

## Original Article

## Evidence of the Lombard effect in fishes

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Noise can be problematic for acoustically communicating organisms due to the masking effect it has on acoustic signals. Rapid expansion of human populations, accompanied by noise that comes with industrialization and motorized transportation, poses a threat for many acoustically communicating species. Although a significant amount of effort has been made exploring the responses of organisms inhabiting marine and terrestrial environments to elevated noise levels, relatively little has been directed toward organisms inhabiting small, lotic, freshwater systems. The aim of this study was to determine what effect elevated noise levels have on acoustic signals and inter-fish distance during sound production in the Blacktail Shiner, *Cyprinella venusta*. We hypothesized, based on the behaviors of other vocal organisms, that *C. venusta* would compensate for elevated noise levels by decreasing distance between sender and receiver, increasing signal amplitude (Lombard effect), or by changing temporal patterns to increase call redundancy. Using an experimental approach, we found that *C. venusta* altered several acoustic components under noisy conditions. Most notably, spectral levels of acoustic signals were increased in background noise, indicating presence of the Lombard effect in fishes. Inter-fish distance was typically not different between noisy and quiet conditions, although one circumstance did show a significantly smaller inter-fish distance under noisy conditions.

**Key words:** acoustic communication, behavior, Cyprinidae, fish, Lombard effect, noise.

## INTRODUCTION

Acoustics are an important mode of communication used by a wide variety of both terrestrial and aquatic organisms. Although organisms will adapt to natural ambient noise conditions, elevations in environmental noise caused by anthropogenic sources can interfere with acoustic signals by decreasing signal-to-noise ratios, masking signals altogether, or altering the behavior of an organism (Vabø et al. 2002; Handegard et al. 2003; Sun and Narins 2005; Sara et al. 2007). The relatively recent development and rapid expansion of mass transportation, urbanization, and industrialization have contributed to the rise and spread of anthropogenic noise (Slabbekoorn et al. 2010).

Animals that are unable to avoid elevated noise levels may compensate for them in several ways according to the predictions of information theory (Shannon and Weaver 1949). Animals may alter spectral components of calls to avoid frequency bands that are more heavily affected by noise (Slabbekoorn and Peet 2003; Fernandez-Juricic et al. 2005; Feng et al. 2006), decrease the distance between sender and receiver, or increase the temporal or spectral redundancy of acoustic signals (Potash 1972; Lengagne et al. 1999; Brumm et al. 2004). By increasing temporal redundancy, 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). Animals may also increase the amplitude 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 (Lane and Tranel 1971). Previous research has shown that in several bird species, the Lombard effect occurs when there is an increase in environmental noise, possibly in an attempt to compensate for the decrease in active area, which in birds, often defines territorial boundaries (Warren et al. 2006). The Lombard effect has also been shown to occur in common marmosets (*Callithrix jacchus*; Brumm et al. 2004), whales (Scheifele 2005; Holt et al. 2008), and humans (Lane and Tranel 1971). To date, the Lombard effect has been documented in birds and mammals, but not in fishes (Brumm and Slabbekoorn 2005).

Sound can be a useful tool for communication, especially in aquatic habitats where turbidity and complex substrates diminish visual signals (Hawkins and Myrberg 1983), and a wide variety of fishes have thus adopted the ability to communicate acoustically (Ladich et al. 2006). Sound production in fishes is used for important life-history events such as reproduction (Myrberg and Lugli 2006), territory defense (Ladich and Myrberg 2006), species recognition, and mate choice (Amorim 2006). Although a good deal of information concerning the effect of noise on marine species, especially marine mammals is available (Richardson et al. 1998; Southall et al. 2007), the effect of this noise on many freshwater organisms is poorly studied.

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Underwater communication through the use of sound does have its own set of limitations 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; Forrest et al. 1993; Mann 2006), attenuate very rapidly. Studies looking at signal propagation in shallow stream habitats have found that signals from both *Cyprinella venusta* (whose main signal frequencies are less than 600 Hz; Holt DE, personal communication) and the gobies *Padogobius martensii* and *Gobius nigricans* (whose main signal frequencies are less than 200 Hz; Lugli and Fine 2003) propagate only several decimeters away from their source, even under ideal conditions. Any elevation in ambient noise could decrease the already limited active area of acoustic signals in these shallow water habitats.

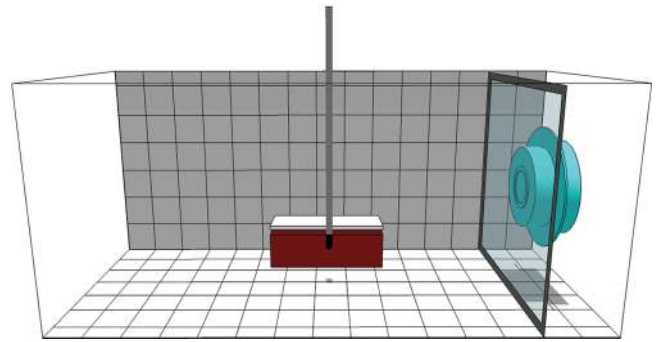
We investigated the effects of elevated noise conditions on the vocal behaviors and signal parameters in *C. venusta*, a common freshwater fish found in streams of the Southeastern United States. Like other species of *Cyprinella*, male *C. venusta* defend crevices where eggs are laid by females during the breeding season (Johnston and Page 1992). Although first described by Delco (1960), Holt and Johnston (2013) found that acoustic signals are produced only by males during aggressive and reproductive behaviors, and they provide a detailed signal description. Although hearing in *C. venusta* has not yet been described, it is an ostariophysian and likely has hearing sensitivity close to that of other closely related ostariophysians (Scholik and Yan 2001, 2002).

Our objectives were to determine whether *C. venusta* was able to compensate for depressed signal-to-noise ratios under noisy conditions by increasing the amplitude of their signals, increasing signal redundancy in the temporal domain, closing the distance between sender and receiver, or a combination of these adaptations. An experimental approach was taken by manipulating background noise levels in a laboratory setting and recording acoustic signals and behaviors of fish under both quiet and noisy conditions.

## MATERIALS AND METHODS

Fish were captured using 10-ft seines from Little Uchee Creek (lat 32.508579°N, long -85.184215°W), Halawakee Creek (lat 32.697579°N, long -85.266951°W), and Wacoochee Creek (lat 32.622799°N, long -85.132685°W), all of which are tributaries to the Chatahoochee River in Lee County, Alabama. Fish were maintained at 26 °C in 76-L aquariums with gravel substrates and hanging external filters. All fish were given a commercial flake food diet and were kept on a 12:12-h light:dark light cycle. A single 76-L experimental tank was setup in an acoustic chamber. The experimental tank contained a brick placed on the wall in the center of the tank, on top of which a tile was propped up about 4mm serving as an artificial nest. Two pieces of white plexiglass with gridlines drawn at 5-cm intervals were placed on the bottom and back of the experimental tank, and a 60-W light bulb was hung approximately 1.5 m above the tank. The bulb cast shadows of the fish on the bottom grid, which in conjunction with the grid on the back of the tank, allowed inter-fish distances to be measured from the video recordings (Figure 1).

Natural ambient noise levels of natural nesting sites were determined by recording 1 min of ambient noise at 10 different active nesting sites (in the absence of fish). A Hi-Tech (HTI-96-MIN, sensitivity -164.4 re 1 V/ $\mu$ Pa, frequency response: 0.002–20 kHz)



**Figure 1**  
Experimental tank setup composed of a 76-L aquarium with 5-cm interval gridlines, artificial nest in the center with hydrophone 5 cm in front, and underwater speaker suspended behind mesh barrier right.

was used in conjunction with a digital recorder (Marantz PMD 661, sampling rate 44.1 kHz). In an effort to minimize flow-induced noise, the hydrophone was placed adjacent to areas of flow, or in the eddy of a rock. Raven 1.4 (Cornell University, Ithaca, NY) was used to analyze sounds, and three, 1-s segments were selected from the recording at each site. A pressure density spectrum of each 1-s segment was calculated using the power spectrum function of Raven (Hamming window, 50% overlap, fast Fourier transformation (FFT) length: 32 768 samples, analysis bandwidth: 1.35 Hz). Spectral levels were calibrated using the sensitivity of the hydrophone and a GW GOS-6xxG dual-trace oscilloscope, and by taking into consideration the gain applied to the signal by the Marantz and when importing sounds into Raven. Excel was used to average the power spectra from the three, 1 s selections at each nest site, and then to average the spectra from all 10 sites.

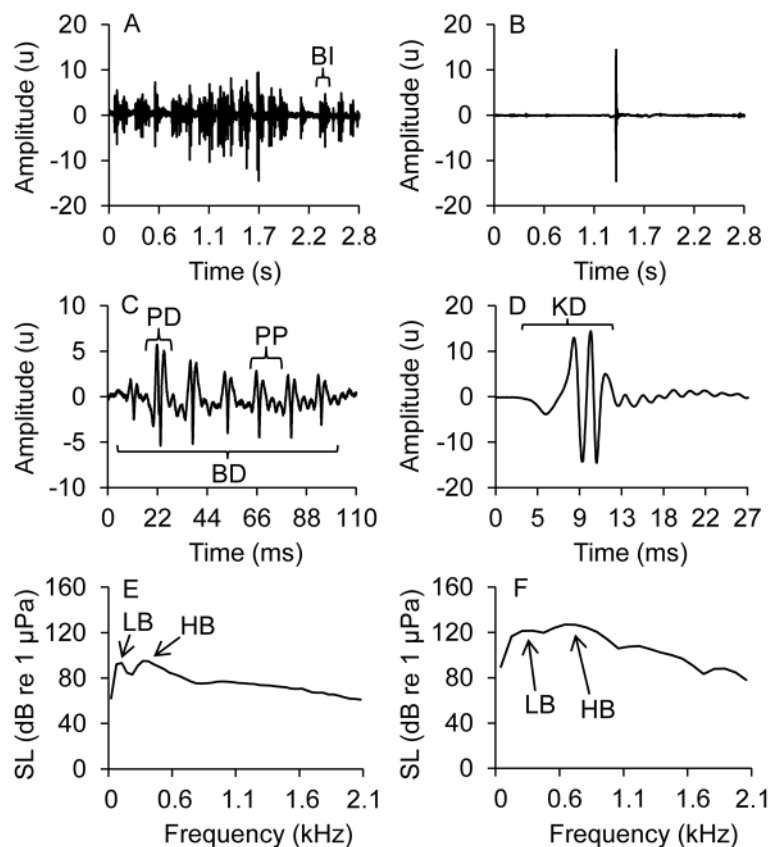
Experiments began by placing previously isolated male and female *C. venusta* in the experimental tank and allowing them to acclimate. This arrangement allowed both courtship (growls) and aggressive (knocks) signals to be recorded. Acclimation periods varied between 20 min and 8 h, but were considered to be over when fish began moving about freely and a male began guarding the artificial nest. A trial was defined as an experimental unit in which a single male was recorded under both noisy and quiet conditions. For each trial, we placed 2 males and 1–2 females in the experimental tank. A total of 78 males and 22 females were used in this study, but only 19 males produced sounds suitable for analysis under both noisy and quiet conditions. Each trial consisted of a quiet period and a noisy period. Each period usually lasted about 2 h, but duration ranged from 17 min to 2.5 h. During noisy periods, band limited white noise generated using SigGen (Tucker Davis Technologies, Gainesville, FL) was amplified using an ART SLA-1, 2-channel stereo linear amplifier, and played through an underwater speaker (UW-30; University Sound, Oklahoma City, OK). During quiet periods, no sound was played through the speaker, and the amplifier was turned off. All trials were video recorded using a Sony Handycam digital HD video recorder (HDR-SR11), and sounds were recorded onto a Dell Optiplex 745 (SoundMAX HD Audio soundcard) using Raven 1.3 (Cornell University, Ithaca, NY), a Brüel and Kjaer 8103 hydrophone, and a Brüel and Kjaer 2635 charge amplifier. The order in which periods were conducted within each trial was random, and noise was always ramped up or down (depending on whether it occurred before or after the silent period) over the course of 5 min to prevent startling the fish.

## Analysis

Because of potential distortion caused by interaction of fish signals with the resonant frequency of the small experimental tank, only acoustic signals that occurred within 15 cm of the hydrophone were analyzed. This distance was based on calculations from Akamatsu et al. (2002) and minimized these distortions. Temporal parameters including durations, intervals, periods, and rates were measured in Raven from the waveform view (Figure 2). A call was defined as all the sound produced during a particular behavior and could be composed of one to several growls or one to several knocks. A growl was composed of a variable number of bursts, and a burst was composed of 2 or more pulses strung together as a pulse train with a relatively constant pulse period. Pulse and knock durations were defined as the duration of the entire waveform and consisted of 2 cycles for pulses, and 3 cycles for knocks. Pulse periods were defined as the duration between the first zero-crossing of 2 adjacent pulses. Burst duration was defined as the duration between the first zero-crossing of the first pulse to the last zero-crossing of the last pulse of a continuous pulse train. Inter-burst interval was defined as the duration between the last pulse of 1 continuous pulse train and the first pulse of the next continuous pulse train. 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).

The number of pulses contained within each growl call type was counted using the waveform view of Raven, and the beginning and end of a call were defined based on the initiation and termination of the accompanying behavior.

Spectral parameters including knock and burst low-band and high-band frequencies were measured from the calculated power spectrum in Raven. The same selections used to measure knock and burst duration were used to generate power spectrums. In order to obtain the most accurate frequency information from the power spectrum, sample sizes of FFT's (Hamming windows, 50% overlap, FFT length: number of samples in selection, analysis bandwidth: variable) were maximized for each knock or burst so that the analysis bandwidth of the power spectrum was as small as possible. Spectral levels of the low-frequency and high-frequency bands of knocks and bursts were also measured from the power spectrum and were adjusted to represent absolute levels by taking into consideration the gain imposed by each unit in the recording chain. Because the spectral level of a sound varies depending on the analysis bandwidth used (larger analysis bandwidths underestimate spectral level of a peak frequency; Richardson et al. 1998), spectral levels of knocks and bursts were corrected for different analysis bandwidths by adding  $10 \log(\text{analysis bandwidth})$  decibel to the measured spectrum level of the low-band and high-band frequencies of each measured signal.



**Figure 2**

Waveform showing a growl (A) and knock (B) under quiet conditions. Amplitude units are arbitrary and are abbreviated as “U”. Burst interval (BI) is indicated in the growl. Waveform showing structure of a single burst (C) and a single knock (D) with brackets showing pulse duration (PD), pulse period (PP), burst duration (BD), and knock duration (KD). Pressure density spectra showing spectrum levels (abbreviated as SL) of burst (E) and knock (F) with low-band frequencies (LB) and high-band frequencies (HB) labeled by arrows. Pressure density spectra generated using Hamming window, sampling rate = 44.1 kHz, FFT samples = 1024, and analysis bandwidth = 43.1 Hz.

Prior to analyses, an average value for each signal parameter (pulse duration, burst duration, burst low-band frequency, and so on) was calculated for each distinct behavior type, for each individual male. Pearson correlations were performed, independently for knocks and growls, to determine if there was a relationship between spectrum level and distance from the hydrophone. Sounds from every behavior type were combined for these correlations. This was performed in an attempt to estimate source levels of the sound (source level defined here as at the vocalizing fish, not 1 m from the source). If spectrum level's were correlated with distance from the hydrophone, source levels were estimated by adding the product of the distance from the hydrophone and the slope of the linear best fit line of the correlation to the original spectrum level of the signal, and were used in further analyses. If no correlation was found, received levels were used. Because movement around the hydrophone was equally random under both noisy and quiet conditions, using received levels for analysis when no correlation was found should be acceptable because any variation in spectrum level caused by proximity to the hydrophone should be equal between noisy and quiet conditions. To ensure this assumption was met, paired *t*-tests were used to determine whether distance from the sound producer to the hydrophone during knock production differed between noisy and quiet conditions. Approach and lateral display behaviors were tested separately, and a Bonferroni correction was applied for multiple comparisons.

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 approach and lateral display behaviors. However, multivariate analysis of variances (MANOVAs) were first run to determine whether vocalizations produced during approach behaviors differed from vocalizations produced during lateral display behaviors. Four MANOVAs were run, independently for knocks and growls under quiet and noisy conditions. Signal parameters including pulse duration, pulse period, pulse rate, number of pulses per call, burst duration, burst interval, burst rate, burst low-band frequency, burst high-band frequency, burst low-band frequency spectrum level, and burst high-band frequency spectrum level were included as dependent variables, and behavior type (approach and lateral display) was used as the fixed factor for growls. Knock duration, knock low-band frequency, knock high-band frequency, knock low-band frequency spectrum level, and knock high-band frequency spectrum level were dependent variables, and behavior type was used as the fixed factor for knocks. Because there was no significant difference between vocalizations produced during the 2 behaviors, for either knocks or growls, they were combined to bolster sample sizes and simplify further analysis between noisy and quiet conditions.

To determine whether *C. venusta* males altered growl acoustic signals under elevated noise conditions, a repeated measures MANOVA design was used. In this design, quiet and noisy conditions were the 2 within subjects' factors, with each factor containing 11 measures (pulse duration, pulse period, pulse rate, burst duration, burst interval, burst rate, burst low-band frequency, burst high-band frequency, burst low-band frequency spectrum level, burst high-band frequency spectrum level, and number of pulses per call). 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 noisy and quiet conditions. All statistical analyses were conducted in PASW 18 statistical software package (IBM SPSS Corporation, Chicago, IL).

Because knocks were recorded from fewer individuals than growls, the 2 types of vocalizations were analyzed separately to maintain the highest possible samples sizes for each analysis. Knocks produced during noisy and quiet trials were compared using repeated measures MANOVA, similarly to the method described previously for growls. For the knock analysis, however, only 5 measures were included (knock duration, knock low-band frequency, knock high-band frequency, knock low-band frequency spectrum level, and knock high-band frequency spectrum level).

A second analysis to test for differences in spectrum level between quiet and noisy conditions was performed because of the concern that softer signals could have been masked during noisy trials. Three bursts with the highest spectrum level's from each quiet trial were therefore averaged, as were the 3 bursts with the highest spectrum level's for each noisy trial. A paired *t*-test was then used to determine whether the average spectrum level of the 3 loudest bursts differed between quiet and noisy conditions. This was done independently for both low-band and high-band frequencies of growls and knocks.

In cases where the Lombard effect was observed, the degree of amplitude modulation was determined. This was performed by first calculating the difference between the average spectrum level of the 3 loudest bursts under noisy conditions and the 3 loudest bursts under quiet conditions for each individual fish. This was done independently for the low-band and high-band frequencies. Each of these differences was then divided by the difference in background noise level between the noisy and quiet trials, measured in one-third octave bands centered at the low-band and high-band frequencies of bursts. The quotients for each individual fish were then averaged to determine the magnitude of amplitude modulation for the low-band and high-band frequencies. The same procedure was performed for the high-band frequency of knocks.

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 for multiple comparisons.

## RESULTS

Pearson correlations indicated that, for bursts, distance from the hydrophone was not correlated with spectrum level under either noisy (low-band Hz:  $R = -0.025$ ,  $P = 0.419$ ,  $N = 1074$ ; high-band Hz:  $R = 0.042$ ,  $P = 0.167$ ,  $N = 1073$ ) or quiet (low-band Hz:  $R = -0.002$ ,  $P = 0.931$ ,  $N = 1397$ ; high-band Hz:  $R = -0.021$ ,  $P = 0.438$ ,  $N = 1397$ ) conditions. For knocks, however, there was a significant negative correlation between spectrum level and distance from the hydrophone under both noisy (low-band Hz:  $R = -0.514$ ,  $P < 0.001$ ,  $N = 157$ ; high-band Hz:  $R = -0.552$ ,  $P < 0.001$ ,  $N = 155$ ) and quiet (low-band Hz:  $R = -0.384$ ,  $P < 0.001$ ,  $N = 143$ ; high-band Hz:  $R = -0.378$ ,  $P < 0.001$ ,  $N = 143$ ) conditions. There was no significant difference in distance from the sound producing male to the hydrophone between quiet and noisy conditions for approach behaviors (noisy:  $156.9 \pm 74.1$  mm,  $N = 14$ ; quiet:  $159.2 \pm 48.4$  mm,  $N = 14$ ; paired *t*-test:  $t_{13} = 0.111$ ,  $P = 0.913$ ) or lateral display behaviors (noisy:  $158.5 \pm 81.5$  mm,  $N = 15$ ; quiet:  $135.7 \pm 59.0$  mm,  $N = 15$ ; paired *t*-test:  $t_{14} = -0.957$ ,  $P = 0.355$ ).

Results from the MANOVA testing for a difference in vocalizations produced during lateral display and approach behaviors indicated that there was no main effect of behavior type on signal parameters associated with growls (MANOVA:  $F_{10,17} = 1.036$ ,  $P = 0.456$ ) or knocks ( $F_{5,24} = 0.816$ ,  $P = 0.550$ ) under noisy



conditions. There was similarly no difference between vocalizations produced during approach and lateral display behaviors under quiet conditions (MANOVA: growls:  $F_{10,19} = 0.495, P = 0.873$ ; knocks:  $F_{5,17} = 0.316, P = 0.896$ ).

Results from the repeated measures MANOVA testing for a difference in vocalizations in response to elevated noise levels showed a significant main effect of background noise level on growl acoustic signal parameters (repeated measures MANOVA:  $F_{11,7} = 9.003, P = 0.004$ ; Table 1). Bonferroni corrected post hoc tests indicated that burst duration under noisy conditions ( $0.0557 \pm 0.0194$  s,  $N = 18$ ) was significantly shorter than under quiet conditions ( $0.0791 \pm 0.0310$  s,  $N = 18; P = 0.001$ ). Burst rate under noisy conditions ( $7.061 \pm 1.583$  bursts/s,  $N = 18$ ) was significantly greater than under quiet conditions ( $6.011 \pm 1.738$  bursts/s,  $N = 18; P = 0.046$ ). Spectral level of both the low-band frequency (noisy:  $105.70 \pm 2.88$  dB re 1  $\mu$ Pa,  $N = 18$ ; quiet:  $99.36 \pm 2.98$  dB re 1  $\mu$ Pa,  $N = 18$ ) and the high-band frequency (noisy:  $104.13 \pm 2.86$  dB re 1  $\mu$ Pa,  $N = 18$ ; quiet:  $94.59 \pm 3.69$  dB re 1  $\mu$ Pa,  $N = 18$ ) of growls was significantly greater under noisy conditions than quiet conditions (Bonferroni corrected post hoc: low-band frequency spectrum level:  $P < 0.001$ ; high-band frequency spectrum level:  $P < 0.001$ ). Figure 3 illustrates the difference in amplitude between pulses produced by a single male under quiet and noisy conditions. The number of pulses per call was also significantly lower under noisy conditions (noisy:  $22.19 \pm 10.51$  pulses/call,  $N = 18$ ; quiet:  $32.20 \pm 13.57$  pulses/call,  $N = 18$ ; Bonferroni corrected post hoc:  $P = 0.001$ ).

A significant main effect of noise level on acoustic signal parameters was also found for knocks (repeated measures MANOVA:  $F_{5,9} = 15.289, P < 0.001$ , Table 1). Bonferroni corrected post hoc tests indicated that the spectrum level of the high-band frequency of knocks was significantly higher under noisy conditions ( $142.65 \pm 4.33$  dB re 1  $\mu$ Pa,  $N = 14$ ) than quiet conditions ( $136.91 \pm 6.13$  dB re 1  $\mu$ Pa,  $N = 14; P = 0.009$ ). No other signal parameters associated with knocks were significantly different.

**Table 1**  
Signal parameters under noisy and quiet conditions

	Noisy	Quiet	<i>P</i>
<b>Growls</b>			
Pulse duration (ms)	7.13 ( $\pm 0.99$ )	7.34 ( $\pm 0.85$ )	0.340
Pulse period (ms)	12.86 ( $\pm 1.37$ )	13.80 ( $\pm 3.27$ )	0.175
Pulse rate (#/s)	84.83 ( $\pm 12.51$ )	81.46 ( $\pm 12.78$ )	0.127
Burst duration (ms)	55.73 ( $\pm 19.43$ )	79.05 ( $\pm 31.03$ )	<b>0.001</b>
Burst interval (ms)	133.39 ( $\pm 62.57$ )	140.85 ( $\pm 53.52$ )	0.647
Burst rate (#/s)	7.06 ( $\pm 1.58$ )	6.01 ( $\pm 1.74$ )	<b>0.046</b>
Burst low-band Hz	84.74 ( $\pm 10.52$ )	81.86 ( $\pm 8.78$ )	0.141
Burst high-band Hz	306.82 ( $\pm 32.62$ )	302.70 ( $\pm 40.07$ )	0.413
Burst low-band SL (dB)	105.70 ( $\pm 2.88$ )	99.36 ( $\pm 2.98$ )	<b>&lt;0.001</b>
Burst high-band SL (dB)	104.13 ( $\pm 2.86$ )	94.59 ( $\pm 3.69$ )	<b>&lt;0.001</b>
Pulses per call	22.19 ( $\pm 10.51$ )	32.20 ( $\pm 13.57$ )	<b>0.001</b>
<b>Knocks</b>			
Knock duration (ms)	13.50 ( $\pm 3.67$ )	15.16 ( $\pm 3.63$ )	0.155
Knock low-band Hz	171.17 ( $\pm 79.32$ )	169.54 ( $\pm 43.74$ )	0.944
Knock high-band Hz	593.57 ( $\pm 75.58$ )	572.81 ( $\pm 69.56$ )	0.170
Knock low-band SL (dB)	136.30 ( $\pm 4.00$ )	135.02 ( $\pm 3.84$ )	0.389
Knock high-band SL (dB)	142.65 ( $\pm 4.33$ )	136.91 ( $\pm 6.13$ )	<b>0.009</b>

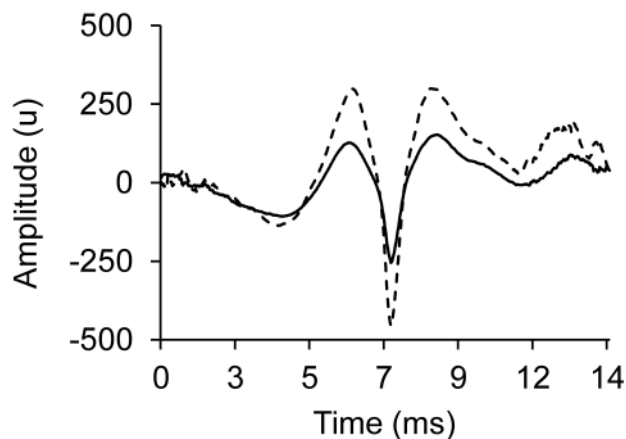
SL, spectrum level.

Mean ( $\pm$  standard deviation) of growl and knock signal parameters for noisy and quiet conditions. Sample size was 18 individual fish for parameters associated with growls and 14 for parameters associated with knocks. *P*-values from Bonferroni corrected post hoc tests are included (*P*-values lower than 0.05 are marked in bold).

Paired *t*-tests comparing the 3 loudest bursts produced under noisy conditions to the 3 loudest bursts under quiet conditions showed that spectrum levels of both the low-band frequency (noisy:  $112.23 \pm 3.16$  dB re 1  $\mu$ Pa,  $N = 18$ ; quiet:  $107.42 \pm 3.29$  dB re 1  $\mu$ Pa,  $N = 18$ ) and high-band frequency (noisy:  $111.08 \pm 4.74$  dB re 1  $\mu$ Pa,  $N = 18$ ; quiet:  $103.92 \pm 4.89$  dB re 1  $\mu$ Pa,  $N = 18$ ) were significantly louder under noisy conditions than quiet conditions (paired *t*-test: low-band frequency spectrum level:  $t_{17} = 5.469, P < 0.001$ ; high-band frequency spectrum level:  $t_{17} = 4.932, P < 0.001$ ). The high-band frequency of the 3 loudest knocks was significantly greater under noisy conditions ( $153.68 \pm 4.97$  dB re 1  $\mu$ Pa,  $N = 14$ ) than under quiet conditions ( $146.38 \pm 6.29$  dB re 1  $\mu$ Pa,  $N = 14$ ; paired *t*-test:  $t_{13} = 4.301, P = 0.001$ ). The low-band frequency of the 3 loudest knocks showed no difference between quiet and noisy conditions.

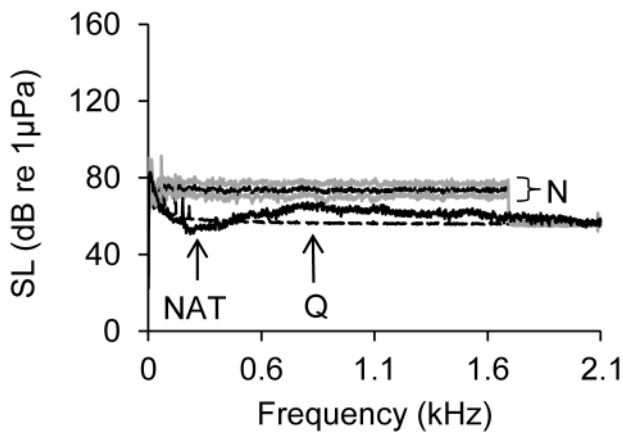
The difference in background noise level between noisy and quiet conditions was 10.20 dB re 1  $\mu$ Pa for the one-third octave band centered at the low-band frequency of bursts (80 Hz), 15.88 dB re 1  $\mu$ Pa at the high-band frequency of bursts, and 16.93 dB re 1  $\mu$ Pa at the high-band frequency of knocks (Figure 4). The degree of amplitude modulation in response to noise ranged from  $0.431 \pm 0.375$  dB re 1  $\mu$ Pa ( $N = 14$ ) per decibel increase in noise for knock high-band frequency to  $0.472 \pm 0.366$  dB re 1  $\mu$ Pa ( $N = 18$ ) for burst low-band frequency. The high-band frequency of bursts showed an amplitude modulation of  $0.451 \pm 0.388$  dB re 1  $\mu$ Pa ( $N = 18$ ).

There was no significant difference in inter-fish distance during growls for either approach behaviors (noisy:  $97.43 \pm 50.33$  mm,  $N = 18$ ; quiet:  $87.24 \pm 52.57$  mm,  $N = 18$ ; paired *t*-test:  $t_{17} = 0.772, P = 0.451$ ) or lateral display behaviors (noisy:  $52.88 \pm 24.45$  mm,  $N = 14$ ; quiet:  $48.86 \pm 14.95$  mm,  $N = 14$ ; paired *t*-test:  $t_{13} = 0.447, P = 0.662$ ). There was also no significant difference in inter-fish distance during knocks for approach behaviors (noisy:  $72.83 \pm 23.75$  mm,  $N = 14$ ; quiet:  $63.37 \pm 29.67$  mm,  $N = 14$ ; paired *t*-test:  $t_{13} = 1.144, P = 0.273$ ). Knocks produced during lateral displays, however, were made at a significantly closer distance under noisy conditions ( $45.55 \pm 14.74$  mm,  $N = 15$ ) than quiet conditions ( $56.10 \pm 10.37$  mm,  $N = 15$ ; paired *t*-test:  $t_{14} = -3.365, P = 0.005$ ).



**Figure 3**

Composite pulses illustrating higher amplitude under noisy conditions. Pulses were constructed by averaging 373 pulses produced under noisy conditions (broken line) and 403 pulses produced under quiet conditions (solid line). All sounds were produced by the same individual.



**Figure 4**

Power spectra showing background noise levels during quiet trials (broken line: Q), noisy trials (solid black line bordered by gray lines: N), and under natural conditions in the field (solid line: NAT). Standard deviations of noise in the laboratory are represented by gray lines above and below the average level (N). Power spectra of tank background noise were generated from a single 10 s selection of background noise containing no contamination, in the absence of fish (Hamming window, sampling rate = 44.1 kHz, FFT samples = 32 768, analysis bandwidth = 1.35 Hz). A total of 20 noisy and 20 quiet trials were included to obtain the average power spectra shown. The power spectrum of natural noise was generated from 10 nesting sites, with three, 1-s clips being averaged at each site (Hamming window, sampling rate = 44.1 kHz, FFT samples = 32 768, analysis bandwidth = 1.35 Hz).

## DISCUSSION

Our results demonstrate the presence of the Lombard effect in *C. venusta*, an important finding due to the fact that, to date, evidence for the Lombard effect has not been found in fishes. The results from the MANOVA including all bursts and paired *t*-tests including only the 3 bursts with the highest spectrum level's were congruent, showing that sounds produced during noisy conditions were significantly louder than under quiet conditions. The Lombard effect has been found in all bird species tested to date and in mammals including whales, cats, bats, rodents, and primates (Brumm and Zollinger 2011). Although the capacity for amplitude modulation has been documented in frogs (Lopez et al. 1988), the only study specifically investigating the Lombard effect in amphibians reported its absence (Love and Bee 2010).

Interestingly, an elevation in spectrum level of knocks under noisy conditions was observed for the high-band frequency, but not for the low-band frequency. Bursts showed a similar pattern in that the high-band frequency showed a greater increase in amplitude under noisy conditions than the low-band frequency (although for bursts both frequencies were significantly louder under noisy conditions). We cannot currently explain this frequency-dependent amplitude regulation, partly because we do not yet understand the mechanism for sound production in this species. Unequal changes in spectral energy of vocalizations under noisy conditions (spectral tilting) have, however, been documented in humans (van Summers et al. 1988).

Relative to other species, *C. venusta* showed a small magnitude of amplitude modulation. Scheifele et al. (2005) compiled a list of species in which the magnitude of amplitude modulation as a function of increased ambient noise had been measured, which ranged from 0.35–3.3 dB per decibel increase in noise. Because propagation of low-frequency sounds in stream conditions is limited due to

shallow water depths and high cut-off frequencies, *C. venusta* may be forced to call near maximum amplitude just to maintain the naturally small active area permitted by their physical environment. Love and Bee (2010) similarly suggested that *Hyla chrysocelis* naturally calls near its physiological limit, not because of the physical environment, but due to the role of call amplitude on mating success (because females are attracted to the loudest signals).

The fact that no correlation was found between burst spectrum level and distance from the hydrophone suggests the precision of our distance measurements was not sufficient to detect the small change in power. This is likely inconsequential, however, because distance from the hydrophone during knock sound production, which could be measured more accurately than bursts because of the isolated occurrence of knocks, did not differ between noisy and quiet trials.

In addition to the Lombard effect, we predicted that when subjected to elevated noise levels, *C. venusta* would attempt to compensate for decreased signal-to-noise ratios by increasing signal redundancy. We were not surprised that the most basic temporal components of *C. venusta* signals (pulse duration, pulse period, and pulse rate) remained conserved, as these probably serve as a reliable source of species identification (Mann and Lobel 1997). Our hypothesis that signal redundancy would increase was not supported by the fact that burst duration decreased 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 results in 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, however, was an increase in burst rate, which may help counteract the decrease in burst duration. However, the fact that there were fewer pulses per call under noisy conditions, along with the observation of shortened burst duration under noisy conditions, suggests that *C. venusta* does not increase and may actually decrease the overall amount of information encoded in the temporal component of their growls. This is an unusual response as both birds and mammals (2 groups well studied in terms of acoustic compensation in response to noise) typically increase temporal redundancy of calls. It should be noted that because acoustic signals of *C. venusta* are almost always associated with some visual cue or behavior and not used for advertisement at long distance as is the case for many birds and mammals, a decrease in temporal redundancy may not be as detrimental to *C. venusta*. However, because the functional significance of *C. venusta* signals remains unknown, it is difficult to make this comparison.

For the most part, inter-fish distance remained unchanged between quiet and noisy conditions, and it was only different for knocks produced during lateral display behaviors. One possible explanation for this is that because lateral displays are a behavior associated with higher motivation (Phillips and Johnston 2008) than approach behaviors, the importance of successfully transmitting the message may be greater. Because knocks are typically associated with aggression (Phillips and Johnston 2008), 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.

Because this study was performed in the laboratory, there were limitations to the degree of reality that we could achieve. Presenting any noise using an underwater transducer in a small glass aquarium, for example, will result in a shaped noise with some frequencies being amplified more than others within the tank. White noise being

played through an underwater transducer in a small glass tank is like nothing these fish would be exposed to under natural conditions. However, the fact that the noise presented in this experiment was not biological and had no temporal patterning (which is typical of biological sounds), leaves us with no reason to believe that *C. venusta* interpreted the noise as biological. We, therefore, believe that the increase in signal amplitude is not part of an escalation in response to aggressive motivation, condition, or other social interaction.

Although *C. venusta* is abundant throughout its large range and is presently in no danger of becoming threatened, other threatened, vocal fish species that do not have the abundance or range of *C. venusta* such as the Pygmy Sculpin (*Cottus paulus*), Lollipop Darter (*Etheostoma neoptermum*), 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. Worldwide anthropogenic noise levels are increasing (Hildebrand 2009; Slabbekoorn et al. 2010) and 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).

In conclusion, this study provides the first evidence for the Lombard effect in fishes, along with insight into the behavioral and acoustic response of a common freshwater fish species to elevated noise levels. Future studies should focus on determining how widespread the Lombard effect is in fishes and how environmental factors, such as natural ambient noise levels, and physiological factors, such as physical constraints on call amplitude play into the ability of fishes to compensate for anthropogenic noise levels.

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## REFERENCES

- Akamatsu T, Okumura T, Novarini N, Yan HY. 2002. Empirical refinements applicable to the recording of fish sounds in small tanks. *J Acoust Soc Am.* 112:3073–3082.
- Amorim MCP. 2006. Diversity of sound production in fish. In: Ladich F, Collin SP, Moller P, editors. *Communication in fishes*. Enfield (New Hampshire): Science Publishers. p. 71–105.
- Brumm H, Slabbekoorn H. 2005. Acoustic communication in noise. *Adv Study Behav.* 35:151–209.
- Brumm H, Voss K, Köllmer I, Todt D. 2004. Acoustic communication in noise: regulation of call characteristics in a New World monkey. *J Exp Biol.* 207:443–448.
- Brumm H, Zollinger SA. 2011. The evolution of the Lombard effect: 100 years of psychoacoustic research. *Behaviour.* 148:1173–1198.
- Delco EAJ. 1960. Sound discrimination by males of two cyprinid fishes. *Tex J Sci.* 12:48–54.
- Feng AS, Narins PM, Xu CH, Lin WY, Yu ZL, Qiu Q, Xu ZM, Shen JX. 2006. Ultrasonic communication in frogs. *Nature.* 440:333–336.
- Fernandez-Juricic ER, Poston R, DeCollibus K, Morgan T, Bastain B, Martin C, Jones K, Treminio R. 2005. Microhabitat selection and singing behavior patterns of male house finches (*Carpodacus mexicanus*) in urban parks in a heavily urbanized landscape in the western U.S. *Urban Habitats.* 3:49–69.
- Forrest TG, Miller GL, Zagar JR. 1993. Sound propagation in shallow water: implications for acoustic communication by aquatic animals. *Bioacoustics.* 4:259–270.
- Handegard NO, Michalsen K, Tjøstheim D. 2003. Avoidance behavior in cod, *Gadus morhua*, to a bottom trawling vessel. *Aquat Living Resour.* 16:265–270.
- Hawkins AD, Myrberg AA. 1983. Hearing and sound communication underwater. In: Lewis B, editor. *Bioacoustics: a comparative approach*. London: Academic Press. p. 493.
- Hildebrand HE. 2009. Anthropogenic and natural sources of ambient noise in the ocean. *Mar Ecol-Prog Ser.* 395:5–20.
- Holt DE, Johnston CE. 2013. Sound production and associated behaviours in blacktail shiner *Cyprinella venusta*: a comparison between field and lab. *Environ Biol Fish.* 1–13. doi:10.1007/s10641-013-0208-5
- Holt MM, Noren DP, Viers V, Emmons CK, Veirs S. 2008. Speaking up: killer whales (*Orcinus orca*) increase their call amplitude in response to vessel noise. *J Acoust Soc Am.* 125:EL27–EL32.
- Johnston CE, Page LM. 1992. The evolution of complex reproductive strategies in North American minnows (Cyprinidae). In: Mayden RL, editor. *Systematics, historical ecology and North American freshwater fishes*. Stanford (CA): Stanford University Press. p. 600–621.
- Ladich F, Collin SP, Moller P, Kapoor BG. 2006. *Communication in fishes*. Enfield (New Hampshire): Science Publishers.
- Ladich F, Myrberg AA. 2006. Agonistic behavior and acoustic communication. In: Ladich F, Collin SP, Moller P, editors. *Communication in fishes*. Enfield (New Hampshire): Science Publishers. p. 121–148.
- Lane H, Tranel B. 1971. The Lombard sign and the role of hearing in speech. *J Speech Hear Res.* 14:677–709.
- Lengagne T, Aubin T, Lauga J, Jouventin P. 1999. How do king penguins (*Aptenodytes patagonicus*) apply the mathematical theory of information to communicate in windy conditions. *Proc Biol Sci.* 266:1623–1628.
- Lopez PT, Narins PM, Lewis ER, Moore SW. 1988. Acoustically induced call modification in the white-lipped frog, *Leptodactylus albilabris*. *Anim Behav.* 36:1295–1308.
- Love EK, Bee MA. 2010. An experimental test of noise-dependent voice amplitude regulation in Cope's grey treefrog (*Hyla chrysoscelis*). *Anim Behav.* 80:509–515.
- Lugli M, Fine ML. 2003. Acoustic communication in two freshwater gobies: ambient noise and short-range propagation in shallow streams. *J Acoust Soc Am.* 114:512–521.
- Mann DA, Lobel PS. 1997. Propagation of damselfish (Pomacentridae) courtship sounds. *J Acoust Soc Am.* 101:3783–3791.
- Mann DA. 2006. Propagation of fish sounds. In: Ladich F, Collin SP, Moller P, editors. *Communication in fishes*. Enfield (New Hampshire): Science Publishers. p. 107–120.
- Myrberg AA Jr, Lugli M. 2006. Reproductive behavior and acoustical interactions. In: Ladich F, Collin SP, Moller P, editors. *Communication in fishes*. Enfield (New Hampshire): Science Publishers. p. 149–176.
- Officier CB. 1958. *Introduction to the theory of sound transmission*. New York: McGraw-Hill.
- Popper AN, Hastings MC. 2009. The effects of anthropogenic sources of sound on fishes. *J Fish Biol.* 75:455–489.
- Potash LM. 1972. Noise-induced changes in calls of the Japanese quail. *Psychonomic Sci.* 26:252–254.
- Phillips CT, Johnston CE. 2008. Sound production and associated behaviors in *Cyprinella galactura*. *Environ Biol Fish.* 82:265–275.
- Richardson WJ, Greene RC Jr, Malme CL, Thomson DH. 1998. *Marine mammals and noise*. San Diego (California): Academic Press.
- Rogers PH, Cox M. 1988. Underwater sound as a biological stimulus. In: Atema J, Fay RR, Popper AN, Tavolga WN, editors. *Sensory biology of aquatic animals*. New York: Springer. p. 131–149.
- Sara G, Dean JM, D'Amato D, Buscaino G, Oliveri A, Genovese S, Ferro S, Buffa G, Lo Martire M, Mazzola S. 2007. Effect of boat noise on the behavior of bluefin tuna *Thunnus thynnus* in the Mediterranean Sea. *Mar Ecol-Prog Ser.* 331:243–253.
- Scheifele PM, Andrew S, Cooper RA, Darre M, Musiek FE, Max L. 2005. Indication of a Lombard vocal response in the St. Lawrence River Beluga. *J Acoust Soc Am.* 117:1486–1492.
- Scholik AR, Yan HY. 2001. Effects of underwater noise on auditory sensitivity of a cyprinid fish. *Hear Res.* 152:17–24.

- Scholik AR, Yan HY. 2002. Effects of boat engine noise on the auditory sensitivity of the fathead minnow, *Pimephales promelas*. *Environ Biol Fish.* 63:203–209.
- Shannon CE, Weaver W. 1949. *The mathematical theory of communication*. Urbana: Illinois University Press.
- Slabbekoorn H, Bouton N, van Opzeeland I, Coers A, ten Cate C, Popper AN. 2010. A noisy spring: the impact of globally rising underwater sound levels on fish. *Trends Ecol Evol.* 25:419–427.
- Slabbekoorn H, Peet M. 2003. Ecology: birds sing at a higher pitch in urban noise. *Nature.* 424:267.
- Southall BL, Bowles AE, Ellison WT, Finneran JJ, Gentry RL, Greene CR Jr, Kastak D, Ketten DR, Miller JH, Nachtigall PE, et al. 2007. Marine mammal noise exposure criteria: initial scientific recommendations. *Aquat Mamm.* 33:411–414.
- Summers WV, Pisoni DB, Bernacki RH, Pedlow RI, Stokes MA. 1988. Effects of noise on speech production: acoustic and perceptual analyses. *J Acoust Soc Am.* 84:917–928.
- Sun JWC, Narins PM. 2005. Anthropogenic sounds differentially affect amphibian call rate. *Biol Conserv.* 121:419–427.
- Vabø R, Olsen K, Huse I. 2002. The effect of vessel avoidance of wintering Norwegian spring-spawning herring. *Fish Res.* 58:59–77.
- Warren PS, Katti M, Ermann M, Brazel A. 2006. Urban bioacoustics: it's not just noise. *Anim Behav.* 71:491–502.