

# The relationship between ambient noise and dominant frequency of vocalizations in two species of darters (Percidae: *Etheostoma*)

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**Abstract** Acoustic communication is used by many freshwater fishes for both courtship and aggressive interactions. Fish that live in shallow streams with fast moving water are often exposed to high levels of ambient noise, which could have an effect on the characteristics of their vocalizations. This paper investigates the aggressive vocalizations produced by two closely related species of darters. We measured ambient noise in their respective microhabitats, and found that both species produce aggressive drum vocalizations with a dominant frequency that corresponds to areas of low ambient noise. Previous studies have found quiet windows in shallow streams that have correlated to the dominant frequency of sounds made by fish that inhabit the streams. This is the first study, however, to show how the dominant frequencies of vocalizations of two closely related species occur in areas of low ambient noise within their respective microhabitats. This study supports other studies which show that ambient noise along with other physical features of the environment can affect vocal characteristics and behaviors in freshwater fish.

**Keywords** Sound production · Communication · Acoustic window · Fishes

## Introduction

Sound production is used by fishes in both aggressive (Johnston et al. 2008) and courtship interactions (Lindstrom and Lugli 2000; Amorim et al. 2003; Ladich 2004; De Jong et al. 2007; Phillips and Johnston 2008). However, in order for acoustic signals to play a role in communication, they must first be transmitted through the environment.

The physical structure of a fish's habitat or even microhabitat can have a tremendous influence on how acoustic signals travel, and whether or not they are successfully perceived. Water depth, for example, can drastically affect the propagation of an acoustic signal. Low frequency sounds propagate great distances in deep water such as the ocean (Au and Hastings 2008), but attenuate very rapidly in extremely shallow water (Bass and Clark 2003; Mann 2006). Other factors, such as substrate composition and ambient noise levels, can also affect the distance at which a sound can be effectively perceived (Forrest et al. 1993; Lugli and Fine 2003).

The amount and characteristics of ambient noise present in an environment may also effect the transmission of vocalizations. Ambient noise can be due to wind, moving water, bubbles and other

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disturbances caused by air from the surface being pushed under the water (Franz 1959; Prosperetti 1988). Microhabitats within one stream can vary greatly with regards to the ambient noise spectrum. Lugli and Fine (2003) found an area of low noise, around 100 Hz, in the ambient noise spectrum of shallow streams in northern Italy. They called this phenomenon a ‘quiet window’. *Padogobius martensii*, a freshwater goby that inhabits this stream, produces sounds for communicative purposes within the frequency range of this quiet window. By utilizing this quiet window, *P. martensii* is able to extend the active area of its acoustic signals. Quiet windows have also been found in other streams (Lugli and Fine 2007; Wysocki et al. 2007).

The objective of this study was to investigate the relationship between ambient noise and acoustic signal structure in two darter (Genus: *Etheostoma*) species with different microhabitat preferences. Darters are small benthic fishes that inhabit shallow freshwater streams. Darters of the subgenus *Catonotus* have been shown to be vocal during spawning, courtship, and aggression (Johnston and Johnson 2000). This paper compares the aggressive vocalizations of the Fringe Darter (*Etheostoma crossopterygum*), a member of the spottail group of *Catonotus* (Page et al. 1992), and of the Fantail Darter (*Etheostoma flabellare*), a member of the fantail group (Porterfield et al. 1999). Both darters are found in shallow freshwater streams, however, they prefer different microhabitats within these streams. *Etheostoma flabellare* prefer habitat in faster moving riffles, while *E. crossopterygum* is known to prefer quiet pools with little to no flow (Page 1983).

## Methods

### Recordings

Fish were collected in the field and brought back to the lab for recording trials. *Etheostoma crossopterygum* was collected from Cowpen Creek (Lauderdale County, Alabama, USA) (34.96273°N, -87.56116°W). *Etheostoma flabellare* was collected from Middle Cypress Creek (Lauderdale County, Alabama, USA) (34.943439°N, -87.755723°W). Collections were made with a 10 foot seine net. Species were kept separate in the lab. When fish were not being recorded they were kept in an aerated, 76 l tank with multiple shelters and 3–4 fish per tank. Recordings were made

in a 76 l tank with a sandy substrate bottom with one ceramic tile for a shelter. Reproductively active males of both species were identified for recording trials by the development of vertical stripes along their bodies, dark fins and dark heads (Page et al. 1992; Page 1983) as well as their aggressive behavior towards other males. Single aggressive males were isolated in a recording tank in the evening and left overnight to acclimate (approximately 12 h). A second reproductive male and a female were then added to the tank at the same time. Trials were conducted in 30 min intervals which began when the fish first interacted (within 5 min of introduction). All recording trials were conducted within 3 days of the fish being brought into the lab.

Since the majority of *Catonotus* sound production occurs in and directly around the nest, the hydrophone was placed directly outside of the shelter. Recordings were made with an omni-directional Bruel and Kjaer hydrophone (Model 8103: sensitivity -211 dB re 1 V/ $\mu$ Pa; frequency response 0.1 Hz–180 kHz) paired with a Bruel and Kjaer charge amplifier, model 2635. Signals were run through a Sony model TC-D5 Pro II stereo cassette recorder, which allowed the observer to listen to the trials, and desktop PC (Dell Optiplex GX754), where signals were recorded and stored for later analysis using Canary version 1.2 or Raven PC 1.2.1 (Laboratory of Ornithology, Cornell University, Ithaca, NY).

Signal analysis consisted of comparing dominant frequency, call duration and the number of harmonic bands between the two species. The call duration was determined using the waveform, the dominant frequency were determined from the generated power spectra (Hanning window, FFT: 10000 samples, bin resolution: 2.7 Hz) and the number of bands was counted using the spectrogram (Hanning window, FFT length: 2466 samples, bin resolution: 10.8 Hz). These sound components were averaged for each individual and these values were used for descriptive statistics and for a multivariate analysis of variance. All statistics were done using SPSS software package (SPSS version 13.0; SPSS inc. Chicago IL).

### Calculating sound pressure levels

Since the sound pressure levels associated with Raven and Canary are not representative of decibels re 1  $\mu$ Pa, the levels obtained from the power spectrum were calibrated using a coefficient which took into account the gain of every piece of equipment in the

recording chain. An oscilloscope was used to obtain voltage values for pure tones. These voltage values were used to calculate decibel levels re 1  $\mu\text{Pa}$ . These ‘true dB levels’ were then compared to measurements made in Raven of the same pure tones, and the power spectrum values were adjusted to reflect the true decibel level (re 1  $\mu\text{Pa}$ ). These adjusted decibel values were then found for calls where the fish was directly next to the hydrophone ( $n=8$  for *E. crossopterus* and  $n=2$  for *E. flabellare*). The average sound pressure level found for *E. crossopterus* was 79.0 dB re 1  $\mu\text{Pa}$  and 87.4 dB re 1  $\mu\text{Pa}$  for *E. flabellare*. This is most likely a conservative estimate of the sound pressure level because although the fish were very close to the hydrophone there was still some sound loss. The fish were also within the calculated attenuation distance of the tank which according to Akamatsu et al. (2002) should result in a signal whose power spectrum that is minimally distorted.

#### Ambient noise measurements

Ambient noise recordings were done in two separate streams outside of Florence, Alabama in April 2010. The first set of recordings was done in Cypress Creek (34.949047 N, -87.694780 W) which is a previous collection site for *E. flabellare*. The second set of recordings was done in Cowpen Creek (34.966163°N, -87.55775°W) which is a previous collection site for *E. crossopterus*. Field ambient noise recordings were performed with a single, preamplified hydrophone (HTI-96-MIN, sensitivity -164.4 re: 1 V/ $\mu\text{Pa}$ , frequency response: 0.002–30 kHz), which fed into a Sony model TC-D5 Pro II stereo cassette recorder. Sounds were later digitized onto a PC (Dell Inspiron 6000), where noise recordings were captured and stored at a sampling rate of 44,100 Hz. Input was acquired onto the computer using Raven 1.2.1 and stored on the hard drive.

Recording sites at Cypress Creek had a substrate consisting of medium to large cobblestone and sand. The first recording site was a run located 3.5 m upstream of a small riffle. The flow in the run was moderate (0.19  $\text{ms}^{-1}$ ) and the water depth at the recording site was 23 cm. The hydrophone was placed at the bottom of the water column and behind a cobble stone. Placement of the hydrophone within a given area was always in a place where flow was low in order to minimize hydrodynamic noises. This

placement of the hydrophone is also biologically relevant because darters are benthic and produce sounds from under and around nesting cavities such as the rocks where we recorded (Johnston and Johnson 2000). Ambient noise was recorded for a period of 1 min. The second location was in the small riffle mentioned above. This location had a water depth of 6 cm, and a flow rate of 0.54  $\text{ms}^{-1}$ . Ambient noise was again recorded for 1 min. The third area was a pool located adjacent to the stretch of stream containing the other two recording sites. The pool had a flow rate of 0.01  $\text{ms}^{-1}$ , and a depth of 49 cm.

Recording sites in Cowpen Creek had a substrate consisting mostly of bedrock, some sand and a few large stones. These sites were chosen to include the habitat requirements of *E. crossopterus* and to be comparable to the Cypress Creek site so again data was collected from a run, a riffle and a pool. The run above the riffle had a depth of 25 cm and a flow rate of 0.34  $\text{ms}^{-1}$ . The riffle has a depth of 12 cm with a flow of 0.63  $\text{ms}^{-1}$ . Finally the pool habitat, which is where *E. crossopterus* persists, had a depth of 30 cm and a flow of 0.14  $\text{ms}^{-1}$ .

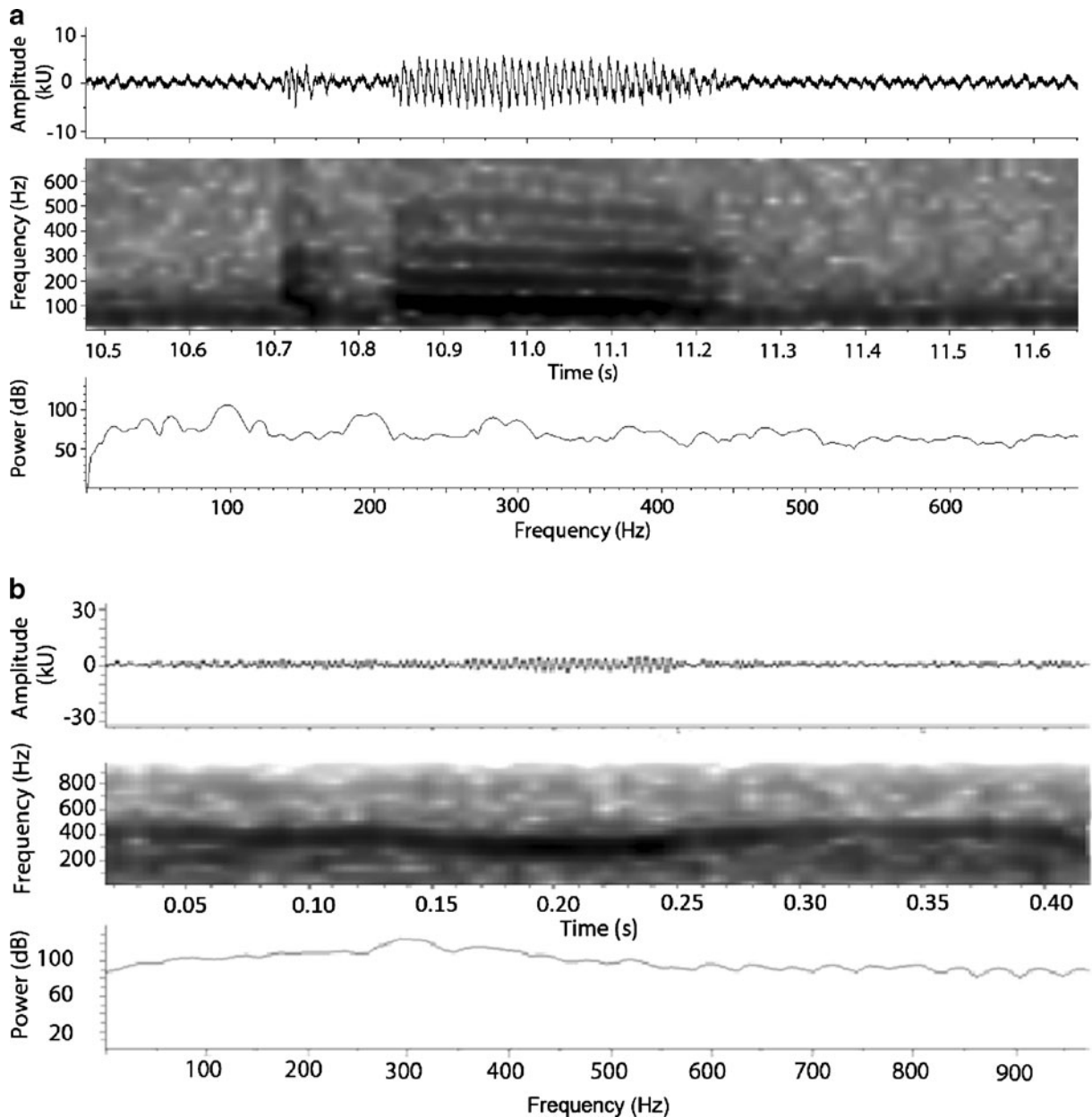
Ambient noise was analyzed using protocol adapted from Lugli and Fine (2003). Three 700 ms segments were randomly selected from each 1 min recording of ambient noise in each location. The pressure density spectra level for each 700 ms segment was computed using the power spectrum function of Raven 1.2.1 (Hanning window, FFT length: 16384 samples, bin resolution: 2.7 Hz). Decibel levels were calibrated to represent absolute levels (dB re 1  $\mu\text{Pa}$ ) by adjusting the power spectrum according to a calibration coefficient that took into consideration the gain of every piece of equipment in the recording chain. From each pressure density spectra, the sound power in 2.7 Hz bands of noise was determined at 30 Hz intervals between 30 and 1501 Hz. Decibel levels from the three 700 ms noise segments were then averaged to generate a single ambient noise pressure density spectrum for each location. Standard error at each 30 Hz interval was measured in order to obtain an estimate of noise variation at each location.

## Results

Both *E. crossopterus* and *E. flabellare* produced vocalizations that included drums (tonal components),

and knocks (pulsed components) during aggression similar to those previously published (Johnston and Johnson 2000). Since very few knocks were recorded for *E. flabellare* ( $n < 5$ ) only the tonal drums were compared for this study. In total, 108 aggressive vocalizations (97 *E. crossopterus* and 11 *E. flabellare*) from 12 males (9 *E. crossopterus* and 3 *E.*

*flabellare*) were included in the analysis. The mean dominant frequency of the *E. crossopterus* aggressive vocalizations was found to be highly variable and ranged from 78 Hz to 496 Hz (Mean = 151 Hz). These vocalizations contained between 2 and 10 harmonics (Mean = 5.6) (Fig. 1a). Although most of the energy of the call was contained in the lower frequencies, the



**Fig. 1** The aggressive drum signal of **a** *Etheostoma crossopterus* and **b** *Etheostoma flabellare* (Hanning window, FFT length: 2466 samples, bin resolution 10.8 Hz). The amplitude is

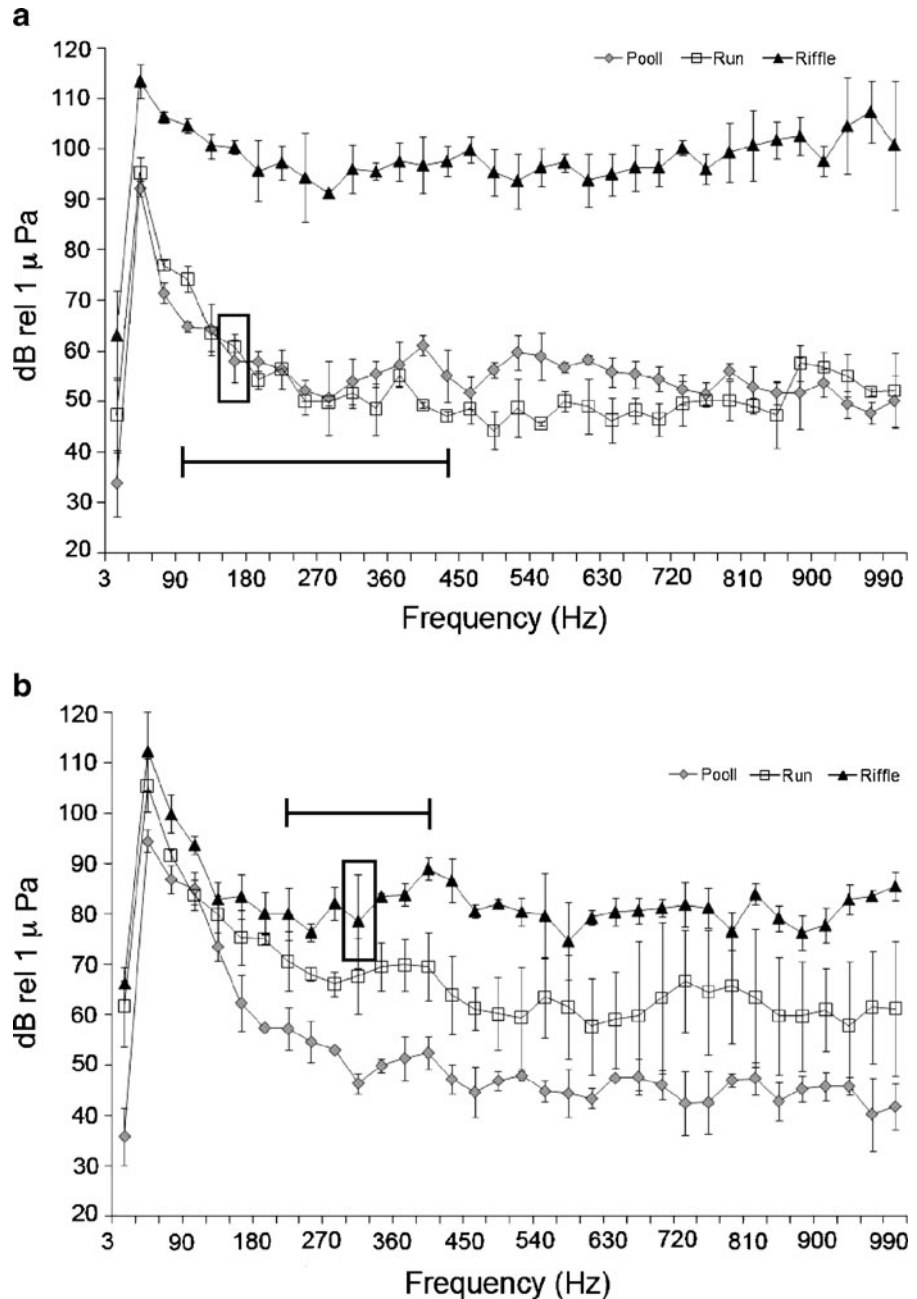
shown in kU which is an uncalibrated arbitrary unit used by the software. Power spectrum levels also represent uncalibrated values

harmonic components of these calls were sometimes present beyond 1 kHz. The mean duration of drums made by *E. crossopterum* was 400 ms. The dominant frequency of *E. flabellare* aggressive calls was 308 Hz (172 Hz–374 Hz) and the mean duration was 400 ms (Fig. 1b). Analysis of variance found a significant difference between species in dominant frequency ( $df=1$ ,  $F=15.404$ ,  $p=0.003$ ), and number of harmonic components ( $df=1$ ,  $F=133.016$ ,  $p<0.001$ ). However

there was no significant difference between species concerning duration of calls ( $df=1$ ,  $F=0.774$ ,  $p=0.40$ ).

The loudest microhabitat in both streams was the fast moving riffle. In Cowpen Creek (Fig. 2a) the loudest part of the ambient noise spectrum of the riffle was around 30 Hz. The ambient noise of the pool, where *E. crossopterum* persists, formed a quiet area around 90 Hz to about 390 Hz. Almost the entire range of the dominant frequency of the *E. cross-*

**Fig. 2** Ambient noise pressure density spectra from three different microhabitats (Hanning window, 16384 FFT samples, bin resolution 2.7 Hz). **a** The ambient noise spectrum of microhabitats in Cowpen Creek, Florence Alabama, the collection site of *Etheostoma crossopterum*. **b** Microhabitats in Cypress Creek, Florence Alabama, the collection site for *Etheostoma flabellare*. In this creek *Etheostoma flabellare* are found in the fast moving riffle habitat. These fish prefer the quiet pool habitat. Run: moderate flow above a small riffle. Pool: A calm pool away from the waterfall with very little to no flowing water. The range of the dominant frequency of the vocalizations for each respective darter is represented by the black line and the mean dominant frequency is indicated by the black box



*opterum* drums falls within this area. The pool habitat then remained consistently below 70 db rel 1  $\mu$ Pa through 1000 Hz.

In the riffle of Cypress Creek, where *E. flabellare* is found, the loudest part of the ambient noise spectrum was around 38 Hz (Fig. 2b). The dominant frequency of the drum vocalization of *E. flabellare* falls into a quiet window of ambient noise between 150 Hz and 330 Hz. The quietest level of ambient noise at 239 Hz is included in the range of the dominant frequency of these vocalizations. In Cypress Creek the loudest level of ambient noise for both the run and the quiet pool is also at 30 Hz. The ambient noise at both of these recording sites stays consistently below that of the riffle. Also, the ambient noise of the pool is consistently lower than that of the run.

## Discussion

Both *E. crossopterum* and *E. flabellare* produce drum vocalizations during aggressive encounters. Sound production has been previously documented in *E. crossopterum* (Johnston and Johnson 2000) but this study is the first to report sound production in *E. flabellare*. There were significant differences between the aggressive vocalizations of these two species with respect to dominant frequency, and the number of harmonics. These inter-specific variances appear to be related to the ambient noise spectra of their environment. This is similar to what has been shown for freshwater gobies (Lugli and Fine 2003).

The dominant frequency of *E. flabellare* drum vocalizations (307 Hz) fall into a quiet window of ambient noise in the riffles of Cypress Creek (150 Hz–330 Hz). Producing vocalizations within this quiet window makes them less susceptible to masking from noise. *Etheostoma crossopterum* produces vocalizations that range from 78 Hz to 426 Hz with a mean of 151 Hz however there is no clear quiet window that corresponds with this dominant frequency. This may be because *E. crossopterum* live in an overall much quieter environment, where the threat of ambient noise masking is much less. Therefore a dramatic quiet window may not be necessary for effective communication. In this microhabitat the level of ambient noise at all frequencies greater than 90 Hz is below 78 dB re 1  $\mu$ Pa which was the calculated sound level of *E. crossopterum* drums.

These results support the hypothesis that both species produce vocalization with dominant frequencies that work within the acoustic constraints of their respective microhabitats.

These two species of darters also differ significantly in the number of harmonic components in their drum vocalizations. *Etheostoma flabellare* consistently has only one harmonic, while there is an average of 5.6 harmonic components found in *E. crossopterum* drums. These multiple harmonic bands extend into the higher frequencies where the sound level of ambient noise in their environment is consistently low. In contrast, *E. flabellare* lives in a much noisier environment, especially in the higher frequencies (above 700 Hz). This noise is attributed to fast moving water and bubbles formed by the breaking of the air-water boundary (Prosperetti 1988). Therefore, it may not be beneficial for *E. flabellare* to make calls with harmonics that spread into the higher frequencies where they could easily be masked.

Another interesting aspect of this data is that in the riffles of Cowpen Creek, where *E. flabellare* do not persist, the quiet window is not as pronounced as in Cypress Creek, where *E. flabellare* does occur. If *E. flabellare* was found in this creek, their vocalizations would most likely be masked, because the ambient noise is consistently above the calculated sound pressure level of their vocalizations (87.4 db rel 1  $\mu$ Pa).

Both of these species of darters have vocalizations with relatively low dominant frequencies (below 500 Hz). These fishes do not have a swim bladder, and have no known hearing specializations. Research done on hearing abilities of closely related fish in the family Percidae (*Perca fluviatilis*; Amoser and Ladich 2005) and within this genus (Speares and Johnston, unpubl. data) indicate that these fishes are most sensitive to hearing lower frequencies. This increased sensitivity to lower frequencies may also assist in successfully using their signals in a noisy environment.

This paper differs from other work done on ambient noise quiet windows, because we did not record vocalizations in the field. *Catnotus* darters readily make vocalizations and spawn in the lab and are difficult to observe in the field. Therefore, we worked with the fishes in a laboratory setting so that acoustic contexts could be observed. We assume that successful spawning is a result of normal behavior similar to what would be seen and heard in the field.

Since these fish produce low frequency vocalizations and we are able keep the hydrophone in close proximity to the fish (within 2 cm), tank distortions should be minimal (Akamatsu et al. 2002). Recording in the lab also allows us to avoid many of the difficulties (finding active nests, accounting for weather, and biotic ambient noises) and inconsistencies inherent with recording in the field.

Although this research does suggest that vocalization characteristics are related to the ambient noise of a fish's microhabitat, there are other factors which could shape the observed variation in fundamental frequency. Both species in this study are from the genus *Catonotus*. *Etheostoma crossopterus*, which has been included in previous studies (along with *Etheostoma nigripinne*) (Johnston and Johnson 2000) is a representative of the spottail the group, while *E. flabellare* belongs to the fantail group of this clade. *Etheostoma crossopterus* vocalizations are more similar with regards to frequency to more closely related species, such as *E. nigripinne* and *Etheostoma oophylax* (Speares and Johnston, unpubl. data) than to *E. flabellare*. *Etheostoma flabellare* are also generally smaller (max size 70 mm, Page 1983) than *E. crossopterus* (83.1 mm; Page et al. 1992). Size is also a characteristic that has been shown to correspond with frequency in many fish species (Lobel and Mann 1995; De Jong et al. 2007).

There are obvious benefits to making vocalizations which are subjected to less environmental masking. Less ambient noise means that the sounds may be perceived at greater distances and are less likely to be distorted. This may be especially critical in shallow streams (<1 m deep), where sounds propagate very short distances (Forrest et al. 1993; Mann 2006). This paper supports other works which suggest that fish in shallow, turbid water have had their vocal behaviors shaped by their environment (Lugli and Fine 2003; Lugli et al. 2003; Lugli and Fine 2007).

Many terrestrial animals have also been shown to adapt their vocalizations to avoid being masked by ambient or anthropogenic noises. Some species change their rate of calling (Sun and Narins 2005) or their timing relative to other noises (Grafe 1996) to prevent masking. Others, like *E. flabellare*, appear to have adapted the frequency of their call in order to avoid masking. Another extreme example of this can be seen in frogs that live in very noisy environments such as waterfalls. These anurans make calls that

extend into the ultrasonic range to avoid being masked by their environment (Feng et al. 2006).

Quiet windows that correspond with the dominant frequency of the vocalizations of the inhabitant fish have been described to occur in other localities (Lugli and Fine 2003; Lugli and Fine 2007; Wysocki et al. 2007). This study is the first however, to correlate the dominant frequency of vocalizations of two separate species with the ambient noise spectrum of two different microhabitats. This finding reiterates the importance that the environment plays in shaping the vocal behavior of fish even at the scale of microenvironments.

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