond dwelling frog species. Researchers have reported that Norwegian spring spawning herring (Vabø et al. 2002) and northern cod (Handegard et al. 2003) performed avoidance behaviors in response to passing ships that were not likely within visual range. Sara et al. (2007) found that exposure to multiple types of boat noise caused behavioral deviations from normal schooling behavior, and increased aggression in the bluefin tuna (*Thunnus thynnus*).

Animals that are unable to avoid elevated noise levels may be required to compensate for them. This can be accomplished in several ways according to the predictions of information theory (Shannon and Weaver 1949). First, an animal may increase the intensity of their signals in order to elevate the signal-to-noise ratio of their call relative to the background noise. This phenomenon is termed the Lombard effect (Lombard 1911). Previous research has shown that in several bird species, the Lombard effect occurs when there is an increase in environmental noise, possibly as an attempt to compensate for the decrease in active space, which in birds, often defines territorial boundaries (Warren et al. 2006). Brumm and Todt (2002) found that the nightingale (*Luscinia megarhynchos*) increases the amplitude of its acoustic signals when exposed to noise in the frequency band of its calls. The Lombard effect has also been shown to occur in common marmosets (*Callithrix jacchus*; Brumm et al. 2004), frogs (Lopez et al. 1988), whales (Scheifele et al. 2005; Holt et al. 2008), and humans (Lane and Tranel, 1971). However, I am unaware of any report, to date, of the Lombard effect in fishes.

Another method that can be used to compensate for elevated levels of ambient noise is altering components of the frequency of calls to avoid frequency bands that are more heavily affected by noise. Feng et al. (2006) showed that the torrent frog (*Amolops tormotus*) has co-evolved an ability to communicate using ultrasonic harmonic frequencies in order to avoid the predominantly low frequency ambient noise produced by the streams around which it lives. Frequency shifts can also be plastic, and can occur in response to transient noise sources, or noise sources that have not been around long enough to have an evolutionary effect. Fernandez-Juricic et al. (2005) found that house finches (*Carpodacus mexicanus*) occurring in public parks of southern Los Angeles County and north Orange County, California, raised the lower frequencies of their calls, possibly to help compensate for the masking effects generated by the higher levels of low frequency ambient noise. Slabbekoorn and Peet (2003) also found that great tits (*Parus major*) living near road traffic shifted the frequency of their calls upward away from the road noise relative to individuals living in quieter areas.

Animals can also compensate for the effects of elevated ambient noise by increasing the temporal or spectral redundancy of acoustic signals. Lengagne et al. (1999) found that king penguins (*Aptenodytes patagonicus*) increase the number of syllables per call at higher levels of ambient noise. Potash (1972) found that the Japanese quail (*Coturnix coturnix japonica*) employs a similar tactic when exposed to higher noise levels. Brumm et al. (2004) also found that although it did not increase the number of syllables per call, the common marmoset (*Callithrix jacchus*) did increase the duration of its calls when exposed to increased levels of white noise. By increasing the call rate or call duration, the probability of receiving a portion of the call containing important information embedded within the acoustic signal is improved by increasing the redundancy of information in the signal (Shannon and Weaver, 1949).

Likelihood of successful acoustic communication may also be increased by decreasing the distance between sender and receiver when producing a sound. This may not be a possibility if signals are being used for advertisement or other purpose in which the location of the potential receiver may not be known. However, if the location of the receiver is known, it should benefit the sender to close the distance under noisy conditions in order to maintain a sufficient signal-to-noise ratio and efficiency of communication.

I investigated the effects of elevated noise conditions on the vocal behaviors and signal parameters in the blacktail shiner (*Cyprinella venusta*), a common freshwater fish found in the southeastern United States. *Cyprinella venusta* is a good species for the current study because it occupies a wide variety of freshwater habitats that are subject to a number of natural and anthropogenic noise sources. *Cyprinella venusta* has also been documented as a sound producing fish (Delco 1960; D.E. Holt and C.E. Johnston, unpublished data), and is easily collected. My specific objectives were to determine whether *C. venusta* was able to compensate for depressed signal-to-noise ratios under noisy conditions by increasing the level of their signals, increasing signal redundancy in the temporal domain, or closing the distance between the sender and receiver. I also looked at whether elevated noise levels resulted in an increase in behavior rate, or an increase in the percentage of behaviors associated with sound. An experimental approach was taken by manipulating background noise levels in a laboratory setting, recording the fish under both conditions, and then measuring sound levels, temporal and spectral parameters, interfish distances, behavior rates, and occurrence of sound production with behaviors.

Methods

Fish were captured by sein from tributaries to the Chatahoochee river in Lee County, East Alabama, specifically Little Uchee Creek (32.508579° N, -85.184215° W), Halawakee Creek (32.697579° N, -85.266951° W, and Wacoochee Creek (32.622799° N, -85.132685° W). Fish were transported back to the lab and stored in 76 l aquariums with gravel substrates. Hanging, external filters were used on all tanks and air stones were used only when necessary. Fish were kept at approximately 26°C, given a 12L:12D hour light cycle, and fed a diet of commercial flake food. Males that had not been tested were kept isolated from other males to prevent dominance hierarchies from being established prior to experimental trials, and females were stored alone, with other females, or with males that were not to be tested. A 20 gallon experimental tank was setup in an acoustic chamber to conduct each experiment. A tile propped up on a brick forming a 5 mm crevice served as an artificial nest, and was placed on the wall in the center of the tank. An underwater speaker (University Sound UW-30, Oklahoma City, OK) was suspended behind a thin mesh barrier on one end of the tank (Figure 2-1).

A white piece of plexiglass with gridlines at 5 cm intervals was placed on the bottom of the tank, and an identical grid was placed on the back of the tank. A 60 W blub was hung approximately 1.5 m above the tank, and a camera was placed in front of the tank in such a way that both the bottom and rear grids were visible. The light bulb above the tank cast shadows of fish in the tank on the bottom grid, allowing the position of the fish along the X and Z axes of the tank to be determined from the video camera. The grid on the back of the tank allowed the position of the fish along the Y axis to be determined from the video camera.

During trials, male and female *C. venusta* were placed in the experimental tank and allowed to acclimate. Acclimation periods varied by trial, and were considered to be over when the fish resumed normal behaviors (swimming freely). Typically, two males and at least 1 female were placed in the tank for each trial. Trials consisted of two periods, one quiet and one noisy. Each period usually lasted about 2 hours, but duration ranged from 17 min to 2.5 hrs. During noisy periods, band limited white noise (100-1600 Hz at 73.3 dB re 1μPa^2/Hz at the hydrophone; Figure 2-2) generated in SigGen(Tucker Davis Technologies, Gainesville, FL) was amplified using an ART SLA-1, two-channel stereo linear amplifier (100 watts per channel), and played through the UW-30 underwater speaker. During quiet periods, no sound was played through the speaker and the amplifier was turned off. Each period was video recorded on a Sony handycam digital HD video recorder (HDR-SR11) and sounds were recorded onto Raven 1.3 (Cornell University, Ithaca, NY, U.S.A) using a Brüel and Kjaer 8103 hydrophone and a Brüel and Kjaer 2635 charge amplifier. Trials were conducted back to back in varying order. When the noisy period began, noise was ramped up to full intensity over the course of 5 minutes to prevent startling the fish.

Analysis

Video of each trial was watched while simultaneously reviewing the audio in Raven. Any time a behavior occurred in the video that was accompanied by a sound that was suitable for analysis (not contaminated by the fish hitting something, or mostly buried under the ambient noise), it was labeled in Raven. A total of 78 males were included in the trials, but only 20 males produced sufficient sounds of acceptable quality to be included in statistical analysis. A total of 1109 calls were produced that were of high enough quality to be analyzed.

All statistical analyses were conducted in PASW 18 statistical software package (IBM SPSS Corporation, Chicago Illinois, USA). Temporal variables were measured in Raven from the waveform view and included duration and interval times for knocks, bursts, and pulses. Burst rate and pulse rate were calculated by dividing the number of bursts within a call by that call's total duration (burst rate) or the number of pulses within a burst by that burst duration (pulse rate). Spectral variables including knock and burst fundamental frequency and first harmonic frequency were measured from the calculated power density spectrum in Raven. In order to obtain the most accurate frequency information from the power density spectrum, sample sizes of fast fourier transformations (FFT's) were maximized for each knock or burst so that the bin resolution of the power density spectrum was as small as possible. Decibel levels of the fundamental and first harmonic frequencies from knocks and bursts were also measured from the power density spectrum, and were adjusted to represent actual levels by taking into consideration the gain imposed by each unit in the recording chain. Decibel levels were also corrected to represent spectrum level (1Hz bands; dB re 1µPa^2/Hz) by subtracting 10 log (bin resolution) of each measured signal (Richardson et al. 1998).

Prior to all statistical analyses of signals recorded under noisy conditions, an average value for each signal parameter (pulse duration, burst duration, burst fundamental frequency, etc.) was calculated for each behavior, for each individual male. Pearson correlation coefficients were calculated to determine if any relationships existed between fish standard length and signal parameters (pulse duration, pulse interval, pulse rate, burst duration, burst interval, burst rate, knock duration, burst and knock fundamental and harmonic frequencies, and burst and knock fundamental and harmonic levels). If a correlation was found, body size was used as a covariate in any analyses involving that signal parameter that was not a repeated measures or paired design. Correlations were also performed to determine if there was a relationship between level and distance from the hydrophone. If levels were correlated with distance from the hydrophone, levels were corrected by multiplying the distance from the hydrophone at the moment of sound production by the slope of the linear best fit line of the correlation, and adding this value to the original level of the signal. This eliminated the correlation without affecting variability around the best fit line. Values that had been corrected for distance from the hydrophone were used in further analysis. Because growls did not show a correlation between distance from hydrophone and level, this correction was only necessary for knocks.

To determine whether signal parameters differed between contexts under noisy conditions, approach and lateral display behaviors were compared between agonistic and courtship contexts. A finding that acoustic signal parameters did not differ between agonistic and courtship contexts would justify the lumping of the contexts to bolster samples sizes. Holt and Johnston (unpublished data) have already shown that no difference exists between agonistic and courtship contexts under quiet conditions and that under quiet conditions, they may be combined. Under noisy conditions, approach and lateral display behaviors were chosen for comparison between contexts because they were the only behaviors that were shared between the two contexts with sample sizes sufficient for statistical analysis. Because an insufficient number of knocks were recorded during approach behaviors performed under courtship contexts, only growls were compared between contexts for approach behaviors. Both knocks and growls were compared between contexts for lateral displays. Multivariate analysis of variance with standard length as a covariate (MANCOVA) was used with context (agonistic and courtship) as the fixed factors, and signal parameters as the dependent variables. A total of 3 MANCOVA's were performed to determine whether sounds differed between agonistic and courtship contexts, one for growls produced during approach behaviors, one for growls produced during lateral display behaviors, and one for knocks produced during lateral display behavior.

To verify that call components (knocks, bursts, and pulses) were distinct under noisy conditions, a repeated measures ANOVA was used to determine whether call components differed in duration. Paired T tests were used to determine whether knocks and bursts differed in fundamental frequency, first harmonic frequency, or level.

The only behaviors that were recorded for the same individual with sufficient sample sizes for statistical analysis comparing signal parameters between quiet and noisy conditions were approaches and lateral displays. Because sounds from these behaviors were shown to not differ between agonistic and courtship contexts, the contexts were combined to bolster samples sizes. Behaviors that did not have sufficient sample sizes were excluded from the analysis. To determine whether *C. venusta* males altered growl acoustic signals under elevated noise conditions, a doubly multivariate repeated measures MANOVA design was used for approach behaviors, and paired t tests with Bonferroni correction were used for lateral display behaviors. A repeated measures MANOVA design was not able to be used for lateral display behaviors because of insufficient residual degrees of freedom resulting from a greater number of measures than cases. In the repeated measures MANOVA used for approach behaviors, the quiet and noisy conditions were used as the two within subjects factors, with each factor containing 10 measures (pulse duration, pulse interval, pulse rate, burst duration, burst interval, burst rate, burst fundamental frequency, burst harmonic frequency, burst fundamental frequency level, and burst harmonic frequency level). If an overall effect of noise was detected in the MANOVA, Bonferroni corrected post hoc tests were run to determine what specific call parameters differed between the noisy and quiet conditions.

Because knocks and growls were not always present together in all behaviors, knocks were analyzed separately from growls. Knocks produced during noisy and quiet trials were compared using a repeated measures MANOVA, similarly to the method described above for growls. For the knock analysis, however, there were only 5 measures (knock duration, knock fundamental frequency, knock harmonic frequency, knock fundamental frequency level, and knock harmonic frequency level).

Paired t-tests were used to test for differences in inter-fish distance during approach and lateral display behaviors between noisy and quiet conditions. Growls and knocks were tested separately and a Bonferroni correction was applied to correct for multiple comparisons.

Behavioral analysis was performed by watching an arbitrarily selected portion of each video and recording each behavior that occurred, and whether or not the behavior was accompanied by sound. The video segment analyzed was not designated by a specific time, but by the length necessary to acquire 100-150 behaviors for each male, under each treatment (noisy and quiet). For each male, under each treatment, the total number of agonistic and courtship behaviors was divided by the duration of the portion of the video in which they occurred to obtain a behavior rate (behaviors/minute) for agonistic behaviors and courtship behaviors. The number of behaviors that were associated with a vocalization was also divided by the total number of behaviors performed by a particular individual to obtain a percentage of behaviors that were associated with sound (% assoc.) under both treatment conditions, for both agonistic and courtship contexts.

A doubly multivariate repeated measures two-way MANOVA was used to test whether a greater number of agonistic and courtship behaviors were performed under noisy conditions than quiet conditions, and whether a greater percentage of agonistic and courtship behaviors were accompanied by acoustic signals under noisy conditions than quiet conditions. Two within-subject factors (treatment and context), each with two levels (noisy and quiet for treatment; agonistic and courtship for context) were included in the model. Additionally, two measures (behaviors/minute and percentage of behaviors associated with sound) were included in the model.

Results

For approach behaviors under quiet conditions, fish standard length showed a significant positive correlation with pulse interval (Pearson correlation = 0.564, P = 0.015, N = 18; Figure 2-3b), and a significant negative correlation with burst harmonic frequency (Pearson correlation = -0.665, P = 0.002, N = 19; Figure 2-4b). Under noisy conditions, many more parameters showed significant correlations with fish standard length including: pulse duration (Pearson correlation = 0.503, P = 0.033, N = 18; Figure 2-5a), pulse interval (Pearson correlation = 0.498, P = 0.035, N = 18; Figure 2-3a), burst duration (Pearson correlation = 0.497, P = 0.036, N = 18; Figure 2-6a), burst rate (Pearson correlation = -0.797, P < 0.001, N = 17; Figure 2-7a), knock duration (Pearson correlation = 0.659, P = 0.014, N = 13; Figure 2-8a), burst fundamental frequency (Pearson correlation = -0.490, P = 0.039, N = 18; Figure 2-9a), burst fundamental frequency dB (Pearson correlation = 0.652, P = 0.003, N = 18; Figure 2-10a), and knock fundamental frequency dB (Pearson correlation = 0.785, P = 0.001, N = 13; Figure 2-11a).

For lateral display behaviors under quiet conditions, fish standard length showed a significant positive correlation with pulse interval (Pearson correlation = 0.638, P = 0.019, N = 13; Figure 2-3d), and a significant negative correlation with burst harmonic frequency (Pearson correlation = -0.660, P = 0.007, N = 15; Figure 2-4d). This was similar to approach behaviors under quiet conditions. Under noisy conditions, however, other signal parameters showed significant correlations with fish standard length during lateral display behaviors including: pulse duration (Pearson correlation = 0.548, P =

0.042, N = 14; Figure 2-5c), pulse interval (Pearson correlation = 0.709, P = 0.010, N = 12; Figure 2-3c), burst fundamental dB (Pearson correlation = 0.595, P = 0.025, N = 14; Figure 2-10c), knock fundamental frequency dB (Pearson correlation = 0.570, P = 0.021, N = 16; Figure 2-11c), and knock harmonic frequency (Pearson correlation = -0.604, P = 0.010, N = 17; Figure 2-12c).

Distance from the hydrophone was not correlated with the level of either the fundamental (Pearson correlation: r = -0.028, P = 0.364) or the first harmonic (Pearson Correlation: r = -0.042, P = 0.170) frequency of bursts under noisy conditions (Figure 2-13). There was, however, a significant negative correlation between the level of the fundamental (Pearson Correlation: r = -0.514, P <0.001) and first harmonic (Pearson Correlation: r = -0.552, P < 0.001) frequencies of knocks and the distance from the hydrophone (Figure 2-14).

There was not a significant main effect of context (agonistic and courtship) on growl signal parameters for approach behaviors (MANCOVA: $F_{10,8}$ = 2.059, P = 0.159) under noisy conditions (Table 2-1). There was also no significant main effect of context on either growl (MANCOVA: $F_{10,2}$ = 0.590, P = 0.767) or knock (MANCOVA: $F_{5,14}$ = 0.796, P = 0.570) signal parameters for lateral display behaviors under noisy conditions (Table 2-2).

An overall difference was found in duration between the three call types for approach behaviors under noisy conditions (repeated measures ANOVA: $F_{2,10}$ = 40.954, P <0.001; Table 2-3). Bonferroni corrected post hoc tests indicated that under noisy conditions, burst duration (mean ± SD; 0.057678 ± 0.022026 sec, N = 12) was significantly longer than both knock duration (0.012270 ± 0.002639 sec, N = 12; Bonferroni corrected post hoc: P < 0.001) and pulse duration (0.007708 ± 0.001124 sec, N = 12; Bonferroni corrected post hoc: P < 0.001). Knocks were also significantly longer in duration than pulses (Bonferroni corrected post hoc: P < 0.001).

Paired t-tests indicated that for approach behaviors, under noisy conditions (Table 2-3), the fundamental frequency of knocks (189.00 ± 90.19 Hz, N = 12) was significantly higher than the fundamental frequency of bursts (83.29 ± 9.16 Hz, N = 12; paired t test: $t_{11} = 3.962$, P = 0.002). The first harmonic frequency of knocks (595.46 ± 99.37 Hz, N = 12) was also significantly higher than the first harmonic frequency of bursts (301.13 ± 30.57; paired t test: $t_{11} = 11.947$, P < 0.001). The level of the fundamental frequency of knocks (96.98 ± 4.70 dB, N = 12) was significantly greater than that of bursts (79.62 ± 4.35 dB, N = 12; paired t test: $t_{11} = 11.766$, P < 0.001), and the level of the first harmonic frequency of knocks (102.82 ± 5.08 dB, N = 12) was significantly greater than that of bursts (77.52 ± 3.08 dB, N = 12; paired t test: $t_{11} = 13.936$, P < 0.001). These results also corroborated findings under quiet conditions.

Results from the repeated measures MANOVA showed a significant main effect of background noise level on growl acoustic signal parameters associated with approach behaviors (repeated measures MANOVA: $F_{10,7} = 9.211$, P = 0.004; Table 2-4). Bonferroni corrected post hoc tests indicated that burst duration under noisy conditions (0.054508 ± 0.020176 sec, N = 17) was significantly shorter than under quiet conditions (0.084586 ± 0.037076 sec, N = 17; Bonferroni corrected post hoc: P = 0.003). Burst rate under noisy conditions (7.347 ± 2.447 sec, N = 17) was significantly greater than under quiet conditions (5.605 ± 2.034 sec, N = 17; Bonferroni corrected post hoc: $P = 0.023$). The level of both the fundamental frequency (noisy; 78.61 ± 3.92, quiet; 75.84 ± 5.53) and the first harmonic frequency (noisy; 77.19 ± 2.90, quiet; 71.03 ± 4.99) of growls was significantly greater under noisy conditions than quiet conditions (fundamental frequency dB; Bonferroni corrected post hoc: $P = 0.037$, first harmonic dB; Bonferroni corrected post hoc: $P < 0.001$).

No significant main effect of noise level on acoustic signal parameters was found for knocks associated with approach behaviors (repeated measures MANOVA: $F_{5,4} = 0.774$, $P = 0.615$), or lateral display behaviors (repeated measures MANOVA: $F_{5,6} = 4.244$, $P = 0.054$; Table 2-5).

Paired t tests with Bonferroni correction showed that during lateral display behaviors (Table 2-6), burst duration was significantly shorter under noisy conditions (0.058557 ± 0.026548 sec, N = 14) than quiet conditions (0.087573 ± 0.040596 sec, N = 14; paired t test: $t_{13} = -4.471$, $P = 0.001$). Paired t tests also showed that the level of both the fundamental frequency (noisy; 78.79 ± 5.17 dB, N = 14, quiet; 74.71 ± 4.51 dB, N = 14) and the first harmonic frequency (noisy; 77.15 ± 3.81 dB, N = 14, quiet; 70.05 ± 4.55 dB, N = 14) of growls was significantly greater under noisy conditions than quiet conditions (fundamental frequency dB; $t_{13} = 3.919$, $P = 0.002$, first harmonic dB; $t_{13} = 6.984$, $P < 0.001$). No other parameters were found to be significantly different.

There was no significant difference in interfish distance during growls for either approach behaviors (noisy; 98.48 ± 52.37 mm, N = 18, quiet; 85.19 ± 51.49 mm, N = 19; paired t test: $t_{17} = 0.763$, $P = 0.456$) or lateral display behaviors (noisy; 52.88 ± 24.45

mm, N = 14, quiet; 48.86 ± 16.29 mm, N = 17; paired t test: $t_{13} = 0.562$, P = 0.584; Figure 2-15). There was also no significant difference in interfish distance during knocks for approach behaviors (noisy; 70.21 ± 30.38 mm, N = 13, quiet; 57.47 ± 23.82 mm, N = 12; paired t test: $t_8 = 0.992$, P = 0.350; Figure 2-15). Knocks produced during lateral displays, however, were made at a significantly closer distance under noisy conditions (39.31 ± 13.18 mm, N = 17) than quiet conditions (60.08 ± 12.66 mm, N = 12; paired t test: $t_{10} = -3.984$, P = 0.003; Figure 2-15).

Behavioral analysis indicated that there was no significant main effect of context (agonistic and courtship; doubly multivariate repeated measures MANOVA: $F_{2,14} = 2.323$, P = 0.134) or treatment (noisy and quiet; doubly multivariate repeated measures MANOVA: $F_{2,14} = 0.190$, P = 0.829) on behavior rate and percentage of behaviors associated with sounds (Figure 2-16).

Discussion

The general description of the basic signal components (knocks, bursts, and pulses) of *C. venusta* calls under noisy conditions agreed with the findings from sounds produced under quiet laboratory conditions and field conditions (D.E. Holt and C.E. Johnston, unpublished data). Knocks, bursts and pulses were all distinct in duration, and knocks and bursts showed significant differences in fundamental and harmonic frequencies and level under noisy conditions, as was seen under quiet conditions. I also found that the correlations between distance from the hydrophone and level were similar for sounds produced under noisy and quiet conditions.

Correlations between fish standard length and signal parameters, although not significant in all cases, typically shared similar trends between noisy and quiet conditions for both approach and lateral display behaviors. Pulse duration, burst duration, knock duration, burst fundamental frequency, burst fundamental frequency level, burst harmonic frequency, knock fundamental frequency level, and knock harmonic frequency all showed a statistically significant correlation with fish standard length for at least one of the four behavior/noise level combinations (approach/quiet, approach/noisy, lateral display/quiet, and lateral display/noisy). Combinations that were not statistically significant did show trends that agreed with the significant correlation, suggesting that their statistical significance may have not been detected due to the high variability among individuals. To explain these correlations, I make the assumption that the non-significant correlations of the signal parameters listed above are probably real due to the fact that they are going in the same direction for both behavior types, under both quiet and noisy conditions, and the simplest explanations entail a physiological mechanism that has no reason to differ between quiet and noisy conditions.

A possible explanation for the correlation of standard length with pulse duration, based on the findings of Connaughton et al. (2000), is that larger males have longer sonic muscles, which take a longer period of time to complete a full twitch. Connaughton et al. (2000) described the sounds produced by male weakfish (*Cynoscion regalis*), who's signal structure is very similar to that of *C. venusta*, and found, as did I for *C. venusta*, a positive correlation between total body length of male *C. regalis* and pulse duration. The same mechanism may also be responsible for the significant correlation between knock duration and fish standard length during approach behaviors under noisy conditions.

The significant correlation between fish standard length and burst fundamental frequency level, as well as knock fundamental frequency level is not necessarily surprising because larger males probably have larger, stronger muscles capable of producing more force, thus producing a louder sound. Complicating the issue is the fact that a significant correlation between standard length and level was observed only for the fundamental frequency, and not for the harmonic frequency of either bursts or knocks. If body size permits the ability to increase levels, why should it be limited to only the fundamental frequency component of the signal, especially when considering that the natural quiet window in the environment corresponds best to the harmonic frequency of *C. venusta*'s signals? I am unsure why, or how male size affects level of the fundamental frequency, and not the harmonic frequency within the same acoustic signal. This question, along with the question of why pulse duration increases with body size, undoubtedly requires a more thorough knowledge of the anatomical and physiological mechanisms responsible for sound production in this species, which are currently unknown.

The positive correlation between fish standard length and pulse interval (the length of time between the first peaks in the waveform of two adjacent pulses) was significant for both behaviors, under both quiet and noisy conditions, and was the only signal parameter that showed a consistent correlation for both behaviors, under both quiet and noisy conditions. The possibility that longer muscle fibers in larger fish require more time to contract (and presumably relax) may also be an explanation for the longer duration of pulse intervals in larger *C. venusta*.

Although it seems intuitive that an increased swim bladder size in larger males would carry a lower resonant frequency (Harris 1964), thus causing the negative correlations seen between standard length and burst fundamental and harmonic frequencies and knock harmonic frequency, swim bladder size does not necessarily correlate with signal frequency. In many fish that produce long duration, tonal calls, sonic muscle contraction rate determines the fundamental frequency of the sound (Cohen and Winn 1967; Bass and Baker 1991), not the size of the swim bladder. Sounds produced by many fishes (including *C. venusta*) have a shorter, more pulsed structure that is probably produced by a small number of muscle contractions or a single muscle twitch (Connaughton et al. 2000). Connaughton suggests that the longer pulse duration of *C. regalis* signals, caused by a slower muscle twitch, is responsible for the negative correlation between fish size and call frequency, which may also be an explanation for the correlation between fish size and burst fundamental and harmonic frequencies, and knock harmonic frequency seen in *C. venusta*.

There are several correlations that I am unable to explain. First, if muscle fiber length is a determining factor for both fundamental and harmonic frequencies in bursts, and the harmonic frequency in knocks, I would expect knock fundamental frequency to show a similar correlation with body size. However, no significant correlations were found. Also, the significant negative correlation between fish standard length and burst rate existed for approach behaviors under noisy conditions only, and was not accompanied by a similar trend under quiet conditions. I am unable to explain this.

Prior to this experiment, my null hypothesis was that when subjected to elevated noise levels, *C. venusta* would attempt to compensate for decreased signal-to-noise ratios using a combination of methods often utilized by other species. Increasing signal redundancy in the temporal domain (by increasing signal duration or signal emission rate) is one method for achieving an increased signal-to-noise ratio with a background noise that randomly changes in amplitude. I was not surprised that the most basic temporal components of *C. venusta* signals (pulse duration, pulse interval, and pulse rate) remained conserved. Mann (1997) found that the basic temporal components (pulse period specifically) of the damselfish's acoustic signals are affected least by propagation, and probably serve as a reliable source of species identification. My null hypothesis that signal redundancy would increase was not supported by the fact that burst duration decreased for both approach and lateral display behaviors under noisy conditions. Shorter burst duration means a decrease in signal redundancy, which goes against the predictions of information theory (Shannon and Weaver 1949), and means that there is a smaller chance that information encoded in the burst structure will fall into a lull in the background noise. Accompanying the shortened duration of bursts under noisy conditions was an increase in burst rate, which could compensate for the shorter duration by maintaining redundancy under noisy conditions. This tradeoff between burst duration and rate was not as consistent as the decrease in burst duration, however, as it was observed in approach behaviors, but not in lateral display behaviors.

The functional significance of burst duration for *C. venusta* is currently unknown. Phillips and Johnston (2008a) found in the whitetail shiner (*C. galactura*), evidence suggesting a potential role of burst duration as an indicator of male quality due to a significant positive correlation between burst duration during agonistic behaviors and fish standard length. A significant correlation was not consistently found for *C. venusta*, but burst duration was significantly positively correlated with fish standard length for approach behaviors under noisy conditions. Phillips and Johnston (2008b) also reported a significant difference in burst duration between agonistic and courtship contexts in *C. galactura*, suggesting that *C. galactura* at least partially utilizes burst duration to convey information associated with different behavioral contexts. The fact that burst duration showed a significant positive correlation with *C. venusta* body size for approach behaviors under noisy conditions suggests that it may be an indicator of male quality in this species. However, because burst duration was highly variable and did not differ between contexts or behaviors, it is unlikely that it is a critical parameter for recognition tasks such as species identification, or to convey different behavioral intentions. It is possible that the decrease in burst duration during noisy trials was an energetic response to the increase in burst rate. However, because the pattern was not found for both types of behaviors, and sound production is thought to be a relatively low energetic investment for fishes (Amorim et al. 2002), I believe this to be an unlikely scenario. Because burst duration decreased for both approach and lateral display behaviors under noisy conditions relative to quiet conditions, and burst rate increased for only approach behaviors, I conclude that *C. venusta* probably does not effectively increase signal redundancy under noisy conditions.

The results of this study show an elevation in the level of growls under noisy conditions, suggesting that *C. venusta* may utilize the Lombard effect to help overcome elevations in ambient noise levels. Both the fundamental and harmonic frequencies of growls showed increased levels under noisy conditions. There is also, however, some evidence suggesting that the difference in level of signals between noisy and quiet conditions may be an artifact of experimental design. The absence of low intensity signals in the noisy trials that were present under quiet conditions (Figure 2-17) may either be a result of *C. venusta* not producing lower amplitude sounds during the noisy trials, or may be a result of the signals being buried in the background noise in the noisy trials. If the latter is the case, then the average level under noisy conditions would not be accurate. This theory is supported further by the fact that the level below which no acoustic signals were detected under noisy conditions was approximately the same level as the background noise.

The fact that the behavior rate between noisy and quiet trials did not differ, and that the percentage of behaviors associated with sound was greater under quiet conditions than under noisy conditions, provides further support for the hypothesis that the softer signals produced by *C. venusta* during noisy trials were not detected by the hydrophone, and that the apparent elevation in level is an artifact. It is also likely that knocks did not show an elevation in level under noisy conditions because all knocks were significantly louder than the background noise during noisy trials, and therefore did not have any masked signals under noisy conditions. The softest knocks detected during either quiet or noisy trials were more than 10 dB greater than the background noise during noisy trials. Because it is unlikely that any knocks went undetected, and no difference was found between the level of knocks produced in noisy and quiet trials, it is a possibility that the difference found for growls is due to a number of softer signals that went undetected during noisy trials.

The results of this study are also mixed as to whether *C. venusta* attempt to close the distance between sender and receiver during acoustic communication under elevated noise conditions. Although the inter-fish distance was significantly smaller under noisy conditions than quiet conditions for knocks produced during lateral display behaviors, distances were not significantly different for growls produced under both approach and lateral display behaviors, or knocks produced under approach behaviors. One possible explanation for this is that because lateral displays are a behavior associated with higher motivation (Phillips and Johnston 2008b) than approach behaviors, the importance of successfully transmitting the message may be greater. Because knocks are typically more associated with aggression (Phillips and Johnston 2008b), and more escalated, physical contests may follow if a dispute is not settled at the lateral display stage, it may afford males to adjust inter-fish distance for knocks produced during lateral display behaviors under noisier conditions. Growls are more typically associated with courtship behaviors, and since there are usually an abundance of females around a nesting site, a single missed spawning opportunity may not be as costly as a physical contest with an equally sized male.

The results of the current study are not conclusive enough to definitively say whether *C. venusta* is able to compensate for depressed signal-to-noise ratios under elevated noise conditions using either temporal redundancy, increased signal levels, or by closing interfish distance. Although the average level increased under noisy conditions, other evidence suggesting that some soft signals may have been masked under noisy conditions leads us to believe that *C. venusta* may not possess the ability to increase signal sound pressure level to compensate for elevated noise levels. Future studies investigating this question should keep in mind the potential problems encountered by the current study, and make attempts to remove the background noise from the recordings while preserving the acoustic signals embedded within the noise. This could potentially be done by adding the inverse waveform of the artificial noise presented during the noisy trials to the final recordings. This would cancel the artificial noise coming from the speaker, but not the sounds being produced by the fishes, and may at least allow the researcher to see if any signals were being masked by the noise.

The possibility that *C. venusta* may not be capable of compensating for increased noise levels is not entirely surprising. *Cyprinella venusta* has evolved in small streams where the ambient noise has probably changed very little. When I consider the consistent presence of a quiet window in the natural soundscape, and how well it fits with the acoustic signals of *C. vensuta*, it seems that selective pressures for dealing with elevated noise levels were probably not strong, preventing the evolution of techniques for dealing with the types of interference that artificial noise sources provide today. Furthermore, because propagation of sounds in stream conditions is so limited to begin with due to the shallow depths and high cutoff frequencies, fish are probably forced to call near maximum amplitude to begin with just to maintain the small active area permitted by their physical environment. Holt and Johnston (unpublished data) have found evidence suggesting that two sources of anthropogenic noise occurring on small, non-navigable streams may affect the signal-to-noise ratio of *C. venusta* signals at distances of up to nearly 900 m. Although *C. venusta* is very abundant throughout it's large range and is presently in no danger of becoming threatened, other vocal fish species that do not have the abundance or range of *C. venusta* such as the pygmy sculpin (*Cottus paulus*), or threatened species that occur in larger rivers that may be exposed to boat traffic such as the pallid sturgeon (*Scaphirhynchus albus*), could be impacted much more by elevated noise levels.

With worldwide anthropogenic noise levels increasing (Hildebrand 2009; Slabbekoorn et al. 2010), so must our understanding of how fishes will respond. Despite a growing concern for the effects of anthropogenic noise on fishes, our understanding of the subject is not unequivocal, and far more questions than answers have been brought to light (Popper and Hastings 2009). The current study provides insight into the relationship between background noise and vocalizations in a common vocal fish found in small freshwater systems. Although I was unable to definitively answer the question of whether *C. venusta* is able to compensate for decreased signal-to-noise ratios, the fact that it did not show obvious signs of compensation may be a red flag for other species of concern.

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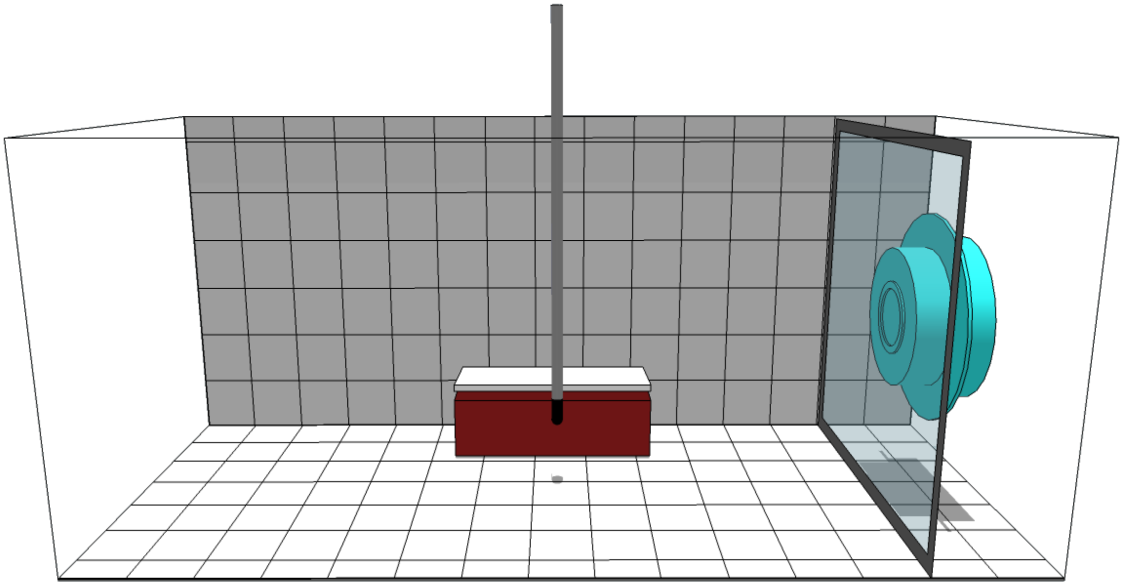


Figure 2-1 – Experimental tank setup. Underwater speaker is suspended behind a mesh barrier on the right hand side of the tank (according to the viewer's perspective). A hydrophone is suspended in front of an artificial nest (composed of a tile propped up on top of a brick), which is placed against the far wall of the tank. A light suspended above the tank cast shadow's onto the bottom grid, which together with the grid on the rear side of the tank allow the position of the fishes to be determined inside the tank.

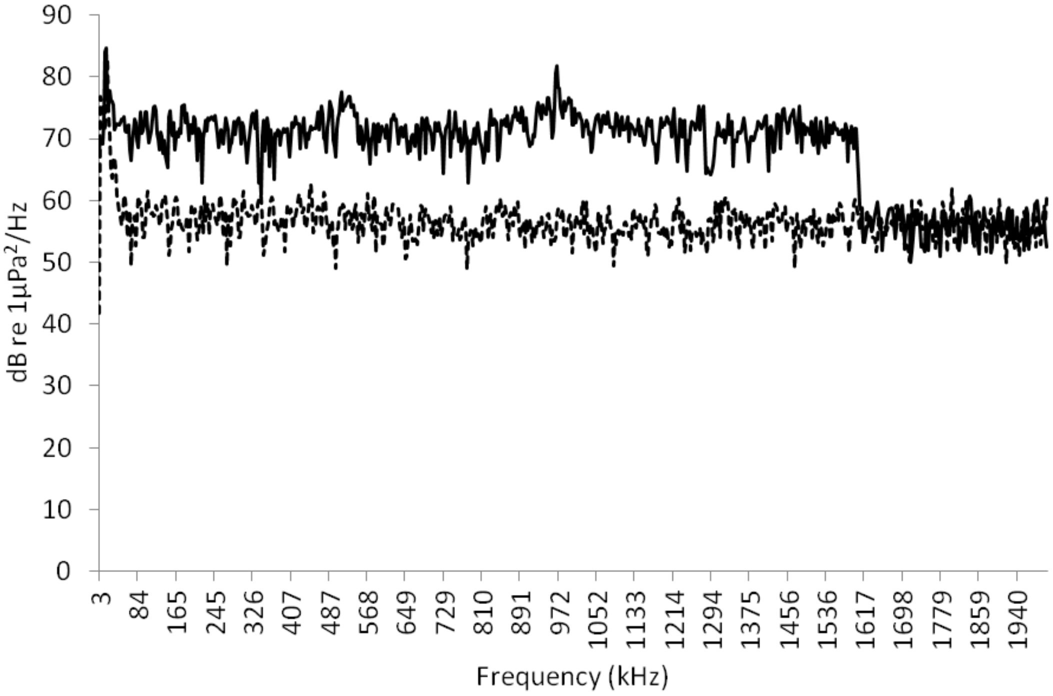


Figure 2-2 – Power spectrums showing background noise levels during quiet trials (broken line) and noisy trials (solid line). Power spectra were generated from a 1 second recording of background noise, in the absence of fish (Hanning window, sampling rate = 44.1 kHz, FFT samples = 16384, bin resolution = 2.69 Hz). Hydrophone placement was approximately 5 cm in front of the nest for both recordings.

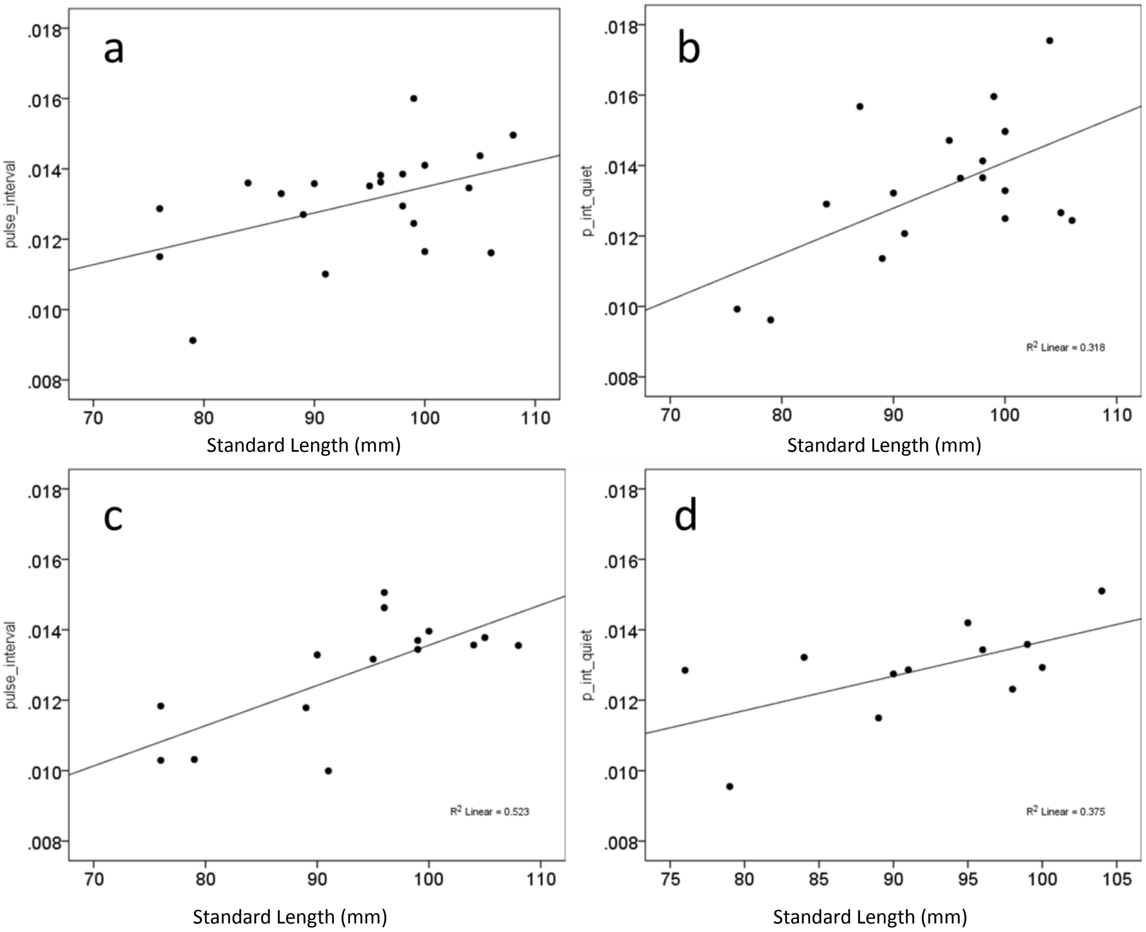


Figure 2-3 – Scatterplots showing the relationships between fish standard length (mm) and pulse interval (sec) for approach behaviors under noisy (a) and quiet (b) conditions, and lateral display behaviors under noisy (c) and quiet (d) conditions. All correlations were statistically significant and positive.

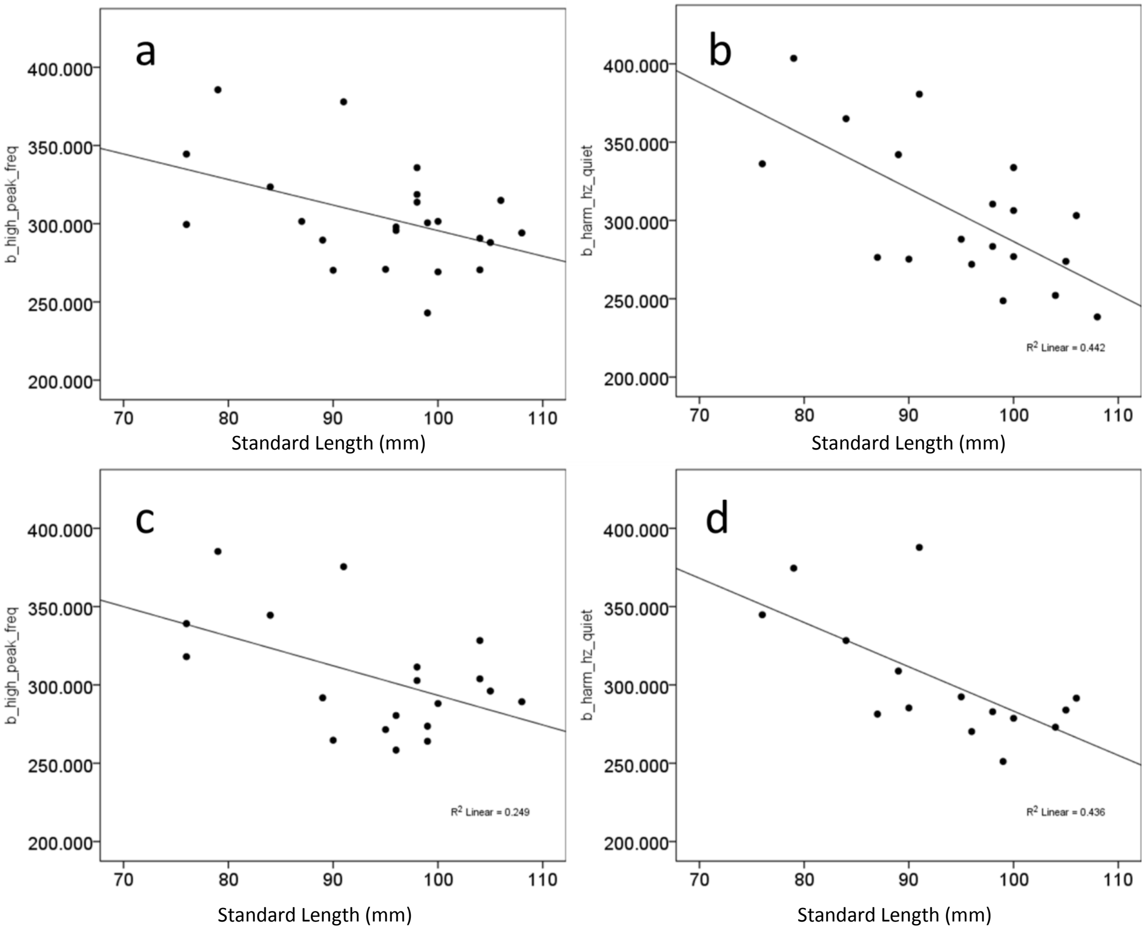


Figure 2-4 – Scatterplots showing the relationships between fish standard length and burst harmonic frequency for approach behaviors under noisy (a) and quiet (b) conditions, and lateral display behaviors under noisy (c) and quiet (d) conditions. Although a negative trend was observed for all conditions, only for approaches under quiet conditions (b) was the correlation statistically significant.

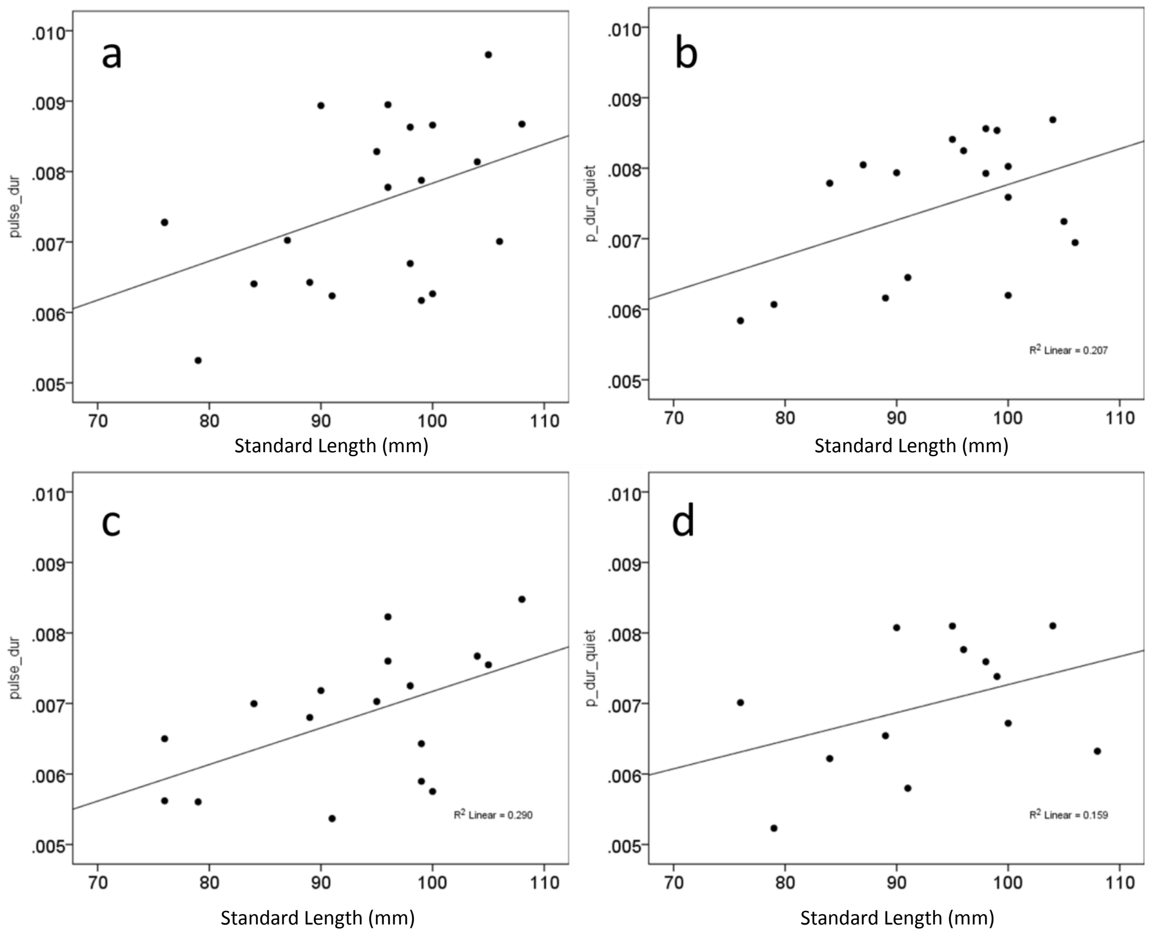


Figure 2-5 - Scatterplots showing the relationships between fish standard length and pulse duration for approach behaviors under noisy (a) and quiet (b) conditions, and lateral display behaviors under noisy (c) and quiet (d) conditions. Although a statistically significant correlation was only observed for approach and lateral display behaviors under noisy conditions, quiet conditions did show positive trends.

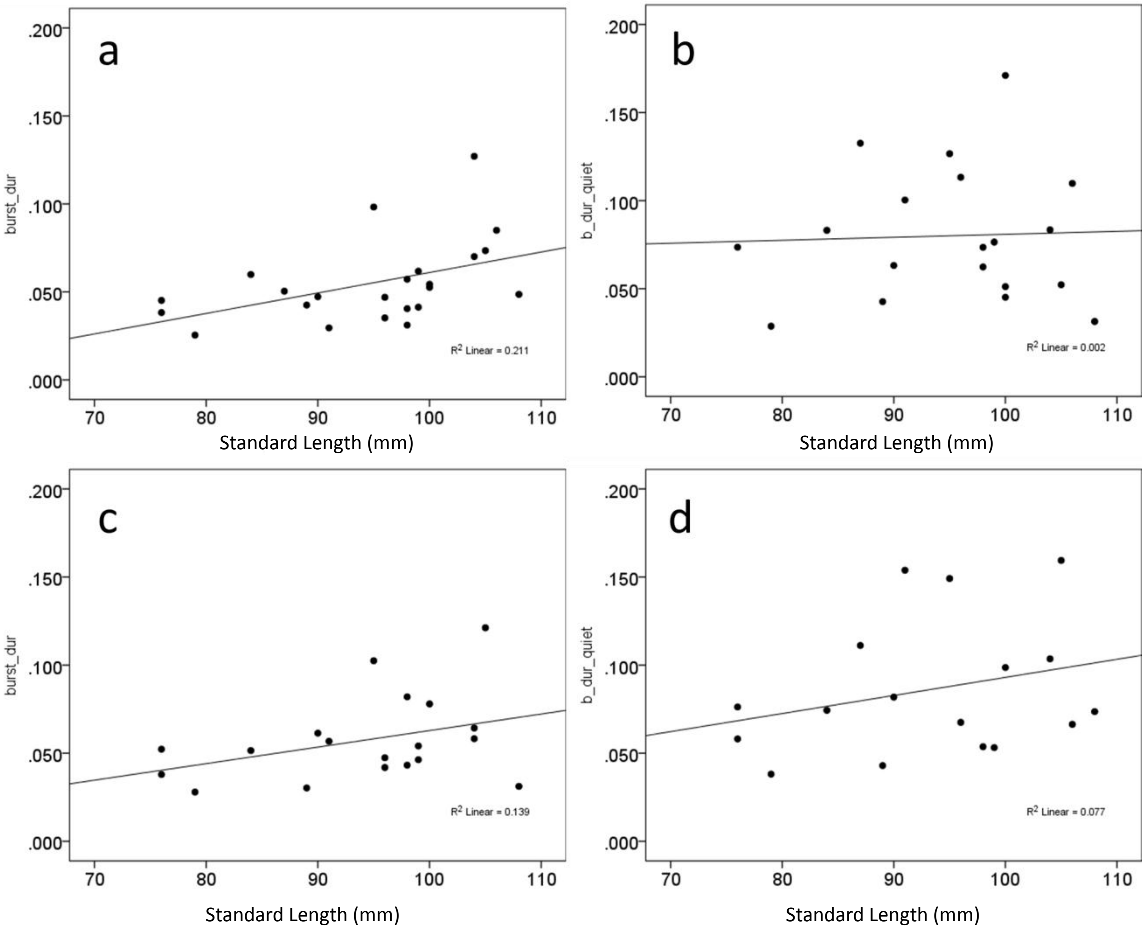


Figure 2-6 – Scatterplots showing the relationships between fish standard length and burst duration for approach behaviors under noisy (a) and quiet (b) conditions, and lateral display behaviors under noisy (c) and quiet (d) conditions. The only condition in which a statistically significant correlation was observed was during approach behaviors under noisy conditions (a).

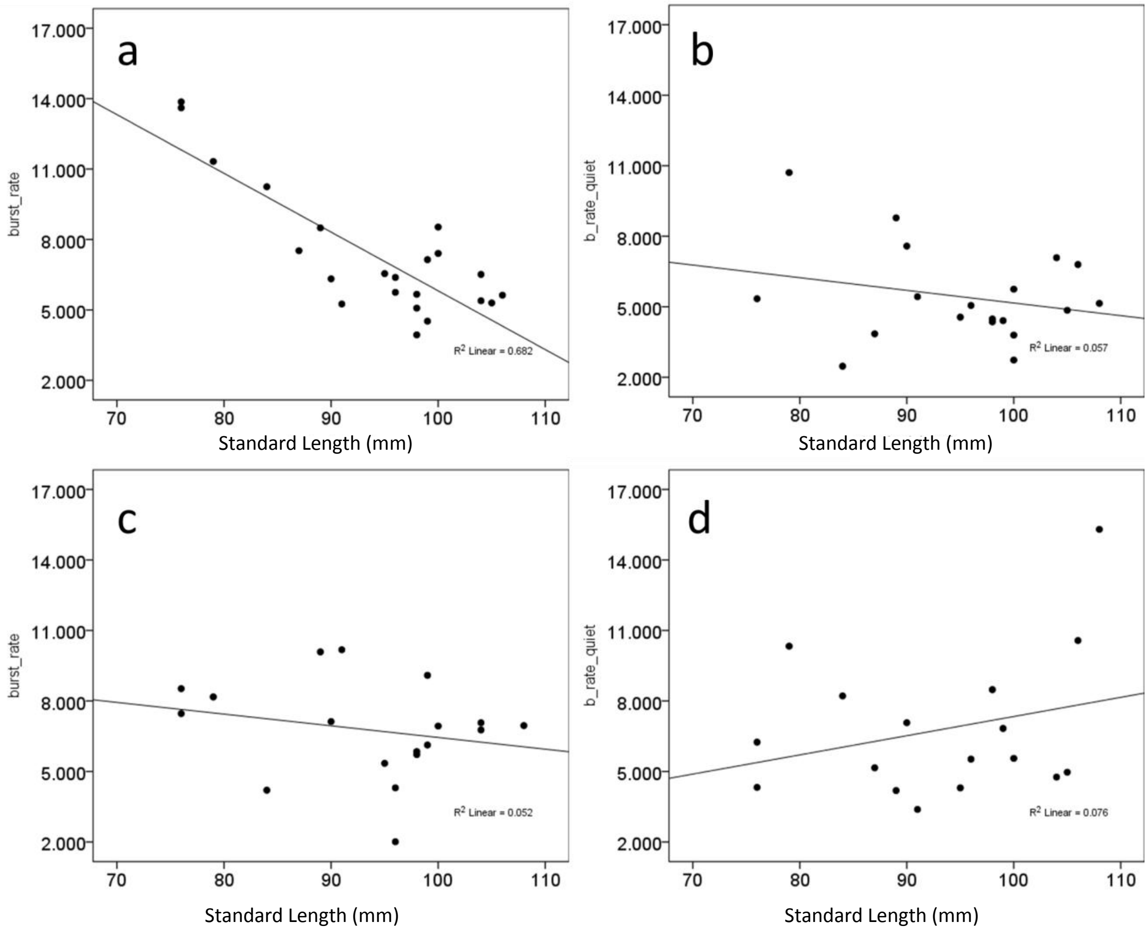


Figure 2-7 – Scatterplots showing the relationships between fish standard length and burst rate for approach behaviors under noisy (a) and quiet (b) conditions, and lateral display behaviors under noisy (c) and quiet (d) conditions.

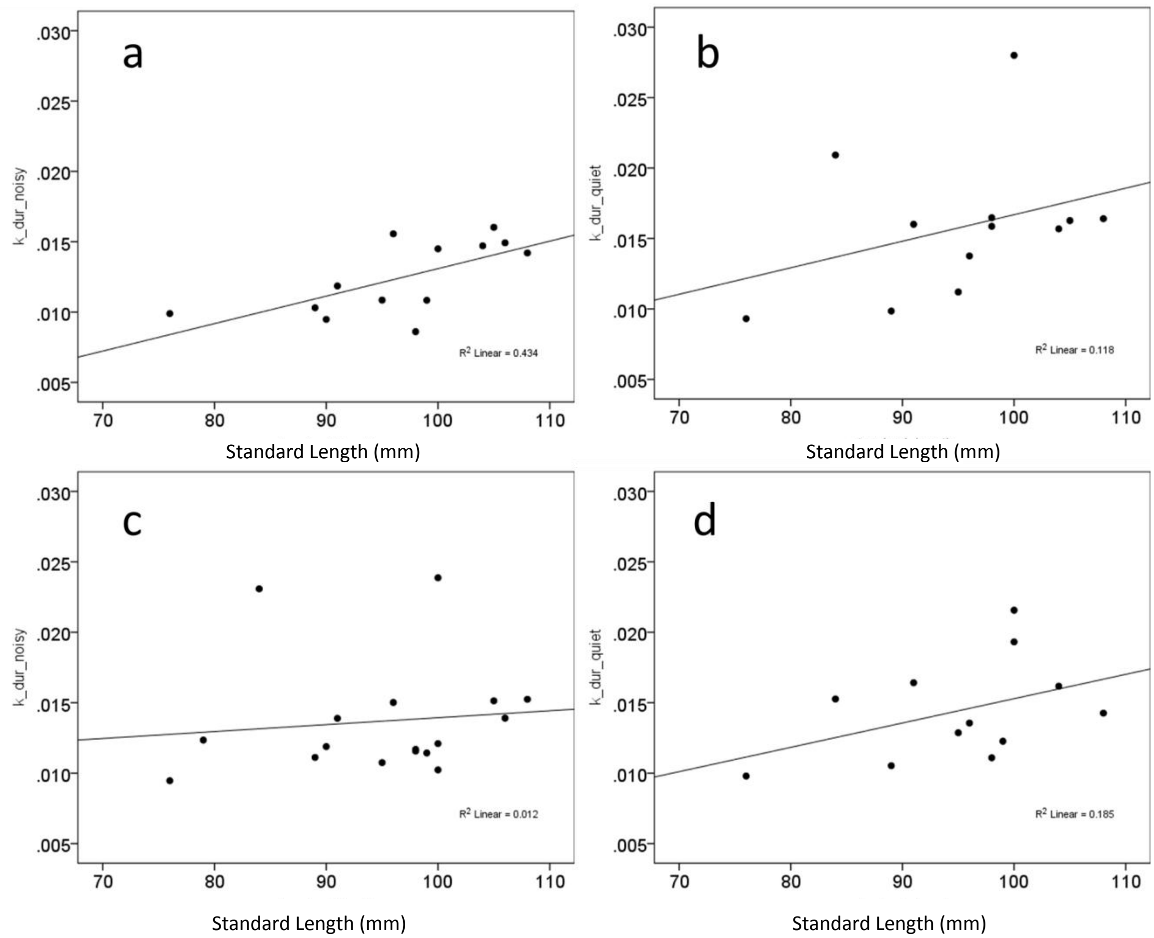


Figure 2-8 – Scatterplots showing the relationships between fish standard length and knock duration for approach behaviors under noisy (a) and quiet (b) conditions, and lateral display behaviors under noisy (c) and quiet (d) conditions.

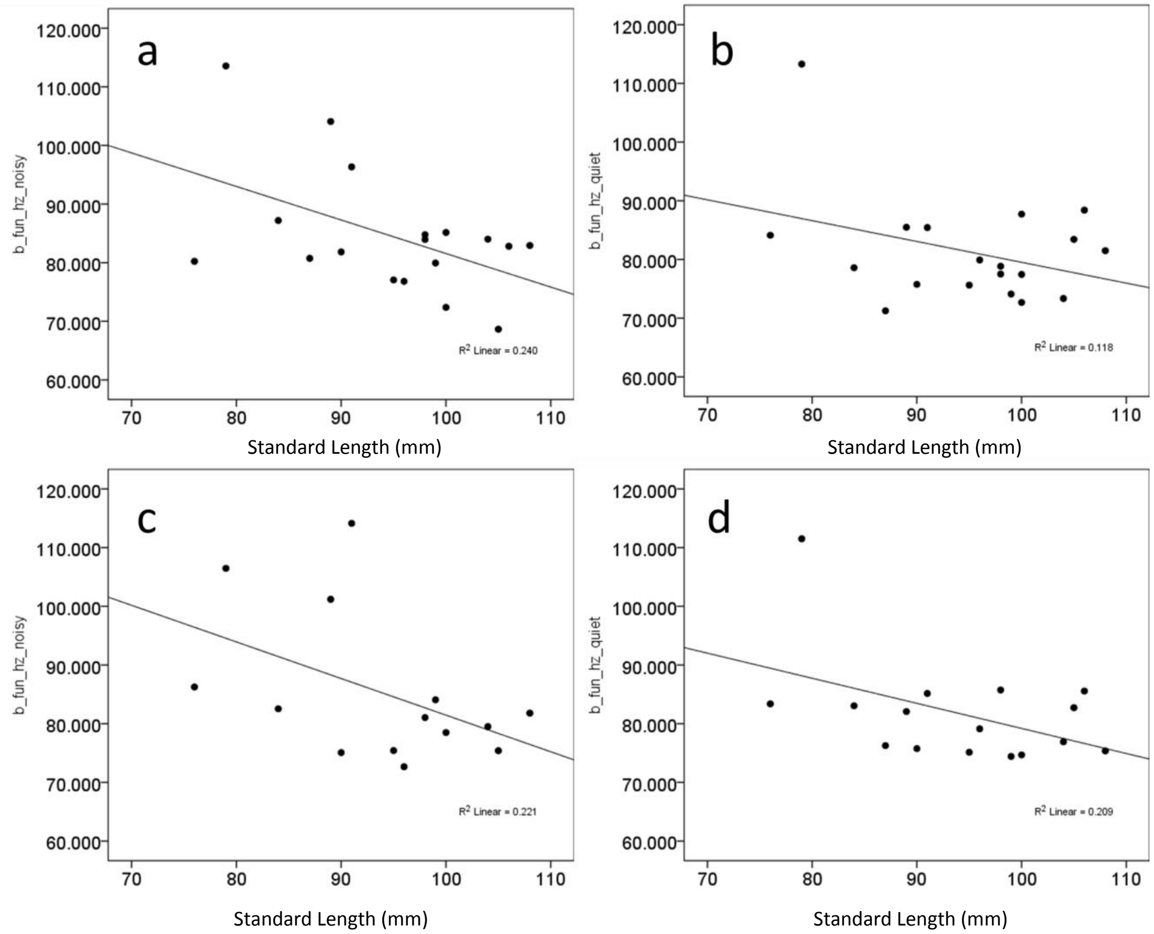


Figure 2-9 – Scatterplots showing the relationships between fish standard length and burst fundamental frequency for approach behaviors under noisy (a) and quiet (b) conditions, and lateral display behaviors under noisy (c) and quiet (d) conditions.

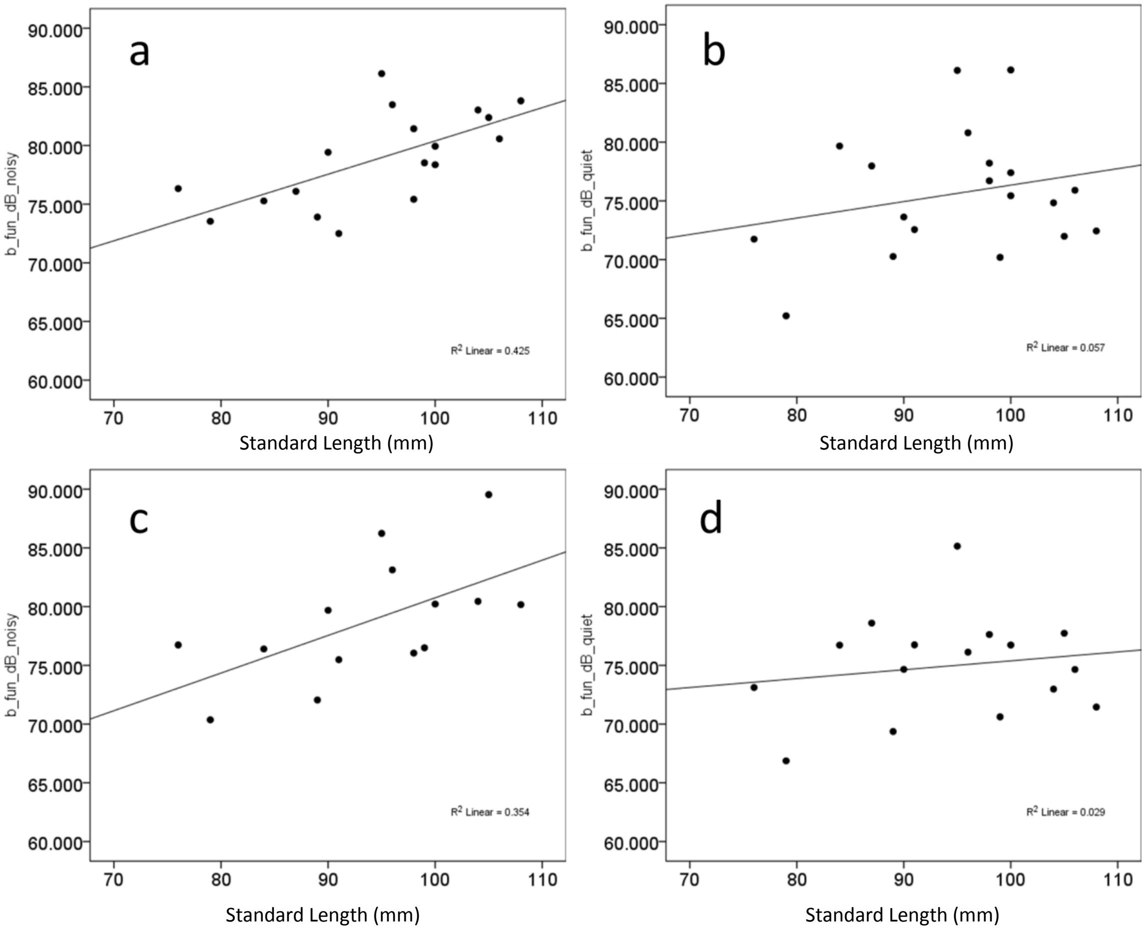


Figure 2-10 – Scatterplots showing the relationships between fish standard length and burst fundamental frequency level for approach behaviors under noisy (a) and quiet (b) conditions, and lateral display behaviors under noisy (c) and quiet (d) conditions.

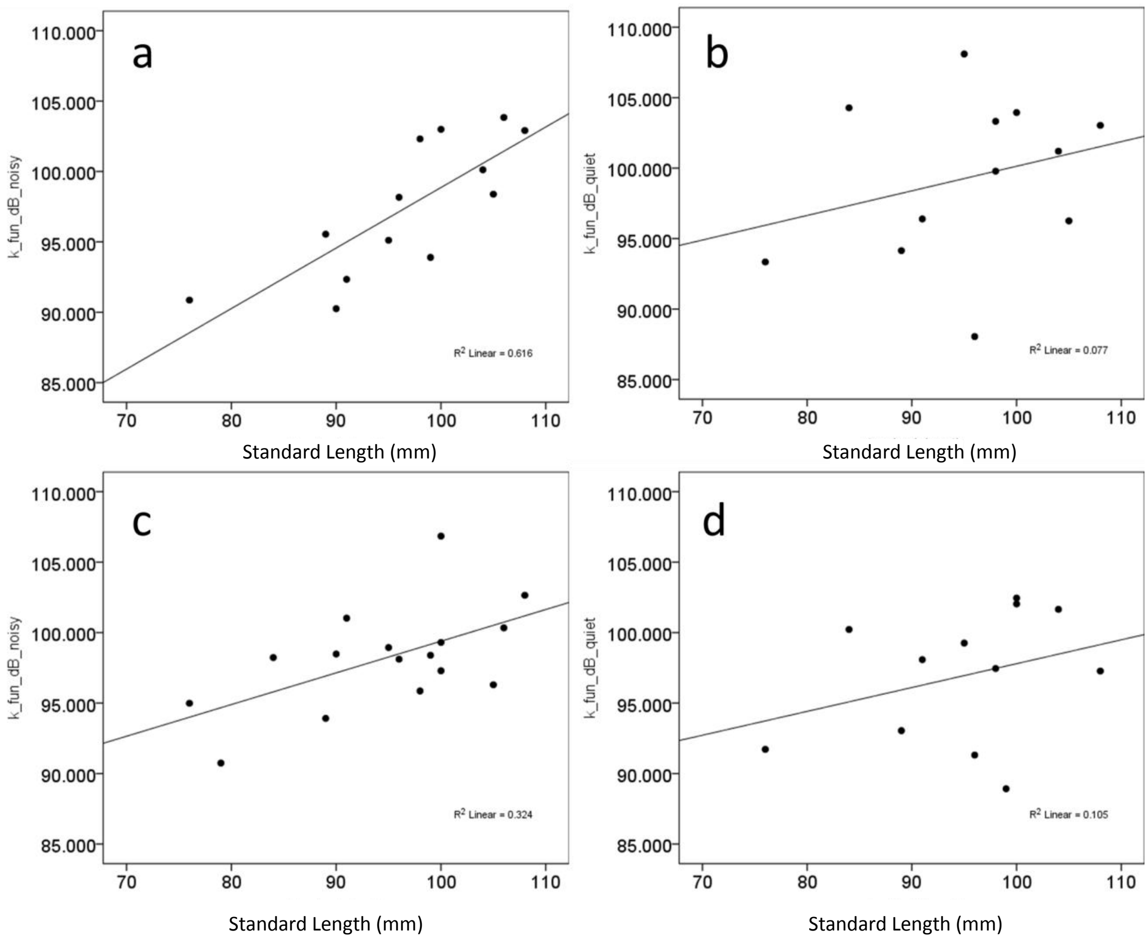


Figure 2-11 – Scatterplots showing the relationships between fish standard length and knock fundamental frequency level for approach behaviors under noisy (a) and quiet (b) conditions, and lateral display behaviors under noisy (c) and quiet (d) conditions.

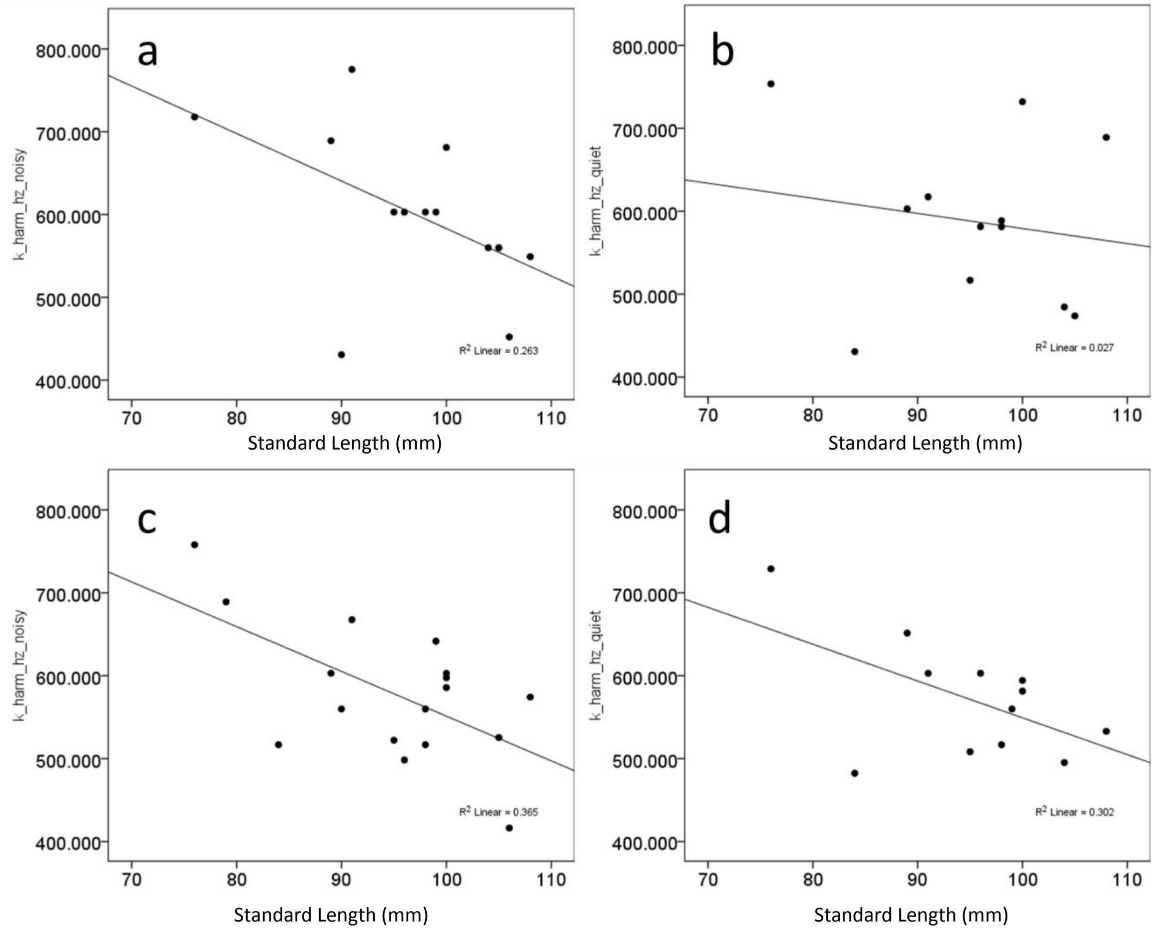
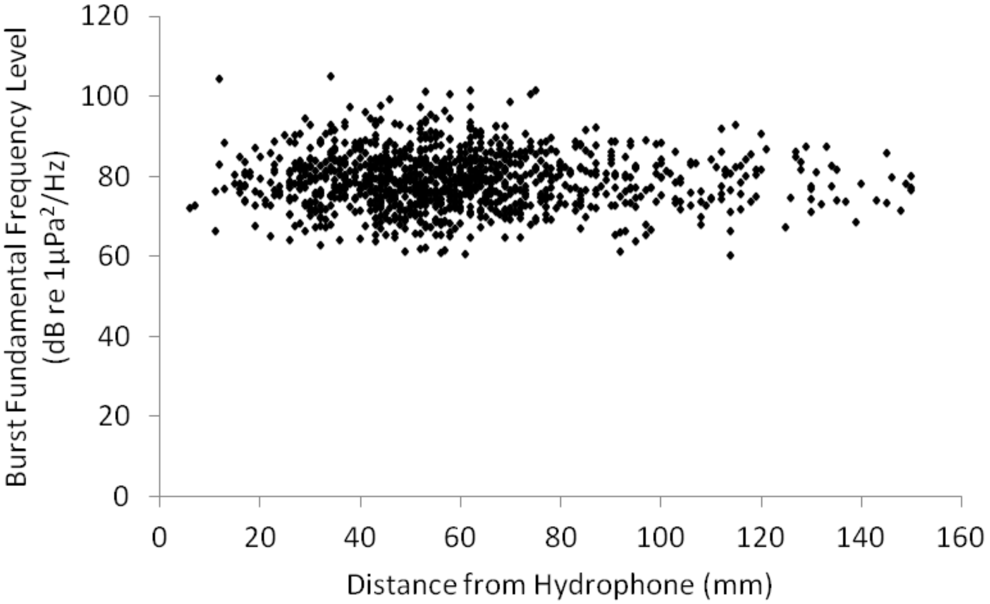


Figure 2-12 – Scatterplots showing the relationships between fish standard length and knock harmonic frequency for approach behaviors under noisy (a) and quiet (b) conditions, and lateral display behaviors under noisy (c) and quiet (d) conditions.



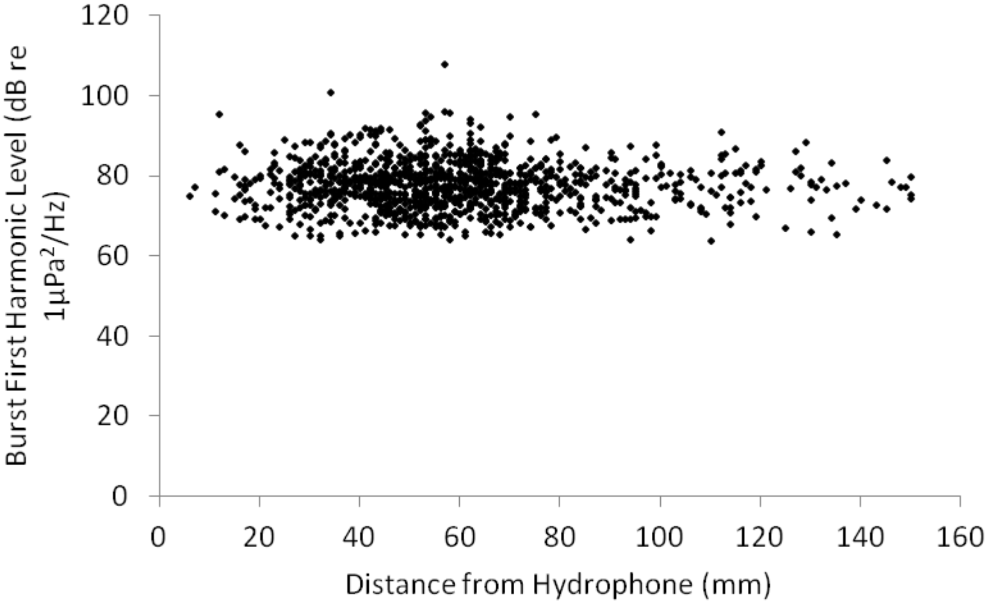


Figure 2-13 – Scatterplot of burst fundamental frequency level (top) and first harmonic level (bottom) with distance of sound producing fish from the hydrophone under noisy conditions. Neither showed a significant correlation (fundamental frequency; Pearson Correlation: r = -0.028, P = 0.364; first harmonic; Pearson Correlation: r = -0.042, P = 0.170).

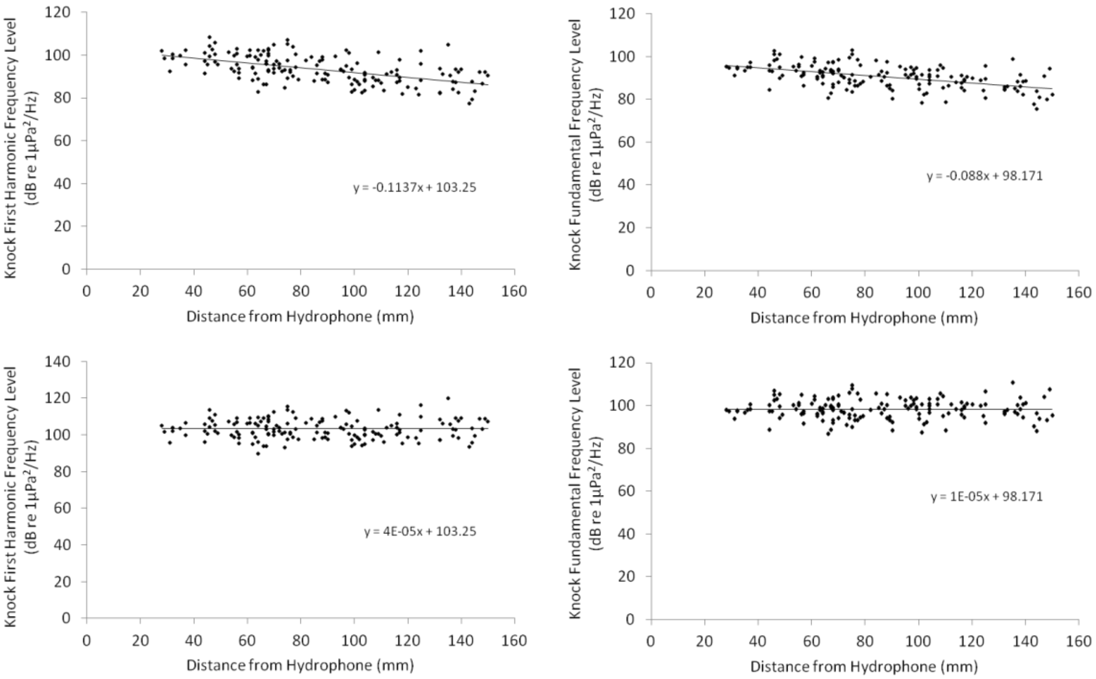


Figure 2-14 – Scatterplot with best fit line (equation included in graph) of fundamental frequency and first harmonic levels of knocks and distance of fish from hydrophone under noisy conditions. There was a significant negative correlation between the level of the fundamental frequency of knocks and distance from the hydrophone (top-right; Pearson Correlation: $r = -0.514$, $P < 0.001$), and the level of the first harmonic frequency of knocks and distance from the hydrophone (top-left; Pearson Correlation: $r = -0.552$, $P < 0.001$). Values were corrected by multiplying the distance from the hydrophone at each point by the slope of the best fit line, and adding the product to the original dB value. Corrected plots for both the first harmonic frequency level (bottom-left) and fundamental frequency level (bottom-right) are provided.

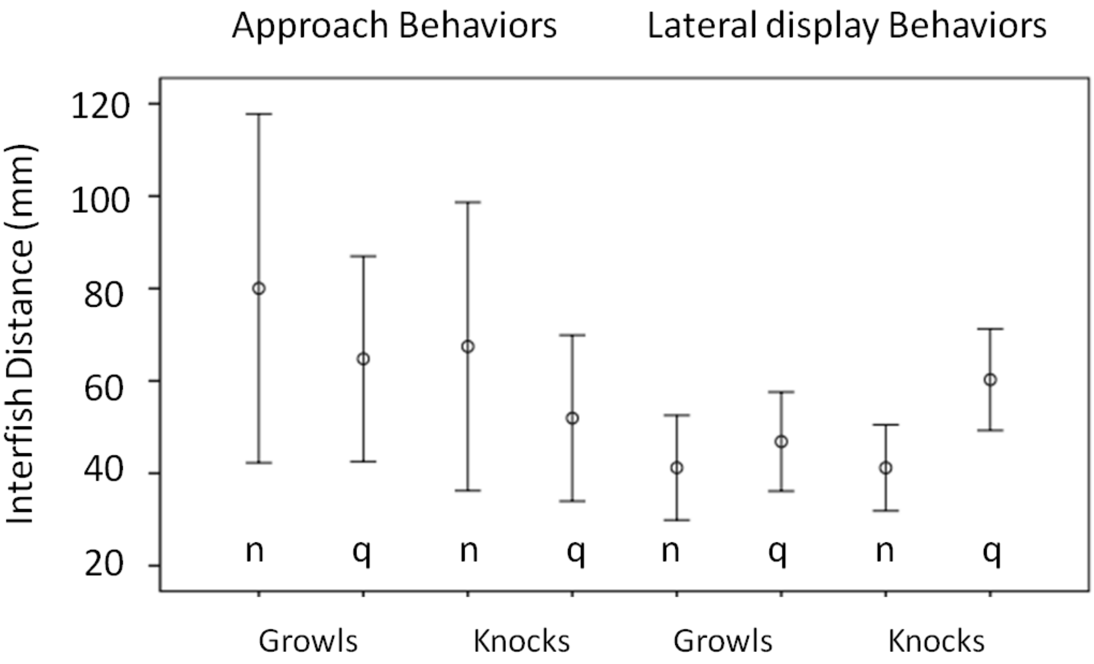


Figure 2-15 – Error bar plots showing interfish distances for growls and knocks, produced during approach and lateral display behaviors, under noisy and quiet conditions. The circles (noisy) and triangles (quiet) represent means, and the whiskers represent the 95% confidence interval around the mean. Knocks produced during lateral display behaviors represented the only situation in which interfish distance was significantly different between noisy and quiet conditions (paired t test: $t_{10} = -3.984$, $P = 0.003$).

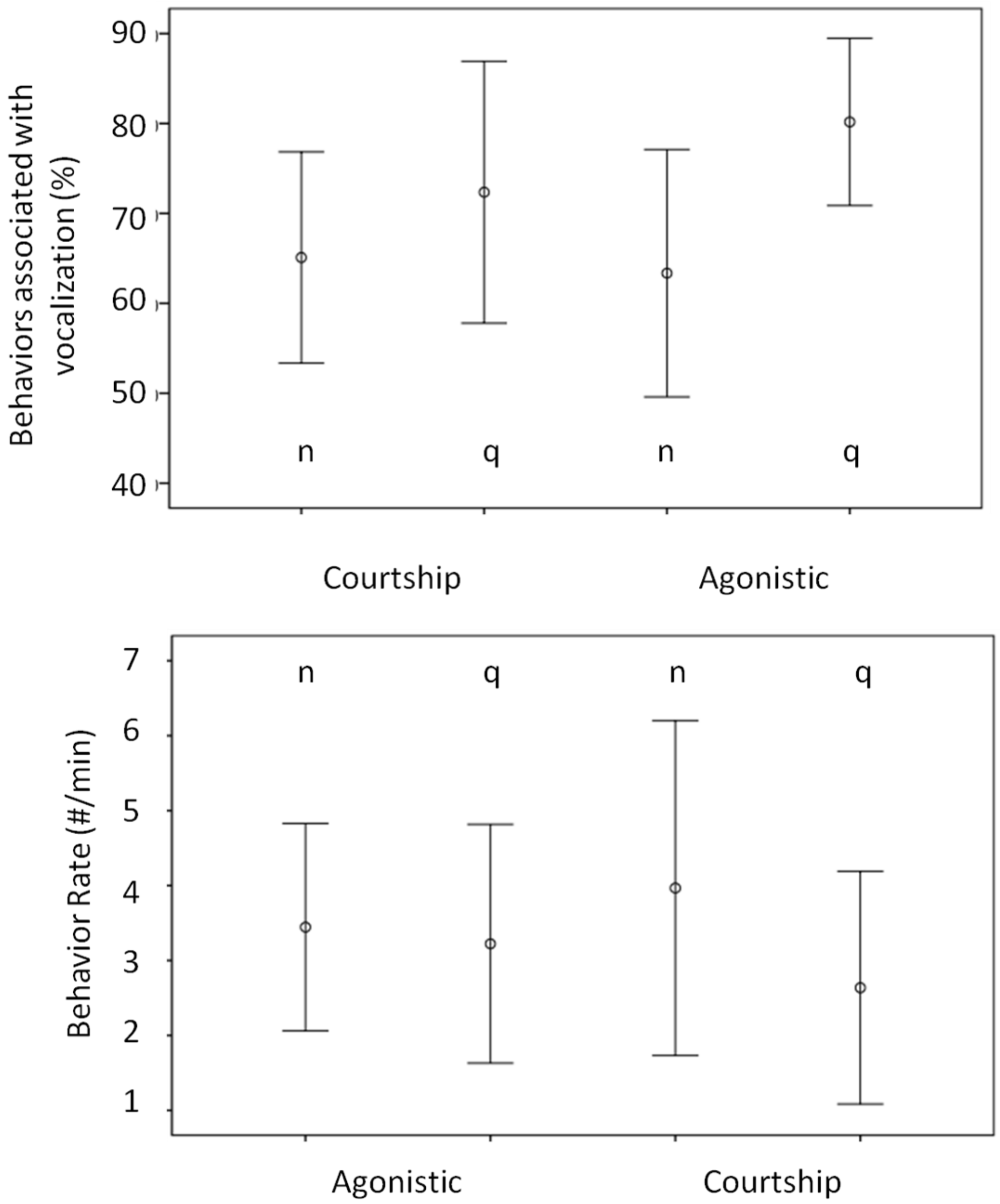


Figure 2-16 – Error bar plot showing the percentage of courtship and agonistic behaviors that were associated with sound production, under noisy and quiet contidions (top), and behavior rates (number of behaviors/minute) for both agonistic and courtship contexts, under noisy and quiet conditions (bottom). The "n" and "q" represent the noisy and quiet trials, respectively. The circles represent means, and the whiskers represent the 95% confidence interval around the mean. Doubly multivariate repeated measures MANOVA indicated that there was no significant main effect of context (agonistic and courtship; $F_{2,14} = 2.323$, $P = 0.134$) or treatment (noisy and quiet; $F_{2,14} = 0.190$, $P = 0.829$) on behavior rate and percentage of behaviors associated with sounds.

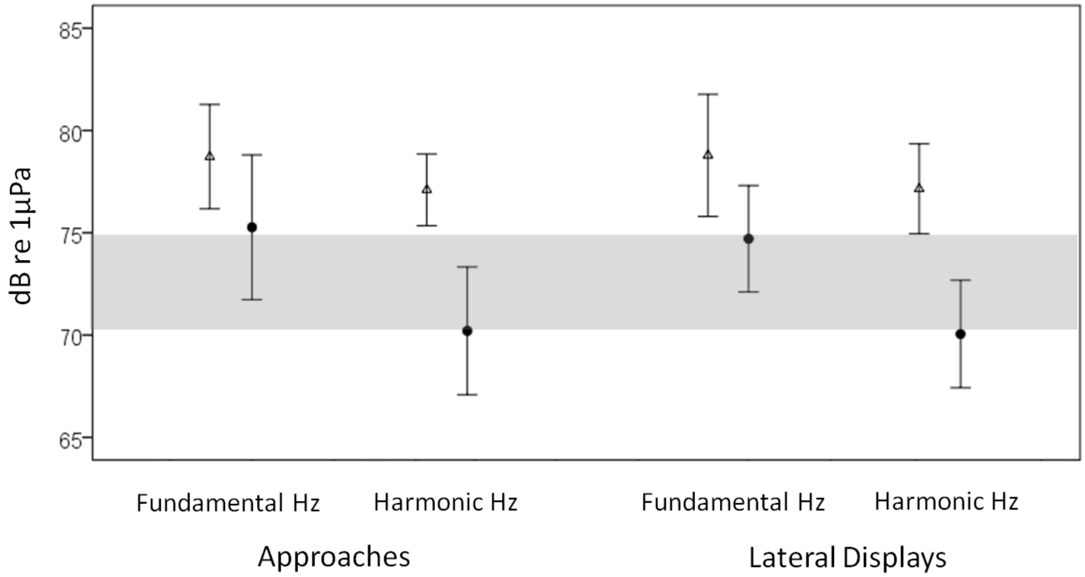


Figure 2-17 – Errorplot showing level of growl fundamental and harmonic frequencies, for approach and lateral display behaviors, under both noisy and quiet conditions. Empty triangles represent the average level under noisy conditions, and filled circles represent the average level under quiet conditions. Whiskers above and below each mean represent the 95% confidence interval around that particular mean. The dark grey bar running across the graph between 70 and 75 dB represents the level of the background noise during noisy trials. Notice that no confidence intervals for the noisy trials fall below the background noise level during noisy trials.

Noisy Conditions Approach behaviors	Agonistic	Courtship
Pulse duration	0.00698 ± 0.001425 (*n*=7)	0.007704 ± 0.000971 (*n*=13)
Pulse interval	0.012843 ± 0.002255 (*n*=7)	0.013015 ± 0.000908 (*n*=13)
Pulse rate	89.828 ± 17.452 (*n*=7)	81.923 ± 7.485 (*n*=13)
Burst duration	0.041803 ± 0.015677 (*n*=7)	0.058638 ± 0.017182 (*n*=13)
Burst interval	0.174018 ± 0.080841 (*n*=7)	0.121138 ± 0.055354 (*n*=13)
Burst rate	7.84 ± 3.469 (*n*=7)	7.401 ± 2.384 (*n*=13)
Burst fund. freq.	88.728 ± 16.34 (*n*=7)	81.493 ± 4.975 (*n*=13)
Burst fund. freq. dB	76.792 ± 3.746 (*n*=7)	79.773 ± 3.293 (*n*=13)
Burst harmonic freq.	326.295 ± 42.44 (*n*=7)	294.995 ± 26.039 (*n*=13)
Burst harmonic freq. dB	76.123 ± 1.552 (*n*=7)	77.861 ± 2.891 (*n*=13)

Table 2-1 – Means and standard deviations of growl parameters produced during approach behaviors under noisy conditions, for agonistic and courtship contexts. Multivariate analysis of co-variance with fish standard length as the covariate indicated no main effect of context on signal structure (MANCOVA: $F_{10,8}$=2.059, P=0.159).

Noisy Conditions **Lateral Display behaviors**	Agonistic	Courtship
Pulse duration	0.006557 ± 0.001159 (*n*=7)	0.006894 ± 0.000908 (*n*=7)
Pulse interval	0.0121 ± 0.002017 (*n*=7)	0.013412 ± 0.000853 (*n*=7)
Pulse rate	123.087 ± 63.547 (*n*=7)	81.094 ± 5.905 (*n*=7)
Burst duration	0.038887 ± 0.010311 (*n*=7)	0.065696 ± 0.019027 (*n*=7)
Burst interval	0.141973 ± 0.096844 (*n*=7)	0.095912 ± 0.039052 (*n*=7)
Burst rate	7.86 ± 2.813 (*n*=7)	6.338 ± 1.151 (*n*=7)
Burst fund. freq.	91.887 ± 16.822 (*n*=7)	78.265 ± 4.151 (*n*=7)
Burst fund. freq. dB	76.211 ± 4.263 (*n*=7)	80.473 ± 3.453 (*n*=7)
Burst harmonic freq.	314.756 ± 51.923 (*n*=7)	285.753 ± 19.137 (*n*=7)
Burst harmonic freq. dB	75.943 ± 2.857 (*n*=7)	79.289 ± 3.141 (*n*=7)
Knock duration	0.013752 ± 0.003668 (*n*=12)	0.013192 ± 0.004249 (*n*=9)
Knock fund. freq.	195.192 ± 77.194 (*n*=12)	209.932 ± 102.426 (*n*=9)
Knock fund. freq. dB	97.504 ± 3.212 (*n*=12)	98.908 ± 5.822 (*n*=9)
Knock harmonic freq.	596.811 ± 86.703 (*n*=12)	564.844 ± 89.416 (*n*=9)
Knock harmonic freq. dB	102.463 ± 2.722 (*n*=12)	105.048 ± 6.784 (*n*=9)

Table 2-2 – Means and standard deviations of growl and knock signal parameters produced during lateral display behaviors under noisy conditions, for agonistic and courtship contexts. Multivariate analysis of co-variance with fish standard length as the covariate indicated no main effect of context on signal structure for either growls (MANCOVA: $F_{10,2}$=0.590, P=0.767), or knocks (MANCOVA: $F_{5,14}$=0.796, P=0.570).

Approach Behavior	Knock	Burst	Pulse	
Duration (s)	0.012270 ± 0.002639 (*n*=12)	0.057678 ± 0.022026 (*n*=12)	0.007708 ± 0.001124 (*n*=12)	ANOVA: $F_{2,10}$=40.954 P < 0.001
Fund. Freq. (Hz)	188.999 ± 90.191 (*n*=12)	83.288 ± 9.162 (*n*=12)	- - -	t_{11}=3.962 P = 0.002
Fund. Freq. (dB)	96.978 ± 4.702 (*n*=12)	79.623 ± 4.349 (*n*=12)	- - -	t_{11}= 11.766 P < 0.001
First Harmonic (Hz)	595.456 ± 99.368 (*n*=12)	301.134 ± 30.566 (*n*=12)	- - -	t_{11}= 11.947 P < 0.001
First Harmonic (dB)	102.820 ± 5.076 (*n*=12)	77.516 ± 3.080 (*n*=12)	- - -	t_{11}= 13.936 P < 0.001

Table 2-3 – Means and standard deviations of frequency and dB properties of the three call types for approach behaviors produced under noisy conditions. The far right column provides statistics (repeated measures ANOVA for duration, and paired-samples t tests for fundamental frequency, first harmonic, and levels of each).

Approach Behaviors	Noisy	Quiet	
Pulse duration	0.007419 ± 0.001206 (*n*=17)	0.007557 ± 0.000938 (*n*=17)	F = 0.261, P = 0.616
Pulse interval	0.012911 ± 0.001422 (*n*=17)	0.013253 ± 0.002029 (*n*=17)	F = 0.834, P = 0.375
Pulse rate	84.832 ± 12.509 (*n*=17)	81.464 ± 12.779 (*n*=17)	F = 2.591, P = 0.127
Burst duration	0.054508 ± 0.020177 (*n*=17)	0.084587 ± 0.037076 (*n*=17)	**F = 11.807, P = 0.003**
Burst interval	0.133218 ± 0.064244 (*n*=17)	0.146928 ± 0.066934 (*n*=17)	F = 0.360, P = 0.557
Burst rate	7.348 ± 2.448 (*n*=17)	5.606 ± 2.034 (*n*=17)	**F = 6.277, P = 0.023**
Burst fund. freq.	84.676 ± 11.044 (*n*=17)	81.782 ± 9.622 (*n*=17)	F = 2.158, P = 0.161
Burst fund. freq. dB	78.606 ± 3.925 (*n*=17)	75.843 ± 5.526 (*n*=17)	**F = 5.181, P = 0.037**
Burst harmonic freq.	307.026 ± 34.105 (*n*=17)	305.522 ± 45.185 (*n*=17)	F = 0.056, P = 0.816
Burst harmonic freq. dB	77.186 ± 2.898 (*n*=17)	71.035 ± 4.987 (*n*=17)	**F = 29.617, P < 0.001**

Table 2-4 – Means and standard deviations of growl signal parameters produced during approach behaviors under noisy and quiet conditions. Multivariate analysis of variance indicated a significant main effect of background noise on signal structure for growls (MANOVA: $F_{10,7}$=9.211, P=0.004). The column on the far right provides P values from Bonferroni corrected post hoc tests for the corresponding signal parameters in the left column. Significant results are in bold.

Approach behaviors	Noisy	Quiet
Knock duration	0.012445 ± 0.002726 (*n*=9)	0.013815 ± 0.002918 (*n*=9)
Knock fund. freq.	151.515 ± 58.925 (*n*=9)	169.856 ± 59.844 (*n*=9)
Knock fund. freq. dB	97.305 ± 4.190 (*n*=9)	97.812 ± 5.933 (*n*=9)
Knock harmonic freq.	628.851 ± 79.729 (*n*=9)	589.781 ± 91.924 (*n*=9)
Knock harmonic freq. dB	103.119 ± 3.462 (*n*=9)	100.569 ± 6.560 (*n*=9)
Lateral Display Behaviors		
Knock duration	0.014161 ± 0.004978 (*n*=11)	0.014266 ± 0.003674 (*n*=11)
Knock fund. freq.	170.913 ± 73.232 (*n*=11)	174.966 ± 55.027 (*n*=11)
Knock fund. freq. dB	98.933 ± 3.641 (*n*=11)	96.524 ± 4.590 (*n*=11)
Knock harmonic freq.	594.726 ± 75.211 (*n*=11)	578.366 ± 70.556 (*n*=11)
Knock harmonic freq. dB	103.815 ± 2.681 (*n*=11)	98.814 ± 5.566 (*n*=11)

Table 2-5 – Means and standard deviations of knock signal parameters produced during approach and lateral display behaviors under noisy and quiet conditions. Multivariate analysis of variance indicated no significant main effect of background noise on signal structure for knocks produced during approach behaviors (MANOVA: $F_{5,4}=0.774$, P=0.615), or lateral display behaviors (MANOVA: $F_{5,6}=4.244$, P=0.054).

Lateral Display Behaviors	Noisy	Quiet	
Pulse duration	0.006832 ± 0.000947	0.006989 ± 0.00094	t_{12} = -0.611, P = 0.552
Pulse interval	0.012679 ± 0.001544	0.014019 ± 0.004057	t_{10} = -1.191, P = 0.261
Pulse rate	100.063 ± 52.561	103.067 ± 68.768	t_{12} = -0.443, P = 0.665
Burst duration	0.058557 ± 0.026548	0.087573 ± 0.040597	**t_{13} = -4.471, P = 0.001**
Burst interval	0.128898 ± 0.076923	0.122995 ± 0.049235	t_{12} = 0.216, P = 0.833
Burst rate	6.971 ± 1.93	6.791 ± 3.264	t_{12} = 0.158, P = 0.877
Burst fund. freq.	85.289 ± 12.747	81.787 ± 9.498	t_{13} = 1.290, P = 0.220
Burst fund. freq. dB	78.786 ± 5.17	74.706 ± 4.506	**t_{13} = 3.919, P = 0.002**
Burst harmonic freq.	306.961 ± 38.232	308.126 ± 42.235	t_{13} = -0.168, P = 0.869
Burst harmonic freq. dB	77.146 ± 3.807	70.051 ± 4.547	**t_{13} = 6.984, P < 0.001**

Table 2-6 – Means and standard deviations of growl signal parameters produced during lateral display behaviors under noisy and quiet conditions. Because of a lack of sample size, repeated measures MANOVA was unable to be performed, and paired t tests with Bonferroni correction were used instead. The column on the far right provides T and P values for the corresponding signal parameters in the left column. Significant results are in bold

Chapter 3

Hearing Sensitivity, Environmental Soundscape, And Propagation Of Acoustic Signals In *Cyprinella venusta*.

In order for an acoustic signal to be an effective source of communication, it must be not only be emitted properly by a sender, but must also be successfully detected by the intended receiver. One potential barrier to acoustic communication is background noise. The habitat of *Cyprinella venusta* varies greatly across it's large range, and in some areas containing riffles, shoals and waterfalls, can become quite noisy. The presence of *C. venusta* in larger, navigable bodies of water and streams with road and train crossings also presents potential anthropogenic noise sources with which *C. venusta* did not evolve. In the present study, I investigated the relationship between the vocalizations, hearing sensitivity, and natural soundscape of *C. venusta*. I also investigated, for the first time, the potential effects of anthropogenic noise from bridge crossings on the ability to communicate acoustically in a small, freshwater fish. The results of this study revealed a particularly close association of a naturally occurring quiet window in the natural soundscape of *C. venusta* and a harmonic frequency within the courtship vocalization of *C. venusta*. I found that *C. venusta*'s acoustic signals propagate very short distances following predictions based on the calculated cutoff frequency of the streams they inhabit, and may be easily disrupted by the noise generated from interstate and train bridge crossings.

Introduction

Sound can be found anywhere a medium in which it may propagate exists. Anything that moves produces sound, and anything with a device able to convert pressure fluctuations or particle displacements into action potentials may be able to detect the sounds. Because sound has the capacity to carry information, can be used intermittently, and does not require a line of sight, it is used extensively by animals as a mode of information transfer. However, numerous factors concerning the properties of the projected sound, hearing abilities of the receiver, constraints imposed by the physical environment, and ambient noise levels of the environment must fit together properly in order for acoustic communication to be effective.

The correlation of sound frequency and intensity with hearing sensitivity is a critical first step in the successful transfer of information through acoustics. If an organism's hearing sensitivity at a specific frequency is not sufficiently below the amplitude of the acoustic signal at that frequency, the sound will not be detected. In many cases, the peak hearing sensitivity of an organism is correlated with the frequency of the sounds they produce for intraspecific communication (Bullock 1977; Capranica 1978; Stabentheiner, 1988; Vasconcelos et al. 2007; Fay and Edds-Walton, 1997; Ladich and Yan, 1998). This correlation will typically maximize the distance at which the sound can be detected by the receiver and minimize the required amplitude for successful detection. However, this correlation is not always the case. Mismatches in peak hearing sensitivity and sound production have been documented in birds (Sachs et al. 1978; Dooling et al. 1978), humans (Geldard 1972), and fishes (Fine 1981; Ladich 1999).

The reason for mismatches is not always known, but is likely the result of conflicting selective pressures. Fine (1981) suggested that in the oyster toadfish (*Opsanus tau*), the mismatch may promote the response of females to relatively louder calls of nearby males, which is often an indicator of male size.

The physical environment can influence the usefulness of acoustic signals. In free field conditions (the deep ocean or open air), sound attenuates according to spherical spreading (a decrease in 6 dB for each doubling of distance). Dense vegetation can have a scattering effect on sound, resulting in reverberations that degrade information encoded in amplitude and frequency modulations (Richards and Wiley 1980). The location of a sound producing individual with reference to the ground or other reflective boundary, as well as the type of reflective boundary can also affect emitted acoustic signals (Forrest, 1994). The medium in which a sound is propagating can also affect transmission. Absorption, the loss of energy from a propagating sound in the form of heat and the relaxation of molecules in the medium (Bass, 1991), is frequency dependent, and is about 100 times greater in air than in water (Michelsen, 1978). However, the close presence of two boundaries in shallow water, the water surface and the substrate, present a unique challenge for organisms attempting to communicate underwater using acoustics. Sounds below a specific cutoff frequency, defined mainly by the water depth and substrate composition (Officier, 1958; Rogers and Cox, 1988), experience severe attenuation in power by a factor of up to 100,000 relative to frequencies above the cutoff frequency (Forrest, 1994).

Ambient noise from both biotic and abiotic sources can also have a large effect on acoustic communication by decreasing the signal-to-noise ratio of signals, making temporal information more difficult to extract (Wysocki and Ladich, 2005), or by masking signals altogether. Abiotic noise can come from sources such as wind, rainfall, or turbulence while biotic noise comes from the acoustic signals of other organisms. Studies on terrestrial species have shown that these environmental noise sources can act as strong selective pressures in the evolution of signal structure (Waser and Waser, 1977; Wiley and Richards, 1982; Narins et al. 2004). Jouventin et al. (1999), for example, found that king penguin, *Aptenodytes patagonicus*, chicks find their parents in a crowded, noisy colony through a highly specialized call that takes advantage of a brief (0.23 s) inflection point containing all the information needed for recognition, along with high degrees of redundancy in time and frequency domains. Narins et al. (2004) also found that an arboreal frog (*Amolops tormotus*) and a leaf warbler (*Abroscopus albogularis*) possess ultrasonic harmonics in their calls, and suggest that their function may be to avoid masking in the wideband river noise of their habitat.

Unlike natural biotic and abiotic noise sources, the relatively recent development and rapid expansion of human activities such as urbanization, shipping, motorized recreational activities, drilling, and seismic explorations (Myrberg, 1990; Popper, 2003) may not be providing the time necessary for the evolution of acoustic signals in some animals. While efforts have been made to determine the effect of anthropogenic noise on marine mammals (Richardson et al. 1995), investigations on the effect on fishes has been sparse. Elevated noise levels have also been shown to reduce egg survival, reduce reproduction and reduce growth rates in fishes (Banner and Hyatt, 1973; Lagardère, 1982). Several studies have also shown that anthropogenic noises can affect hearing abilities or fish behaviors (Fernandes et al. 2000; Vabø et al. 2002; Handegard et al. 2003), which can have detrimental effects on fitness. Amoser et al. (2004) found, for example, that noise from powerboats racing on an alpine lake was loud enough to be detected by otophysine fishes (fishes possessing a hearing specialization; see Popper and Fay, 2010) at up to 400 m away. Vasconcelos et al. (2007) found that the noise from ferry boats in the Tagus River estuary (Portugal) caused significant hearing threshold shifts in the Lusitanian toadfish (*Halobatrachus didactylus*), and that females ability to detect male signals would be significantly diminished under ship noise.

Despite the efforts that have been made elsewhere, I am currently unaware of any study that has looked at potential anthropogenic noise sources in small streams, and how these noise sources may impact the ability of small, vocal cyprinids to communicate acoustically. The current study was aimed at describing the interaction between the natural soundscape and acoustic repertoire and hearing of the blacktail shiner (*Cyprinella venusta*) in east Alabama, and examining the effect of two anthropogenic noise sources that occur in low order streams that are not typically exposed to motorized boat traffic. The results provide a better understanding of how small, freshwater fishes cope with the seemingly noisy natural soundscape of their environment, and how two common sources of anthropogenic noise may affect communication.

Methods

ABR

Hearing thresholds were determined using the auditory brainstem response (ABR; aka auditory evoked potential , AEP). Methods and equipment were identical to those used in Holt and Johnston (2011) with a few exceptions that will be mentioned here. Test subjects (seven *C. venusta* and five *C. auratus*) were wrapped in bandage gauze and pinned (through the gauze) to a flat bed of clay in lieu of restraining the fish with clay alone. This was done in response to concerns that the heavy clay would affect the sound field at the fish's body. Also, frequencies used in this experiment included 100, 200, 300, 400, 600, 800, 1000, 2000, and 3000 Hz.

Natural and Anthropogenic Ambient Noise Measurements and Propagation

A large shoal on Little Uchee Creek (Lee Co., AL, U.S.A., 32.508579° N, -85.184215° W), a tributary of the Chattahoochee River, was chosen to describe the natural soundscape of *C. venusta*. This location was chosen because it offered a wide variety of suitable spawning habitats for *C. venusta* such as pools, runs, and chutes. During the reproductive summer months, the water is also periodically shallow and clear enough to allow a researcher to locate the exact locations of nests by watching the fish behave from the bank. A total of 9 active nest sites were identified by observation from the creek bank using polarized sunglasses. Nest sites were typically found at the confluence of a chute or waterfall and a pool. However, spawning aggregations were also observed directly within rapidly flowing chutes, and pools with negligible flow.

A hydrophone (Hi-Tech HTI-96-MIN, sensitivity -164.4 re 1V/µPa, frequency response: 0.002-30 kHz) and digital recorder (Marantz PMD 661, sampling rate 44.1 kHz) were used to record 1 minute of ambient noise in each of the 10 sites. In sites with substantial flow, an effort was made to place the hydrophone in a still area adjacent to the flow, or in the eddy of a rock to reduce hydrodynamic noises. Sounds were imported into Raven 1.4 (Cornell University, Ithaca, NY), where three, 1 sec segments were randomly selected from the recording made at each site. Two pressure density spectra of each 1 sec segment were then calculated using the power spectrum function of Raven (Hanning window, FFT length: 2048 samples, bin resolution: 21.5 Hz and Hanning window, FFT length: 512 samples, bin resolution: 86.1 Hz). Decibel levels were corrected to represent actual levels by taking into consideration the gain applied to the signal by the Marantz recorder and the sensitivity of the hydrophone. Decibel levels were also corrected to represent dB re $1\mu Pa^2/Hz$ by subtracting 10log(sampling rate/FFT sampling size) dB. The pressure density spectra levels were exported into an excel file, where the three, 1 sec segments from each nest site were averaged to produce a single power density spectra for each of the 10 nest sites, which were then averaged to generate a single power spectrum for the natural ambient noise. Kendall's concordance test was used to determine whether the spectrum shape (using the power density spectrum curve with bin resolution of 21.5 Hz) of natural ambient noise between 21.5 and 1999.5 Hz was significantly different across active nesting sites (Lugli and Fine 2003).

Source levels and propagation of train and road noise were measured at the crossing of Interstate-85, and a CSX Railroad crossing at Uphapee Creek (Macon County, AL, interstate: 32.47441°N, -85.68656°W, train: 32.48429°N, -85.70605°W). Both crossings were beam bridges with at least one piling exposed to the water in the creek. For all recordings at both the train and interstate crossing, the hydrophone was placed approximately 10 cm off the substrate. This depth was chosen because the substrate was mostly sand and gravel, and most potential nests (logs, rocks, or other items that could present suitable crevices) in this type of habitat are usually close to the substrate. Also, when spawning aggregations were observed, behaviors that were associated with sound were usually performed near the nest.

At the interstate crossing, a hydrophone was fixed 1.3 m upstream of the piling that entered the water. A second hydrophone was moved 2, 4, 6, 9, 12.2, and 14 m upstream of the piling and both hydrophones recorded simultaneously on two channels of the Marantz digital recorder. Because of hydrophone cable length constraints, measurements at 22, 48.8, and 82.3 m upstream of the piling were performed with a single hydrophone and levels were compared to the average semi-trailer truck levels at 1.3 m. Recording was performed for several minutes at each distance, and the time at which semi-trailer trucks passed was noted. A total of three semi-trailer truck passes were selected from the recordings at each distance. To determine the noise level of the semi-trailer trucks at the source, two power density spectra were generated (bin resolution of 21.5 and 86.1 Hz) for each measurement made at 1.3 m away from the bridge (n=17). Data were exported to excel and the same corrections to level that were used in the natural ambient noise at nesting sites was applied. The 17 spectra were then averaged to generate two final power density spectra (one at a bin resolution of 21.5 Hz and one at 86.1 Hz) representing semi-trailer truck noise 1.3 m downstream of the bridge (Figure 3-7).

To calculate attenuation of noise at the interstate crossing, three semi-trailer truck passes at each distance were selected from the audio files. Pressure density spectra were calculated for each of the three passes using Raven (Hanning window, FFT length: 32768 samples, bin resolution: 1.35 Hz). The pressure density spectra were exported to Excel where they were adjusted so that they represented dB re 1µPa^2/Hz. The average power in 1/3 octave bands was then calculated for bands with center frequencies between 63.1 and 5011.9 Hz for each of the semi-trailer truck passes. Attenuation of the noise in each of the 1/3 octave bands was calculated by subtracting the level between the two hydrophones for each 1/3 octave and averaging this attenuation across the three semi-trailer truck passes at each distance.

At the train crossing, a source level was taken at 3 m downstream of the piling that entered the water. Subsequent recordings were taken at 8, 12.5, and 18 m downstream of the piling. All recordings lasted between a few seconds before the locomotive started upon the bridge, to a few seconds after the last car had left the bridge. Similar methods were used to determine levels at the source, and attenuation of the train noise as interstate noise, except that instead of selecting three semi-trailer truck passes at each distance, five, 1 sec segments were selected from the train noise at each distance. One addendum was that the portion of the sound that included the locomotive crossing was excluded due to its transient nature relative to the duration of the rest of the train crossing, and that it's level was significantly different than the rest of the train.

Bin resolutions of 21.5 and 86.1 were included in the analysis to allow for signal-to-noise ratios (SNR's) comparison between *C. venusta* acoustic signals and the different noise sources. When comparing SNR's, it was necessary to analyze all sounds to be included in the analysis at the same frequency resolution. The bin resolutions of 21.5 and 86.1 Hz result from the typical duration of growls and knocks. Decibel levels of knocks and bursts were measured in the lab, and further details describing the acoustic signals of *C. venusta* will be presented in a forthcoming paper.

Propagation of *C. venusta* acoustic signals

Propagation of *C. venusta* signals was measured both directy from the fish, and by playing the sounds back through an underwater speaker. To measure propagation directly, a nest guarding male was found, and a hydrophone was placed as close to the nest as possible without chasing the sound producing male away. A second hydrophone was placed 20 – 30 cm away from the first hydrophone, and both recorded simultaneously to the Marantz digital recorder. Video of the sound producing male at the nest site was recorded using an underwater camera (Aqua-Vu ZT 60, Nature Vision Inc., Brainerd, MN, U.S.A) linked to a mini digital video recorder (Super Circuits, MDVR25). Raven 1.4 was used to analyze the sounds. Sounds included in the analysis of propagation were limited to those that were made when the male was within 1-2 cm directly above the hydrophone closest to the nest. The sound level at this hydrophone was considered the source level of the sounds. Using Raven, the individual burst that occurred when the male was positioned directly above the source hydrophone was isolated on both channels. The pressure density spectra level of the burst was calculated at both hydrophones using the power spectrum function of Raven (Hanning window, *n*-point FFT where *n* is the number of points in the signal, bin resolution: 44100/*n*), and was corrected to represent actual dB re 1µPa^2/Hz as described previously. The average power was then calculated in 13 one-third octave bands (center frequencies from 63 to 1000 Hz) at both hydrophones. Attenuation in each one-third octave band, at each distance was calculated by subtracting the level at the distant hydrophone from the source hydrophone.

Propagation was also measured by playing the sounds back through an underwater speaker (University Sound UW-30, Oklahoma City) and recording at different distances. A sound clip consisting of a growl preceded and followed by a single knock was generated in Raven (the knocks were from agonistic behaviors and the growl was from a courtship behavior). The sound was 2.2 sec in duration, and the dominant frequency of the knocks was 17 dB louder than the dominant frequency of the growl (the typical difference in level between growls and knocks). The sound was played from a Blackberry curve 8520, and amplified by a Pioneer GM-X372 240W amplifier. One hydrophone was placed 5.08 cm directly in front of the speaker, and a second hydrophone was moved away from the first in increments of 5.08 cm up to 96.52 cm. At some nest sites, measurements were not able to be taken along the entire 96.52 cm, and analysis was limited to distances that were included at all sites. The generated *C. venusta* sound was recorded by both hydrophones at each distance and attenuation was calculated using the same methods described above for propagation of fish-produced sounds.

Results

The audiogram of *C. venusta* was very similar to that of *C. auritus* (Figure 3-1). Evoked potential traces at 100 and 200 Hz appeared different than traces at higher frequencies (Figure 3-2). Lower frequencies (100 and 200 Hz) were associated with longer latencies, and showed multiple spikes within a longer dip and rise. Frequencies above 200 Hz generally showed a cleaner evoked potential that lacked the series of smaller spikes following the initial dip and rise.

The pressure and particle acceleration audiograms for *C. venusta* and *C. auratus* both showed a U shaped sensitivity curve typical of hearing specialists (Figure 3-1a,b). *Cyprinella venusta* sensitivity decreased relatively linearly from 100 Hz (95.7 dB; $2.5e^{-3}$ m s^2) to 300 Hz (87.1 dB; $9.2e^{-4}$ m s^2), increased slightly to 89.3 dB ($1.2e^{-3}$ m s^2) at 400 Hz, then dropped to the peak sensitivity of 83.6 dB ($6.5e^{-4}$ m s^2) at 600 Hz. Above 600 Hz, thresholds increased relatively linearly to 116.4 dB ($3.0e^{-2}$ m s^2) at 2000 Hz, which was the highest attainable threshold for *C. venusta*. *Carassius auratus* differed from *C. venusta* in slightly lower thresholds at 100 and 400 Hz.

At frequencies above 100 Hz, background noise was typically between 45 -50 dB, more than 30 dB lower than the lowest threshold. Spikes in the background noise were found at 60, 120, 180, 240, 300, 420, 540, 660, and 780 Hz.

Noise levels of semi-trailer trucks crossing Uphapee Creek at Interstate-85 were relatively high at the source (1.3m upstream of the bridge piling), with the greatest power occurring at low frequencies below 180 Hz. The average power between 0-56, the 63 Hz band, and the 80 Hz band at 1.3 m from the bridge was 103.8, 108.8, and 105.7 dB re 1µPa, respectively. Power declined linearly from 106.2 dB at the 100 Hz band, to 69.6 dB at the 500 Hz band at a rate of approximately 0.1 dB/Hz. Power increased to 71.2 dB at the 800 Hz band, and then gradually decreased until levels were comparable to natural background levels at Uphapee Creek (approximately 47 dB) at 2.4 kHz and above. Attenuation of semi-trailer truck noise showed some variation with frequency, but generally followed attenuation rates between spherical and cylindrical theoretical rates (spherical spreading based on 20log(distance from source); cylindrical spreading based on 10log(distance from source);Figure 3-3).

At the train crossing, noise levels were considerably higher than the semi-trailer truck noise. Train noise showed more variation than semi-trailer truck noise at frequencies below 540 Hz. The average power between 0-56, the 63 Hz band, and the 80 Hz band at 3 m from the bridge was 115.3, 111.9, and 109.4 dB respectively. There was a distinct gap in the train noise spectrum at the 400 and 500 Hz one-third octave bands where the levels were 97.7 and 101.9 dB respectively. Surrounding this window were spikes in the spectrum of 109.0 dB at the 250 Hz band, and 104.1 dB at the 630 Hz one-third bands. Between the 630 and 1250 Hz bands, power levels dropped relatively linearly at a rate of approximately 0.054 dB/Hz. Above the 1250 Hz band, levels continued to drop, but very slowly, and stayed about 7.4 ± 3.5 dB above the ambient noise levels up to and beyond 4.0 kHz. Attenuation of train noise generally followed attenuation rates between spherical and cylindrical theoretical rates, similar to semi-trailer truck noise (Figure 3-4)

The peak level of natural ambient noise averaged across 9 active nesting sites was 73.6 ± 5.6 dB at 30 Hz. Above 30 Hz, power levels dropped rapidly at a rate of approximately 0.13 dB/Hz into a quiet window between 180 – 330 Hz, where the average power was 54.3 ± 1.3 dB (average power in 1.35 Hz bands). Above this quiet window, power levels increased at approximately 0.026 dB/Hz to a second peak at 690 Hz, where the average power was 66.9 ± 15.5 dB. Power levels gradually declined above 690 Hz to levels comparable to the quiet window at 2.2 kHz and above. Significant concordance in spectrum shape of naturally occurring ambient noise between 21.5 and 1999.5 Hz was found among the active nesting sites at Moffits Mill (W=0.428, chi-square=232.64, P<0.001, df=8).

Attenuation rates of natural *C. venusta* calls were very similar to those played through the speaker (Figure 3-5). Assuming losses from cylindrical spreading as 3 dB/distance doubled, the distance from 0 to 40.6 cm (with the first measurement at 5.08 cm) should result in a transmission loss of 12 dB, which equates to 16 dB/m. Although some variation with frequency was apparent (Figure 3-6), attenuation at all frequencies was much more rapid than what cylindrical spreading would predict, and follows what would be expected for signals below the cutoff frequency. A linear best fit line plotted through the average attenuation of all frequencies for knocks and growls showed an attenuation of 55.9 dB/m. At the 1000 Hz one-third octave band, the frequency at which attenuation was the slowest, the slope of the best fit linear line was 38.9 dB/m. At the 315 Hz one-third octave band, where attenuation was the most rapid, the slope was 62.8 dB/m.

The sounds produced by *C. venusta* are broadband, with the greatest power existing at lower frequencies. With growls, the dominant frequency typically occurs around 70 Hz (which is the pulse repetition rate), but can also be found around 300 Hz (which is typically the dominant frequency of an individual pulse). Knocks are also broadband in nature with the majority of their power in the low frequency range. The dominant frequency of knocks is also variable and often occurs between 70 and 300 Hz or 500 and 700 Hz. In the laboratory, under quiet conditions, bursts are produced with levels of 70.42 ± 7.05 dB at 86 Hz and 67.41 ± 8.08 dB at 301 Hz (bin resolutions of 21.5 Hz; Figure 3-7). Knocks are produced with levels of 96.89 ± 5.08 dB at 172.2 Hz and 97.16 ± 8.24 dB at 602.7 Hz (corrected for hydrophone distance, bin resolutions of 86.1 Hz; Figure 3-8).

Applying attenuation rates of *C. venusta* signals observed in the field to the average level of sounds produced by *C. venusta* in the lab, and the average power spectra of naturally occurring noise their environment, I was able to approximate the potential active area of the signals under different noise conditions. Fishes are generally unable to detect pure tones with signal-to-noise ratio below 15 - 20 dB (10 - 15 dB if the signal and noise are coming from the different directions; Buerkle, 1969; Chapman, 1973; Chapman and Sand, 1974; Fay, 1974; Hawkins and Sand, 1977; Fay 1988; Mann and Lobel, 1997). At 172.2 and 602.7 Hz, the natural noise is 49.91 ± 4.91 and 51.96 ± 14.12 dB, respectively. Based on the average knock levels and attenuation rates,*C. venusta* knocks should propagate approximately 50.73 cm at 172.2 Hz and 65.19 cm at 602.7 Hz before reaching a signal-to-noise ratio of 15.0 dB (SNR calculated as Signal level – Noise level, which is equilivant to 20log(signal voltage/noise voltage)). At 86 and 301 Hz, the natural noise is 53.75 ± 3.82 and 43.19 ± 10.02 dB, respectively. Based on the average burst levels and attenuation rates, growls should propagate approximately 2.99 cm at 86 Hz and 16.49 cm at 301 Hz before reaching a SNR of 15 dB.

Losses based on spherical spreading show that in the presence of semi-trailer truck noise at the interstate crossing (96.64 dB at 86 Hz and 67.82 dB at 301 Hz; 1.3 m away from the bridge piling), the growls should emerge from the noise with a SNR above 15 dB at distances beyond 117.8 m from the bridge at 86 Hz and 5.9 m from the bridge at 301 Hz . At 172.2 and 602.7 Hz, the semi-trailer truck noise is 87.98 and 50.94 dB, respectively. The 172 Hz portion of knocks should reach a SNR above 15 dB at 2.0 m from the bridge. At 602.7 Hz, knocks already have a SNR of 31.76 dB at the closest distance to the bridge that semi-trailer truck noise was measured (1.3 m).

Three meters away from the bridge piling at the train crossing, the noise level was 105.64 and 101.67 dB at 86 and 301 Hz, respectively. Based on spherical spreading rates of the train noise, the 86 Hz portion of growls should reach a SNR above 15 dB at distances beyond 324.3 m from the bridge. The 301 Hz portion of growls should reach a

SNR above 15 dB at distances beyond 290.4 m. Noise levels were 102.14 and 90.76 dB at 172.2 and 602.7 Hz at the train crossing, respectively.   The 172.2 Hz portion of knocks should reach a SNR above 15 dB at distances beyond 10.3 m. The 602.7 Hz portion of knocks should reach a SNR above 15 dB at distances beyond 2.7 m.

The values presented above only indicate at what distance the signals should be detectable over the semi-trailer truck and train noise at the point source of the biological sound (aka, at the sound producing fish). It may be more meaningful to report the distance at which anthropogenic noise levels should attenuate to levels comparable to natural noise, which would be the point at which the signals regain the active area observed under natural conditions. Semi-trailer truck noise should attenuate to natural noise levels in 142.7 m at 86 Hz, 52.8 m at 172 Hz, 17.0 m at 301 Hz, and 1.8 m at 602.7 Hz. Train noise should attenuate to natural levels in 393.1 m at 86 Hz, 269.5 m at 172 Hz, 839.5 m at 301 Hz, and 178.6 m at 602 Hz (Table 3-1).

Discussion

It was surprising that attenuation of the four highest one-third octave bands of semi-trailer truck noise followed attenuation rates intermediate to spherical and cylindrical spreading. Because all of the bands were below the calculated cut-off frequency of the stream, my null hypothesis was that the frequencies would have shown attenuation values much greater than spherical or cylindrical spreading, and more similar to the attenuation rates of *C. venusta* signals. One possibility for the persistence of the sounds is that they are traveling through the air and transmitting into the stream at farther distances. The critical angle of transmission from the air into the stream is 76.6° (based on Snell's law; $\cos\theta_c = c_1/c_2$). Only sounds with incident angles less than the critical angle are capable of being transmitted into the stream. The bridge is 8.5 m above the surface of the water, meaning that the incident angle of airborne sounds at the air/water boundary relative to the vertical would become greater than 76.6° at 35.7 m away from the bridge. Because an elevation in attenuation beyond this distance is not observed, the sounds must also be propagating through the substrate and re-emerging into the water column. Because the composition of the bottom below the immediate substrate (sand) is unknown, I cannot address propagation in this respect beyond what was observed, which was attenuation between spherical and cylindrical spreading. Because the train noise was not measured beyond a distance of 18 m, I cannot address the transmission from air into the water at longer distances. However, because both the train and interstate bridges had pillars entering the water, were similar heights, and attenuation of noise was similar between the two bridges, I assume that the method of propagation is similar.

The spawning strategy of *Cyprinella venusta* involves the deposition of demersal eggs into crevices formed by bedrock, woody debris, or other structure along the substrate. This strategy is an effective method of protecting the eggs from many potential predators, but does not allow fanning by the parental fish, a technique used by many fish species that removes silt and circulates oxygenated water around the developing eggs. It is not surprising, therefore, that nest sites associated with flow are preferred by *C. venusta* (Baker et al. 1994). All of the active nesting sites observed at

Moffits Mill were associated with substantial flow, and occurred within or just below a chute, or in a run just below a waterfall or riffle, an observation that corroborates findings of Baker et al. (1994). Under natural conditions in shallow stream habitats, noise levels are usually dependent on hydrological factors such as water speed, volume, sediment transport, and cavitation (Wysocki et al. 2007). Quiet areas typically occur where flow is minimal and the water surface is unbroken (Lugli and Fine 2003). The presence of considerable flow and pieces of substrate breaking the surface of the water at *C. venusta* nesting sites most likely contributes to elevated levels of natural ambient noise at spawning sites.

The low frequency spike in natural ambient noise around 20 Hz is caused mainly by turbulence from water flowing around submerged objects such as rocks (Strasberg 1979; Lugli and Fine 2007). The broadband elevation in the natural ambient noise around 690 Hz is probably due to bubble noise caused by the surface being broken by turbulence or objects, forcing pockets of air underwater (Lugli and Fine 2003). Lugli and Fine (2003) suggested the quiet window found in the shallow streams in which they recorded was formed by the attenuation of the low frequency turbulence noise before the emergence of bubble noise at higher frequencies.

Despite the presence of elevated natural noise at nesting sites, *C. venusta* are able to maintain a SNR above 15 dB at both the dominant, and sub-dominant frequencies of both call types, albeit at different distances. Knocks showed levels that were drastically above the ambient noise at all frequencies relevant to *C. venusta* with SNR’s of 43.36 dB and 51.44 dB at 172 Hz and 602.7 Hz, respectively. The presence of a quiet window in the natural noise power spectrum around approximately 300 Hz was an important finding of the current study. Because of large SNR of knocks at all frequencies, this quiet window is irrelevant with reference to knocks. The subdominant frequency of growl type calls, however, occurs directly within this quiet window with a SNR of 24.22 dB. This quiet window is especially relevant due to the low SNR of growls relative to the natural ambient noise at frequencies outside the window (Figure 3-7). The dominant frequency of growls, for example, is 3 dB greater than the subdominant frequency, but does not overlap as well with the quiet window, and is thus only detectable up to 2.99 cm, which is 13.5 cm less than the subdominant frequency.

Several other studies have found quiet windows in the natural background noise of stream environments (Wysocki et al. 2007; Speares et al. 2011; Lugli and Fine 2003; Amoser 2006; Crawford et al. 1997). Lugli et al. (2003) found a match between the peak hearing, quiet window, and dominant frequencies utilized during sound production in two freshwater gobies (*Padogobius martensii* and *Gobius nigricans*). Speares et al. (2011) found that the dominant frequency of two darter species (*Etheostoma flabellare* and *E. crossopterum*) falls within a relatively quiet area of the natural ambient noise spectrum. Crawford et al. 1997 also found that the sounds produced by the mormyrid *Pollimyrus isidori* fit into a very broad quiet window (200-3000Hz) in the Niger River (Mali, Africa). Because, for the most part, hydrology dictates natural environmental noise, and fish are often bound to certain habitat types due to specific life history strategies (such as for *C. venusta*), the utilization of a naturally occurring quiet window in the ambient noise is probably an adaptation for intraspecific communication. The almost perfect match between growl subdominant frequency and the naturally occurring quiet window in *C. venusta* habitat may be considered as potential evidence that the environmental soundscape acted as a selective pressure in the evolution of signal structure for *C. venusta*.

Detection distances of the different call types do not appear to be arbitrary and seem to fit appropriately with the behaviors they accompany. The ability of *C. venusta* to theoretically detect knocks at over half of a meter away indicates that males are capable of relatively long distance acoustic communication under normal ambient noise conditions. This correlates well with the types of behaviors that are typically associated with knocks. Knocks are usually produced by males during aggressive encounters with other males around the nesting site. Knocks were also observed during chases between nest guarding males and sunfish that would attempt to eat eggs from the nest. If knocks serve as an honest signal of male fitness to other males, a larger active area of the sound should benefit the sound producer. Because there are often many other reproductive males at a given nesting site, a territorial male should benefit from sending honest signals about their intentions and motivational level to both the intruding male and peripheral males simultaneously.

Growls are produced more often during courtship behaviors with females. During courtship, males will often approach and circle a potential female quickly, making frequent trips back and forth between the nest and the female while all the time producing growls. Sound production continues up to the moment of spawning, ceases during the 1 – 2 sec of actual spawning, then immediately resumes. During the course of this courting behavior, the distance between male and female varies, but is often close (within 15 cm). During the period immediately prior to and during spawning, the male is very close (within 1-2 cm) to the female. Due to the small active area of growls, it is unlikely that they are used as advertisement to distant females. They are most likely strictly intended for the individual female being courted, possibly for spawning synchronization, or as a signal of male fitness.

Semi-trailer truck and train noise was shown to have the ability to diminish the normal active area of both growls and knocks, depending on the frequency of concern and the distance from the noise source (Table 3-1). Growls showed the potential to be more severely affected than knocks, due to their lower amplitude and greater overlap with anthropogenic noise frequency spectrum. The location of the subdominant frequency fits ideally within the natural ambient noise spectrum, as might be expected in a natural system. However, both sources of anthropogenic noise significantly increase the noise levels within the quiet window.

It is difficult to assess just how much the anthropogenic noise sources may affect communication and overall reproductive success of *C. venusta*. It is not known whether there is a difference in the information content of the dominant and subdominant frequencies, which are affected differently by the anthropogenic noises. Several studies have shown that dominant frequency is correlated with male size, which can be considered an indicator of male fitness. In the case of *C. venusta*, the first harmonic frequency is negatively correlated with fish standard length while the fundamental frequency is not. This, along with the fact that the quiet window corresponds more closely to the first harmonic frequency, suggests that the fundamental frequency may not carry as much importance as the first harmonic frequency. Other signal parameters such as call duration have also been found to be correlated with male condition (Amorim et al. 2010). If call duration carries such information in *C. venusta*, a smaller active area of growls induced by anthropogenic noise events could cause growls to be heard for a shorter period of time by a female, which could diminish perceived male fitness and have adverse effects on reproductive success. Unfortunately, I was unable to measure call duration as the fish were often moving in and out of hydrophone range while vocalizing, frequently causing the beginnings and ends of calls to be undefinable.

It is also possible that the periodic nature of both semi-trailer truck and train noise allow sufficient communication between noise events. However, the ability to effectively communicate even between noise events may require that hearing is not significantly affected by exposure to the noise. Numerous studies have shown that exposure to elevated noise can cause temporary threshold shifts (TTS's) in fishes (Smith et al. 2004; Popper and Clarke, 1976; Scholik and Yan, 2001; Amoser and Ladich, 2003). However, most of these studies have looked at relatively long term exposure to constant noise at very high levels, with the shortest exposure times being 10 minutes at 122 dB re 1 $\mu Pa^2/Hz$ (after which *C. auratus* exhibited a 5 dB TTS; Smith et al. 2004). Although the recovery time is not specified for that particular exposure, recovery from a greater TTS followed an exponential curve with initial rapid recovery rates slowing as the threshold neared original levels. It is not known how hearing thresholds would be affected by continued exposure to relatively low level transient noise sources such as the anthropogenic sources investigated in this paper. Lugli et al. (2003), however, found that the hearing thresholds of *P. martensii* and *G. nigricans* matched (albeit about 30 dB higher) the upper standard deviation spectrum level curve of the stream locations with the highest ambient noise levels. This suggests that periodic elevations in ambient noise could dictate hearing thresholds more than average levels. In the current study, background noise levels at 301 Hz are elevated to 67.82 dB at 1.3 m by semi-trailer truck crossings, and 101.67 dB at 3 m by train crossings. Although these are not exceptionally high levels, and values will decrease as distance is increased from the bridge, numerous studies have found that threshold shifts in response to a white noise are greatest at frequencies where hearing sensitivity is best (Smith et al. 2004; Popper and Clarke, 1976; Amoser and Ladich, 2003), which most likely occurs within the quiet window (around 300 Hz) for *C. venusta*. Further research focused on the effect of these particular noise sources and the avoidance rate of fishes around the bridges is needed for a more clear understanding of how these noise sources affect hearing.

The ABR's in this study provide similar audiograms between *C. venusta* and *C. auratus*. Descriptions of hearing sensitivity in fishes using both behavioral and electrophysiological methods such as the ABR have shown very high variation between researchers, even for identical species. *Carassius auratus* is probably the most extensively tested species of fish, and it has been suggested by Higgs et al. (2003) that authors describing the hearing sensitivity of a new species include an audiogram of *C. auratus* using the same system to facilitate interspecific comparisons. Because of the high degree of variation in audiogram sensitivity seen across numerous studies, the notion that ABR provides a conservative estimate of sensitivity, the fact that the greatest sensitivity on the audiogram produced for *C. venusta* in the current study is 13 dB above the average peak dB of growls, and the fact that the audiograms for *C. venusta* and *C. auratus* were so similar, I feel that a more realistic picture of *C. venusta* hearing abilities may be obtained by looking at historical *C. auratus* audiograms obtained through behavioral methods. Popper (1971) found an audiogram with a shape similar to the current study with a peak sensitivity of 52 dB re 1µPa at 500 Hz and 54 dB at 300 Hz. Jacobs and Tavolga (1967) also found a similarly shaped audiogram with a peak sensitivity of 55 dB at 500 Hz. I believe that because of physiological similarities, similar audiogram shapes, and the fact that hearing sensitivities should be below the level of the acoustic signals, it is not unreasonable to assume that hearing sensitivities of *C. venusta* should more closely resemble the behavioral audiograms found historically for *C. auratus*.

The findings of the current study offer a new perspective at which to look at reproductive behaviors in *C. venusta*. The noisy environment in which they often live has been shown to have a convenient window in the noise spectrum, which has been exploited by *C.venusta* for the purpose of communication with females during reproductive behaviors, albeit at very short distances. Two sources of anthropogenic noise were determined to have potentially detrimental effects on the communicative ability of *C. venusta* during critical periods in it’s life history. This work is the first to explore the effect of bridge crossing anthropogenic noise sources on the acoustic communication of fishes in a small, unnavigable stream. Future work should attempt to determine whether *C. venusta* are avoiding areas impacted by such noise and if not, what affect the noise is having on hearing ability. Investigations should also look into the effect of different types of stream crossings on noise levels, and whether more sedentary species such as darters are affected.

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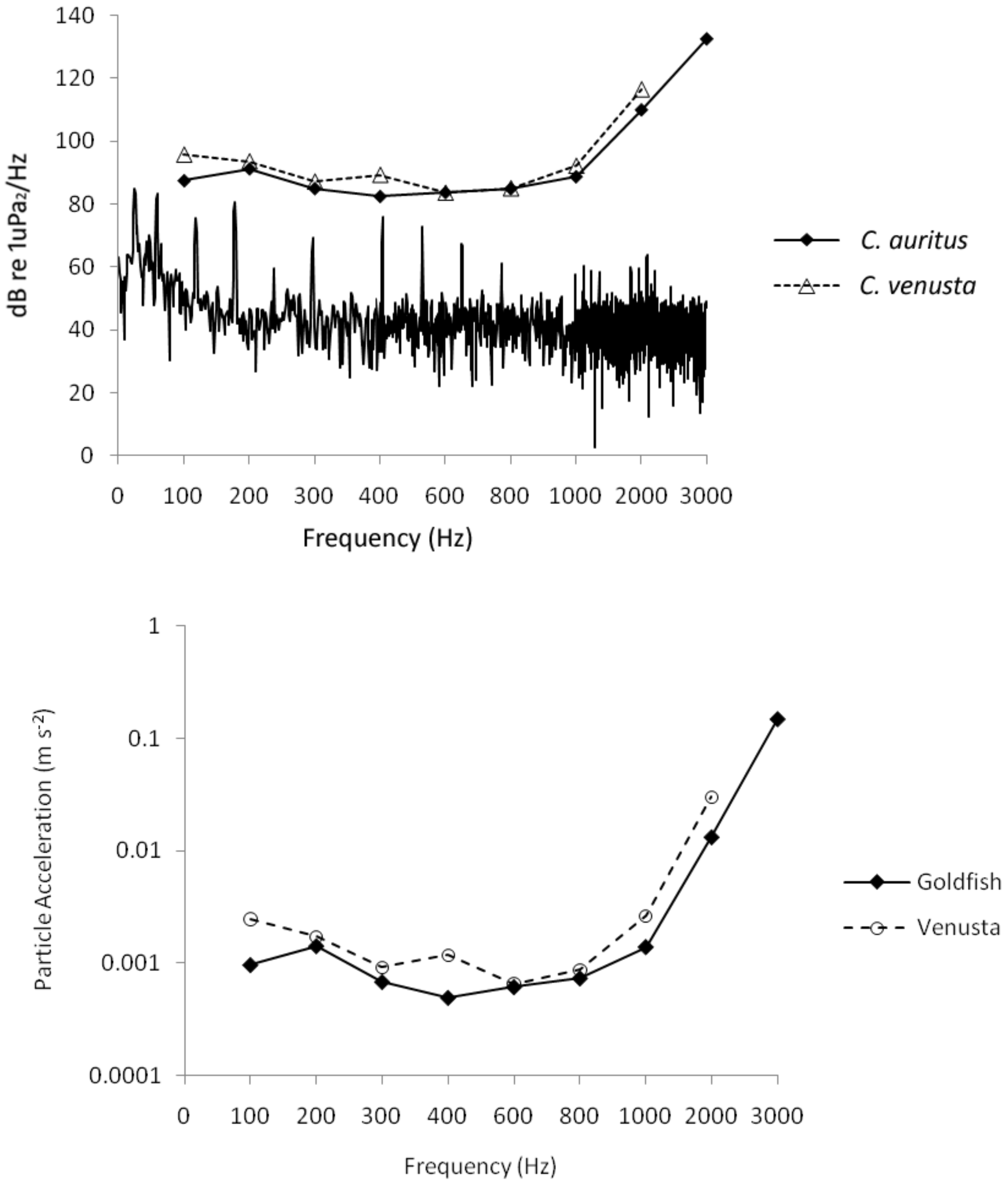

Figure 3-1 – Audiograms of *Carassius auratus* and *Cyprinella venusta* presented in (a)pressure and (b) particle acceleration. Baseline noise recorded at the position of the fish's head within the ABR chamber is shown as the power spectrum in (a).

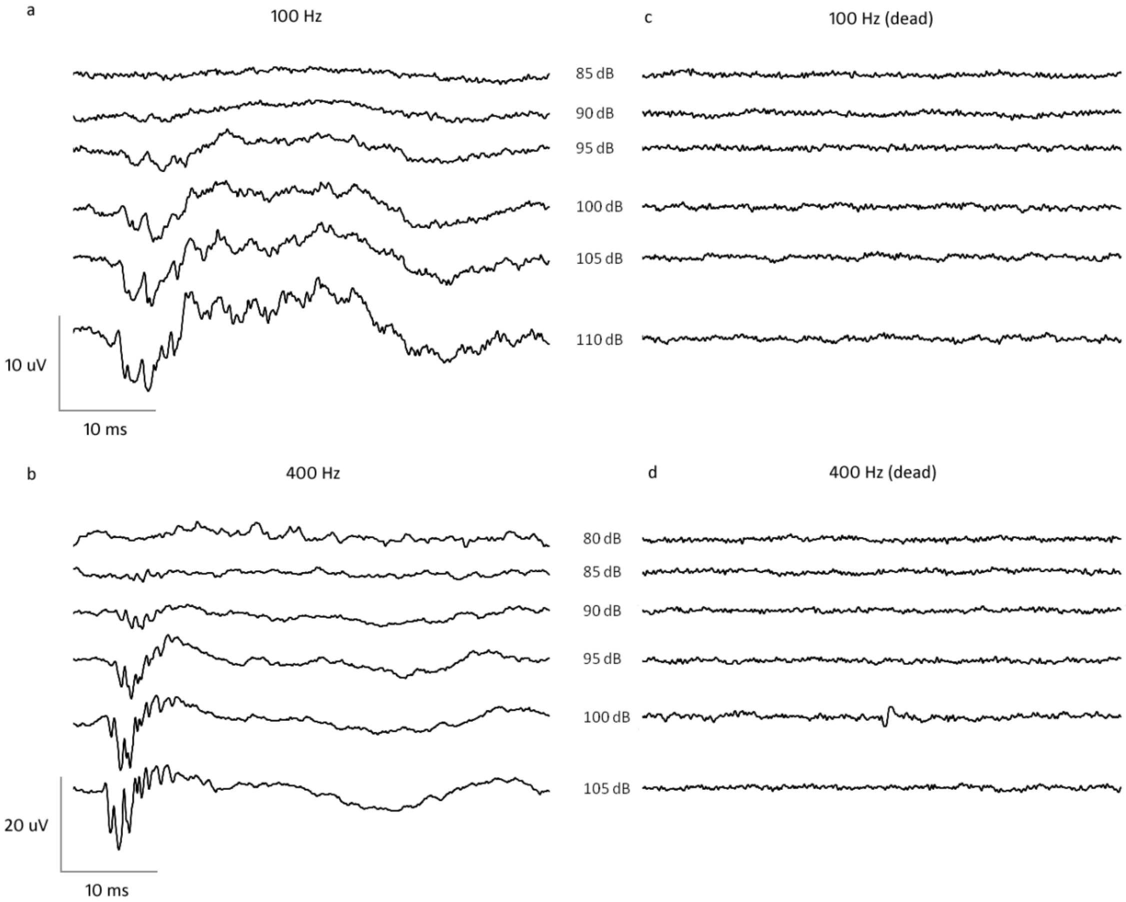

Figure 3-2 – Evoked potential traces at threshold, 5 dB below threshold, and 20 dB above threshold to (a) 100 Hz tone burst and (b) 400 Hz from *C. venusta*. Plates (c) and (d) are traces from the same individual being presented with the same stimulus, but after the individual had been sacrificed.

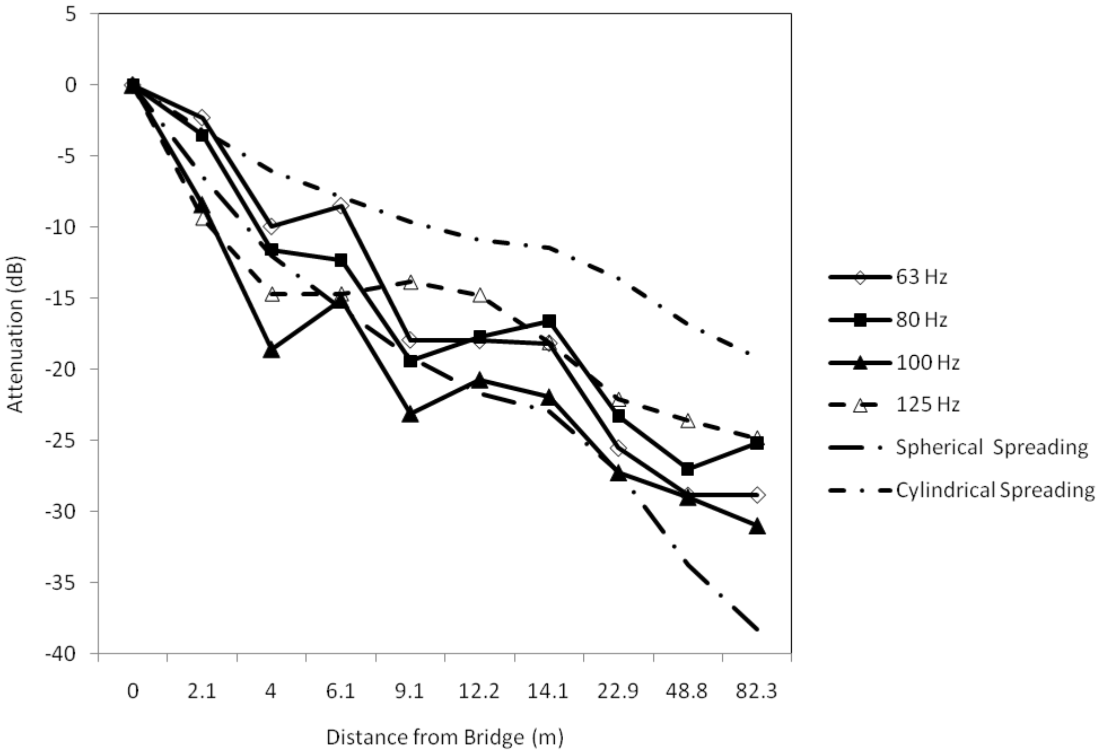

Figure 3-3 – Attenuation of semi-trailer truck noise at the crossing of Interstate-85 over Uphapee Creek. The four one-third octave bands above 60 Hz with the highest energy are shown. Attenuation of the 315 and 630 Hz bands was highly irregular due to interference from ambient noise. Theoretical attenuation values for spherical and cylindrical spreading are shown for reference.

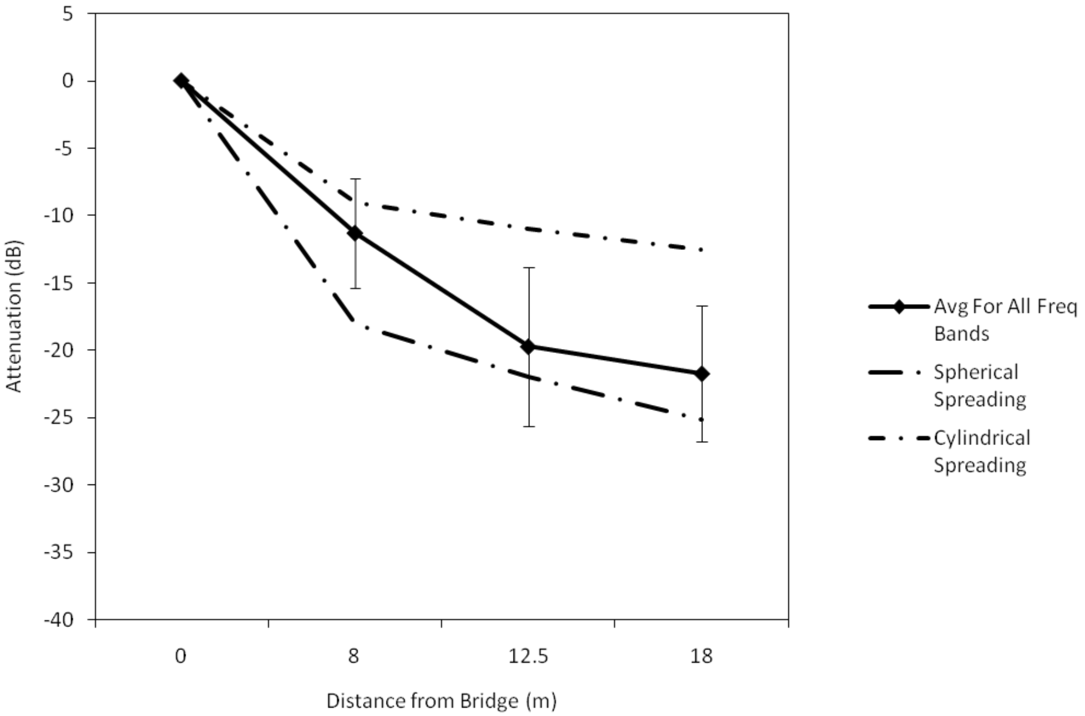

Figure 3-4 – Attenuation of train noise at the CSX crossing of Uphapee Creek. Attenuation of all one-third octave frequency bands from 63 to 1000 Hz were included in the average. Error bars represent a standard deviation. Calculated theoretical attenuation values from spherical and cylindrical spreading are presented for reference.

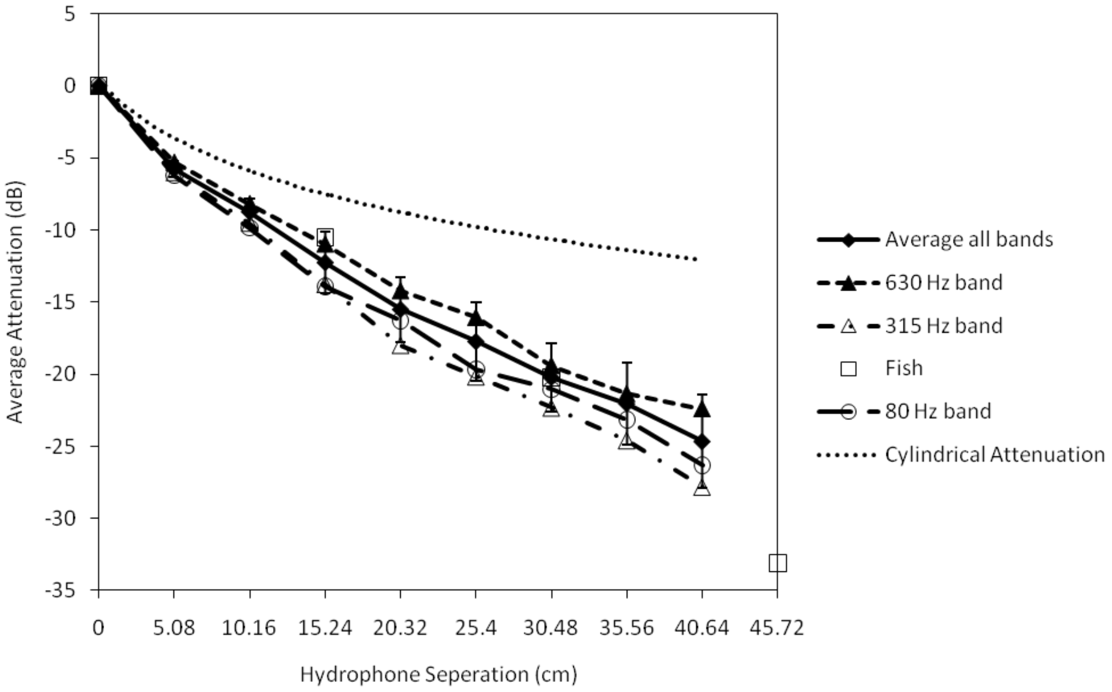

Figure 3-5 – Attenuation of actual and speaker playbacks of *C. venusta* signals. The solid line with standard deviation bars represents the average attenuation of the 13 frequency bands between 60 and 1000 Hz for knocks and bursts played back by the speaker at five sites. The frequency bands containing the dominant frequencies of knocks (80 and 630 Hz) are shown, as well as for growls (80 and 315 Hz). The calculated theoretical attenuation rate based on cylindrical spreading (3 dB/distance doubled) is also shown for reference. Average attenuation across the 13 frequency bands of signals actually produced by real fish are shown as empty squares occurring at hydrophone separations of 15.24, 30.48, and 45.72 cm. Measurements of signals from actual fish were taken at one distance per site at three different sites.

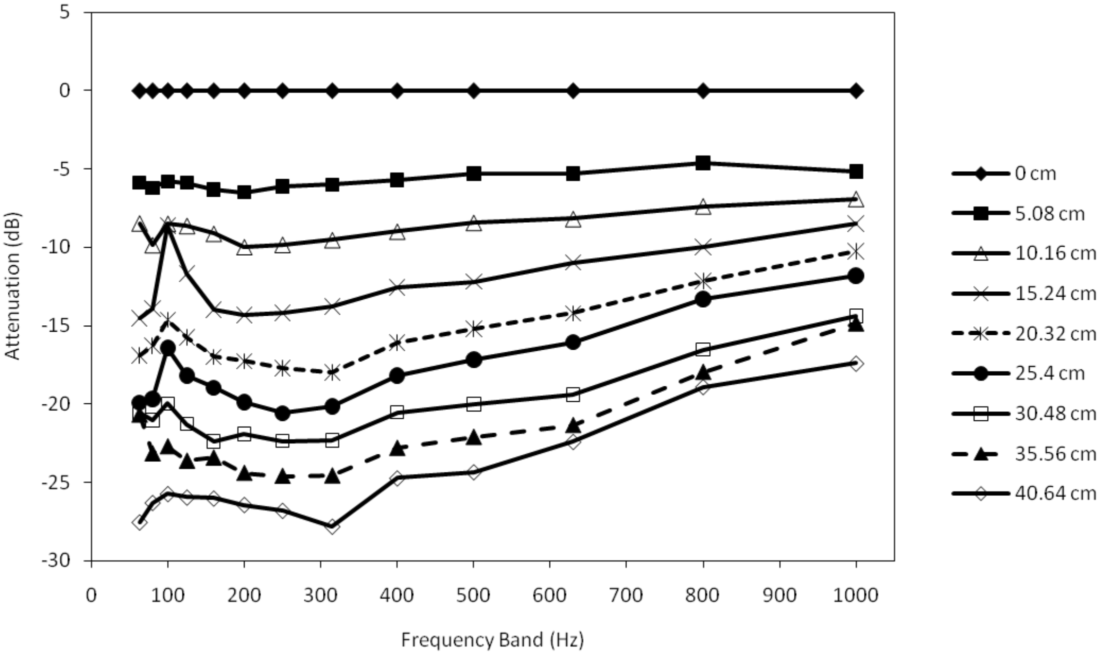

Figure 3-6 – Attenuation of one-third octave bands from speaker playbacks of *C. venusta* signals.

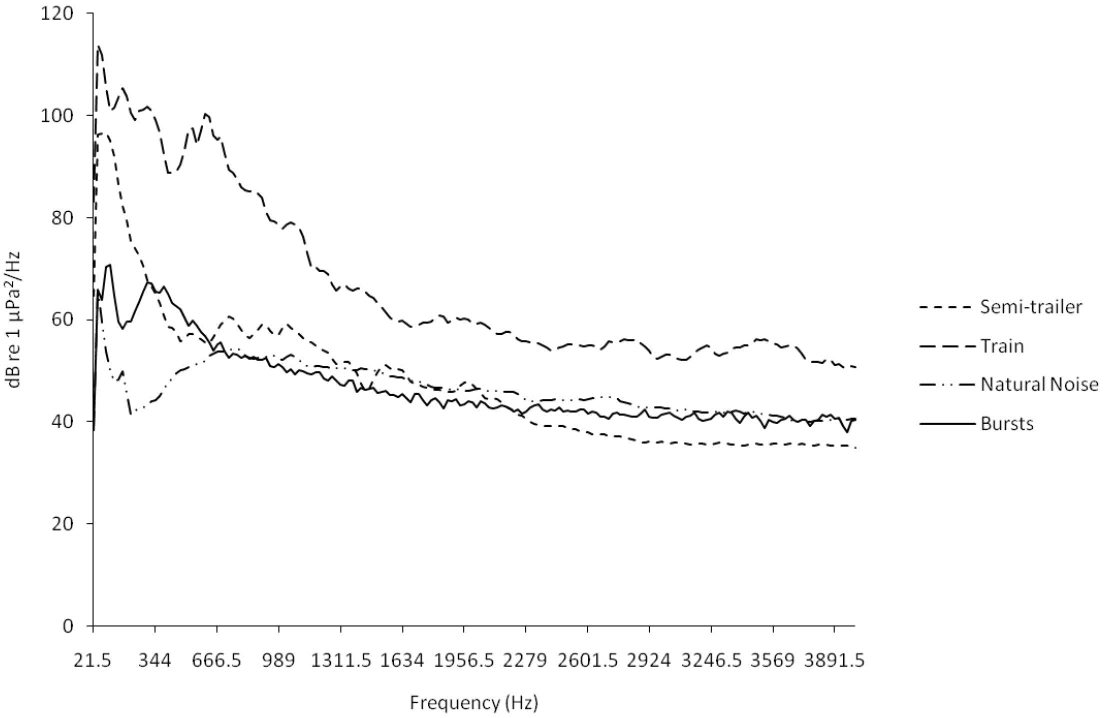

Figure 3-7 – Solid line shows average power spectra of bursts under quiet conditions in the laboratory. Other lines represent the average power spectra of semi-trailer truck noise and train crossings, and the average level of natural ambient noise at active nesting sites. A bin resolution of 21.5 Hz was used for all power spectra and SNR's for bursts were calculated based on these levels.

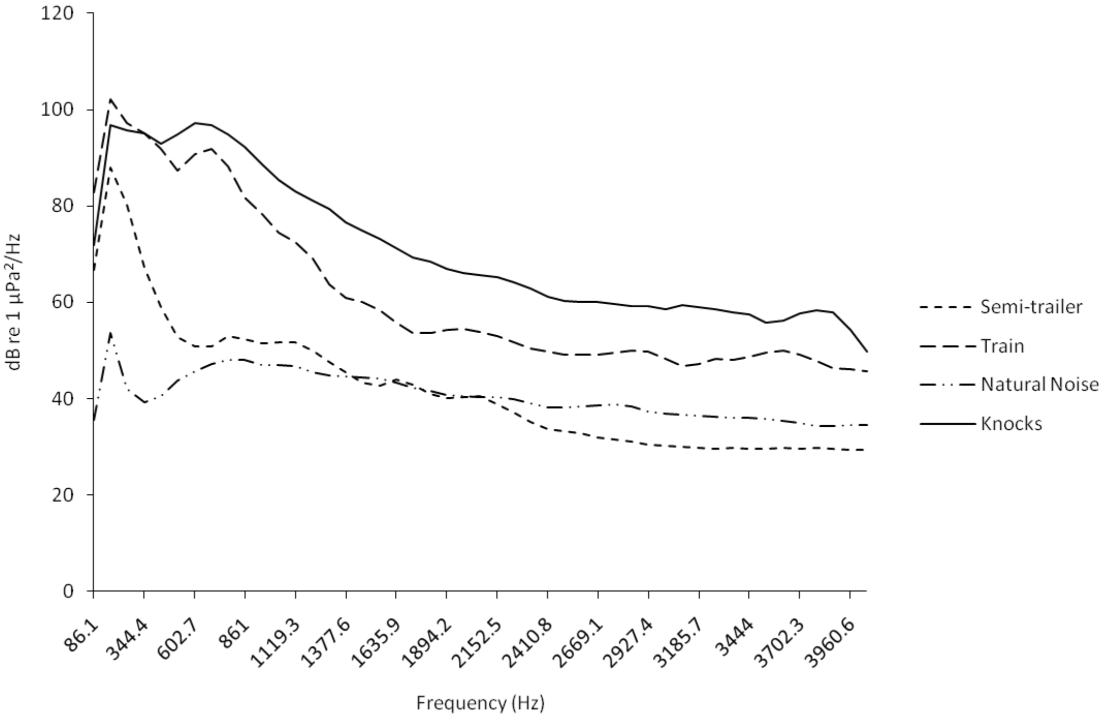

Figure 3-8 - Solid line shows average power spectra of knocks under quiet conditions in the laboratory. Other lines represent the average power spectra of semi-trailer truck noise and train crossings, and the average level of natural ambient noise at active nesting sites. A bin resolution of 86.1 Hz was used for all power spectra and SNR's for knocks were calculated based on these levels.

	Growls		Knocks	
	Dominant Hz	Subdominant Hz	Subdominant Hz	Dominant Hz
Frequency (Hz)	86	301	172.2	602.7
A.A. in Natural Noise (m)	0.0299	0.1649	0.5073	0.6519
Distance from semi-trailer truck noise (m)	117.8 (142.7)	5.9 (17.0)	2.0 (52.8)	--- (1.8)
Distance from train noise (m)	324.3 (393.1)	290.4 (839.5)	10.3 (269.5)	2.7 (178.6)

Table 3-1 – The active area (A.A.) of the dominant and subdominant frequencies of both call types from *C. venusta* in natural noise conditions as measured in 9 active nesting sites at Moffits Mill. Active area is based on a threshold of detection at a signal-to-noise ratio of 15 dB. For the rows containing a set of numbers, the top numbers show the closest distance to the noise sources at which the signal frequency component would theoretically be detectable (i.e. above a SNR of 15 dB). Numbers in parentheses show the distances at which active areas of the dominant and subdominant frequencies should re-establish themselves at levels seen under natural noise conditions due to attenuation of truck and train noise. Attenuation rates of semi-trailer truck and train noise are based on spherical spreading, which is a conservative estimate of attenuation based on the results of the current study. Distance from semi-trailer truck noise at 602.7 Hz is blank because knocks were already above a SNR of 15 dB at the source of the noise.