2020-02-27

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Blair, J

<http://hdl.handle.net/10026.1/15419>

10.1080/01650424.2020.1726963

Aquatic Insects

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RESEARCH ARTICLE

**The call of the squeak beetle: bioacoustics of *Hygrobia hermanni*
(Fabricius, 1775) revisited (Coleoptera: Hygrobiidae)**

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Astract

Hygrobiidae, or squeak beetles, originated in the Triassic-Jurassic and exhibit a relictual distribution in the Palaearctic, Oriental and Australasian regions. Hygrobiids are well known for their sound-producing abilities, although studies of their bioacoustics remain limited. Here we describe sound producing organs and bioacoustics of the Palaearctic *Hygrobia hermanni* (Fabricius, 1775). Plectra and *pars stridens* were examined in both sexes, and sound characteristics analysed. Despite small differences between male and female last abdominal ventrites, plectra were identical. *Pars stridens*, however, differed subtly, tooth ridges being wider in females. Calls of both sexes were harmonic, with peak frequency at 6.1 kHz, and secondary peak at 10.9 kHz; males exhibiting longer inter-chirp intervals. Calls changed with time in the laboratory, this possibly condition-related effect being more apparent in males. The differences found between sexes suggest that sound production in squeak beetles may function in intraspecific communication, in addition to being an antipredator device.

KEYWORDS: stridulation; *pars stridens*; bioacoustics; *Hygrobia hermanni*; squeak beetle

Introduction

Sound production is widespread in insects, being found across almost all orders (Alexander 1963; Bailey 1991) and frequently resulting from stridulation. This typically involves one structure (the scraper or plectrum) being moved across a finely-ridged surface (the file or *pars stridens*) or vice versa, resulting in sound output as it does so (Arrow 1942). Structures involved in sound production clearly relate to the characteristics of the sound produced (Endler 1992; Casaretto, Picciulin, and Hawkins 2016), although not all studies of insect bioacoustics link these two aspects. Stridulatory files are present on a wide range of body surfaces, including wings, legs and elytra, and stridulation is used by insects in a variety of contexts including defence, competition and reproduction (Alexander 1967; Masters 1980; Lyal and King 1996; Mason 1996; Smith and Harper 2003). In some cases, it may serve to repel predators by initiating a startle response – so called disturbance stridulation. Masters (1979) noted that wolf spiders showed greater attack persistence on silenced compared to phonic individuals of *Tropisternus* Solier, 1834 (Hydrophilidae). Masters (1979) also compared mutillid wasps that were allowed to stridulate with muted individuals, noting that predators persisted for longer and killed more wasps when stridulation was prevented, all suggesting that sound production acts as a deterrent (Haskell 1961). Similarly, Bauer (1976) found that the carabid *Elaphrus cupreus* Duftschmidt, 1812 was more persistently attacked and frequently eaten by predators if its stridulatory apparatus had been removed (see also Thiele 1977).

For intraspecific interactions, specifically sexual ones, stridulation may be critical in mate choice. Differences in the calls between sexes may serve to distinguish gender, saving time and energy pursuing individuals of the wrong sex, as well as encoding information about the quality of an individual (Simmons and Ritchie 1996). Analysing the characteristics of stridulation can provide insights on the function of sound production, since an interspecific function, e.g., defence, will have not been selected for sexually (Hall, Howard, Smith, and Mason 2015).

Investigations of insect bioacoustics to date have mostly been conducted on terrestrial taxa, particularly orthopterans and cicadas (Drosopoulos and Claridge 2005), despite the fact that the relatively low visibilities in many freshwaters may select for the use of sound in information transfer within and between species. Most studies of freshwater insect bioacoustics deal with sound production by Corixidae (e.g., Janssen 1973; Aiken 1982, 1985; Prager and Streng 1982; Theiss 1982; Bailey 1983) and include what is arguably the loudest animal by

body size, *Micronecta scholtzi* (Fieber, 1860) (Sueur, Mackie, and Windmill 2011). Work on the acoustic behaviour of other freshwater taxa often deals with terrestrial adults (e.g., Tierno de Figueroa, Luzón-Ortega, and López-Rodríguez 2019), and studies of water beetles are very limited, although there is evidence that a number of taxa produce sounds in both the Adephaga (Smith 1973; Miller and Bergsten 2014; Greenhalgh 2018) and Polyphaga (e.g., Balfour-Browne 1958).

The Hygrobiidae (Coleoptera: Adephaga) are commonly known as squeak beetles, due to the audible sound these insects produce when captured, by rapid back-forth movement of the last abdominal ventrite against *pars stridens* on the underside of the elytral apices (Balfour-Browne 1922). Squeak beetles are a relictual family of water beetles, with six extant species, one each in the Palaearctic and Oriental regions and four in Australia, which apparently originated in the Upper Triassic to Middle Jurassic around 184 Ma (Hawlitschek, Hendrich, and Balke 2012). The best-known species by far is *Hygrobia hermanni* (Fabricius, 1775) (Figure 1a), distributed widely in the western Palaearctic from Scotland to North Africa, east to the Ukraine (Dettner 1997). Balfour-Browne (1922) stated that *H. hermanni* ‘used to be sold in St Martin’s Lane, London, under the name of the “Squeak beetle”, owing to its being able to make a loud, strident noise...’, and indeed the insect remains well-known to non-entomologists. Despite being one of the best-known sound producing beetles in Europe, studies of the bioacoustics of *H. hermanni* remain limited. Balfour-Browne (1922) provides a simple description of the *pars stridens*, and Beutel (1986) includes scanning electron micrographs indicating the location of this file and a waveform, but without any quantitative analyses of sound characteristics. These observations are repeated by Dettner (1997, 2016) in his accounts of the family. Sound production by *Hygrobia* is most often associated with handling (e.g., Balfour-Browne 1922) and is considered to primarily constitute a disturbance stridulation or startle response, which may serve to repel predators including fish, known to predate adult aquatic Adephaga (e.g., Åbjörnsson, Wagner, Axelsson, Bjerselius, and Olsén 1997), although this assertion has never been tested experimentally in *Hygrobia*. Whilst *H. hermanni* possesses both pygidial and prothoracic glands, the antimicrobial secretions of the former are not involved in defence, and the role of secretions from the latter remains unknown (Dettner 2019). Balfour-Browne (1922) noted in captivity that ‘if one individual tried to seize a piece of worm upon which another was feeding, the latter “squeaked”, the squeak in this case presumably being equivalent to the growl of a dog with a bone’, an observation which suggests that sound production may also serve interspecific functions. One of us (DTB) has observed similar

behaviour in captive *H. hermanni*, as well as hearing beetles squeaking repeatedly whilst half-buried head down in tank sediment, in the absence of food items. Whether *Hygrobia* possess a tympanum remains unknown (see Yager 1999), but such observations raise the possibility that stridulation in *H. hermanni* also functions in intraspecific communication, conveying information about the sender to the recipient (Ewing 1989; Bradbury, and Vehrencamp 1998).

Here we explore the sound producing organs and bioacoustics of *H. hermanni* in detail for the first time, characterising the plectrum, *pars stridens* and temporal and spectral properties of sounds produced by captive beetles. In addition, we compare the sound producing structures and calls of males and females, to determine whether the sexes differ in their bioacoustic organs and calls, something which may be anticipated if sound production in these animals also serves an intraspecific function.

Material and methods

Specimen collection and maintenance

Specimens of *Hygrobia hermanni* were collected using a D-framed pond net with 1 mm mesh in November 2016. Post-teneral adults were netted from a muddy, semi-permanent pond frequented by livestock near Yelverton, Devon, UK (50°31'04.42"N, 4°02'12.37"W, 369 m). Beetles used in bioacoustic studies were free from visible peritrich ciliate infection (which has the potential to affect stridulatory behaviour) and were maintained in six litre tanks of artificial pond water (APW; pH ~7.3–7.5) at $15 \pm 1^\circ\text{C}$ with a 12 h light/dark regime. They were fed *ad libitum* on a diet of chironomid larvae (Cuppen 2000). Sexes were distinguished by eye on the basis of fore-tarsal morphology (Dettner 1997).

Morphology of sound producing structures

The last abdominal ventrites and right elytra were removed from five individuals of each sex, preserved in 70% ethanol, to study the plectrum on the ventrite apex and *pars stridens* on the interior elytral face. Both elytra and ventrites were imaged with a Canon EOS 5D camera attached to a Leica Z6 Apo microscope, fitted with a 2X objective lens. Specimens were illuminated using a Leica LED5000 HDI dome illuminator to avoid shadow. Image stacks were produced by hand, and combined using Zerene Stacker software (www.zereneystems.com). Elytra were mounted on metal stubs using double-sided carbon conducting tape and air dried at $35 \pm 1^\circ\text{C}$ for 48 hours. An Emitech K550 sputter coater was then used to coat with gold, prior to imaging using a JEOL JSM-6610LV scanning electron microscope. Images of the *pars*

stridens for each individual were obtained at magnifications of 90X and 350X. *Pars stridens* measurements were made from 350X images using the ‘straight’ dimension tool, calibrated to the scale of each image, in ImageJ (Schneider, Rasband, and Eliceiri 2012). The widths of five teeth, spacings, ridges and furrows (see Figure 1e for details), were measured at three locations, spaced evenly across the centre of the *pars stridens*. Data met assumptions of homogeneity and normality and a series of *t*-tests were used to explore possible differences in *pars stridens* morphology between sexes. Statistical analyses were conducted in R studio version 0.99.491 (R Core Team 2014).

Bioacoustic recording and set up

Recordings took place underwater at 15°C in a glass aquarium (30 x 20 x 20 cm) filled to 16 cm with APW and a 1 cm layer of fine aquarium sand to act as acoustic buffing. Water temperature was within the range commonly observed in the field when beetles were active (DTB, personal observations). The tank was placed on a trolley in the middle of the room to avoid vibration from the walls, and was mounted on a 4 cm thick expanded polystyrene foam mat. The theoretical attenuation distance was calculated from Akamatsu, Okumura, Novarini, and Yan (2002; equations 2, 7 and 11), in order to help minimise the effects of reverberation, distortion and internal reflection on recordings. Fine watchmakers forceps were used to hold beetles and induce stridulation. Individual beetles were always grasped by their right mid-leg, to produce a consistent disturbance effect and positioning relative to the hydrophone. Forceps were fixed in position in the tank using a bench vice, minimising manual disturbance. Recordings were taken during daytime, at a depth of 9 cm, 8 cm away from an HTI-96-Min hydrophone (with inbuilt preamplifier, manufacturer-calibrated sensitivity -165 dB re 1 V μ PA; frequency range 0.002–30 kHz, High Tech Inc., Gulfport, MS, USA) and Linear Sony PCM-M10 recorder (96 kHz sampling rate, Sony Corporation, Tokyo, Japan; recording level calibrated using pure sine wave signals from a function generator with measured voltage, recorded in line on an oscilloscope). Sounds were recorded from eleven individuals of each sex, calls from each individual being recorded three times at 7–10 day intervals (‘time of recording’ in analyses).

Bioacoustic analyses

Beetle song terminology follows Alexander (1967). Call parameters were documented in Avisoft SAS Lab Pro version 5.2.05 (Specht 2004). The third call in each beetle recording was selected for acoustic analysis since the first calls in a recording were often incomplete or highly

variable (see Lengagne, Voituron, and Gomez 2017). Temporal parameters were measured manually from oscillograms and included duration of first chirp, duration of second chirp, duration of inter-chirp interval and the total duration of a call (see Figure 2b). Peak frequency (the frequency of maximum power) was also recorded, taken as the maximum amplitude of elements within a spectrogram, using default parameters in Avisoft.

Statistical analyses were carried out in R studio version 0.99.491 (R Core Team 2014). *lme4* (Bates, Mächler, Bolker, and Walker 2015) was used to generate linear mixed effect models of the effect of sex and time of recording on a given chirp component (see Figure 2b). In the model, sex and time of recording (and their interaction) were the fixed effects, with the intercept as the random effect. Where visual inspections of residual plots were unsatisfactory for normality and homoscedasticity, data were Log₁₀ transformed. Spectral parameters however were unable to satisfy these assumptions despite transformation. *lmerTest* (Kuznetsova, Brockhoff, and Bojesen Christensen 2015) was then used to run an Analysis of Variance (with Kenward-Roger approximation for degrees of freedom) on the linear mixed effects models.

Results

Morphology of sound producing structures

The *pars stridens* of *H. hermanni* is located on the underside of the elytral apex, being a raised structure containing many small, flattened teeth (Figure 1b–c, f–g). In both sexes, the teeth possess a double-ridge like structure (Figure 1g); each tooth consisting of two ridges separated by a shallow furrow. Stridulatory teeth are struck using the plectrum on the last abdominal ventrite, formed from the flattened apex of the ventrite (Figure 1d–e). Despite differences in ventrite morphology between males and females, plectra appeared identical in their morphologies. The last ventrite is moved rapidly back and forth in an apical to basal direction during sound production in live animals.

Inter-tooth spacing increases apically down the *pars stridens* in both sexes (Figure 1c–d), but is relatively constant across the central portion of the file (Figure 1c–e). Ridges were the only structures whose size apparently differed between sexes. In females, these are thicker

($t = 2.8174$, $P = 0.023$); mean width $8.43 (\pm 0.26 \text{ SE}) \mu\text{m}$ compared to $7.34 (\pm 0.29 \text{ SE}) \mu\text{m}$ in males. The mean width of individual teeth was $29.93 (\pm 0.47 \text{ SE}) \mu\text{m}$ and $28.49 (\pm 1.06 \text{ SE}) \mu\text{m}$ in males and females respectively, but these did not differ significantly. The mean length of spacing between teeth was $12.10 (\pm 0.98 \text{ SE}) \mu\text{m}$ in males and $10.48 (\pm 0.38 \text{ SE}) \mu\text{m}$ in females, but again did not differ significantly between sexes. The mean widths of furrows between the two ridges of individual teeth were $14.69 (\pm 0.48 \text{ SE}) \mu\text{m}$ in males and $13.78 (\pm 0.72 \text{ SE}) \mu\text{m}$ in females, again not significantly different between the sexes.

Bioacoustics

The call of *Hygrobia hermanni* is biphasic, consisting of two chirps each made up of a series of pulses (Figure 2). These chirps correspond with the forward and backward stroke of the 7th abdominal ventrite against the pars stridens, respectively (Dettner 1997). Chirp 1 in both sexes shows amplitude modulation which appeared slightly stronger in females. The waveform also reveals that chirp 1 has a higher amplitude and is more distinctly pulsed than chirp 2 (Figure 2). *Hygrobia hermanni* calls show a broad frequency spectrum, but with some harmonious structure; containing a peak frequency of just over 6 kHz (Figure 2a) in both chirp 1 and chirp 2, this not differing between sexes. A second dominant peak occurs in both sexes at approximately 10.9 kHz. Some signal was also visible at higher frequencies, particularly in the first chirp (up to ca. 45 kHz), outside the flat response range of the hydrophone.

The mean duration of a complete call in *H. hermanni* was $0.746 (\pm 0.038 \text{ SE}) \text{ s}$ in males and $0.656 (\pm 0.035 \text{ SE}) \text{ s}$ in females, these durations not differing significantly (Table 1). Temporal variability was observed across recordings, however, males having longer calls during their final recordings, whereas females were more consistent (Table 1; Figure 3). Across recordings, the mean duration of chirp 1 was $0.338 (\pm 0.014 \text{ SE}) \text{ s}$ and $0.322 (\pm 0.015 \text{ SE}) \text{ s}$ in males and females respectively, but these timings did not differ significantly (Table 1). However, within males, chirp duration did differ significantly across time of recording, being longer during the last recording interval (Table 1; Figure 3). Chirp 2 was shorter than chirp 1 (Figure 2), lasting $0.250 (\pm 0.018 \text{ SE}) \text{ s}$ in males and $0.209 (\pm 0.018 \text{ SE}) \text{ s}$ in females, although not significantly different between sexes or recordings.

Interval duration differed significantly between sexes, lasting $0.158 (\pm 0.017 \text{ SE}) \text{ s}$ in males and $0.125 (\pm 0.011 \text{ SE}) \text{ s}$ in females (Table 1). Furthermore, interval duration differed over time in both sexes, increasing from first to last recording (Table 1). In females, interval

duration increased from 0.112 (± 0.016 SE) s to 0.141 (± 0.020 SE) s whilst in males it more than doubled, from 0.090 (± 0.017 SE) s to 0.233 (± 0.037 SE) s (Figure 3).

Discussion

Our study details the bioacoustic apparatus and sound production of squeak beetles. We provide quantitative analyses of the *pars stridens* and the spectral and temporal characteristics of *H. hermanni* stridulation for the first time, including an explicit attempt to determine whether the sexes differ in their sound producing apparatus and behaviour. Interestingly, both spectral and temporal parameters reported here contrast with those described by Beutel (1986), which is the only other study of *Hygrobia* bioacoustics to date. Beutel (1986) stated that *H. hermanni* exhibited a peak frequency of 0.5 kHz in both chirps of a call, with a second peak at around 1.5 kHz; some 12 times lower than the values obtained here. Furthermore, total call durations in our study were some 1.3–1.5 x longer than the *ca* 0.5 s of Beutel (1986). Accurate comparisons between these investigations are difficult, since Beutel (1986) provides few details of the experimental set-up, although the use of a ‘Brüel & Kjaer Hydrophone Type 8101’ suggests that recordings were also undertaken in water. It is possible that the recording devices used by Beutel under sampled higher frequency parts of the call (see, e.g., Robillard, ter Hofstede, Olivé and Vicente 2015), or that the tank setup influenced results. Alternatively, it is not impossible that there are regional differences in the call of this species. Consistent in both studies is the occurrence of two peak frequencies within the call spectrum (see Figure 2). Our observations of the stridulatory apparatus may explain why these two peaks occur. The *pars stridens* of *H. hermanni* possesses teeth with two ridges (see Figure 1), which essentially double the number of effective teeth between a break. By doing so, these structures potentially act as a frequency multiplier, which could account for the high frequency peak (10.9 kHz) of the call spectrum. The lower dominant peak frequency (6.1 kHz) would in turn be produced from the striking of the teeth as a whole, analogous to the situation described in the cricket *Eneoptera guyanensis* Chopard, 1931, which also possesses dual peak frequencies and a double toothed *pars stridens* (Robillard and Desutters-Grandcolas 2011).

Our results are consistent with the possibility that stridulation in *H. hermanni* serves additional functions besides defence. The call spectrum, with strong peak frequencies, is consistent with a use in sexual advertisement (Gerhardt and Huber 2002; Guerra and Morris 2002; Forrest, Lajoie and Cuswick 2006), although in many such cases peak frequency differs between sexes (e.g., Hyder and Oseto 1989; Gray 1997), which was not the case here. This is

surprising, since male and female *H. hermanni* do show differentiation in the width of ridges on the *pars stridens*, and in insects it is often the case that spectral characteristics are directly related to the morphology of the sound producing organs (Claridge 1974; Hyder and Oseto 1989; Robillard and Desutters-Grandcolas 2011; Robillard et al. 2015). However, in the case of phaneropterid bush crickets, Heller and von Helverson (1986) found that despite dramatic differences in the type of apparatus ('pegs' vs 'teeth'), as well as the size of the *pars stridens* in conspecific males and females, frequency spectra were remarkably similar. Determining why peak frequencies are the same in both sexes of *H. hermanni*, if stridulation serves a sexual function, is difficult as there are no data on the reproductive behaviour of this species. Heller and von Helverson (1986) suggested that in the phaneropterid bush crickets, spectral parameters could serve as species identifiers, with the receptor organ being most sensitive to the frequencies of conspecific sounds. In males, coevolutionary matching of transmitter and receiver may be due to intrasexual rivalry. As a result, a responding female would then have to modify the spectrum of her calls in order to be heard by males, meaning that sexual selection would favour females producing similar sounds to males. Exploration of the auditory interneurone system in *H. hermanni* would be instructive here and would support an intraspecific function for stridulation if spectral tuning was found to match conspecific calls (see Dobler, Stumpner and Heller 1994; Stumper 1997).

The limited differences found here between male and female calls, particularly spectrally, could also partly result from the context under which stridulation occurred. In this study, beetles were grasped to induce stridulation. The sounds were therefore likely to mimic those used to repel predators, and it remains possible that other intraspecific calls exist in the species repertoire that could not be observed here (see Hall, Mason, Howard, Padhi and Smith 2013). Furthermore, if both sexes are producing disturbance calls in response to the same predators, these calls would be selected to be the same. Claridge (1974), for example, found no differences between sexes in the defensive stridulations of the ground beetle *Cychrus caraboides* (Linnaeus, 1758), but noted that this did not rule out an intraspecific function of stridulation in this species due scant knowledge of its behaviour, something which also applies to *H. hermanni*. Calls may also be modulated if *Hygrobia* produces them whilst partially buried in the substrate, which has been observed (see above) (Roberts and Elliott 2017).

Temporally, calls did differ between sexes; male call intervals being 1.25 x longer than those of females (Table 1). This suggests that stridulation may be sexually selected in this species, as such differences would not be expected in an anti-predator response, if both sexes

are under the same selection pressures. Since the temporal characteristics of song are controlled by muscular activity (Ryan 1988; Prestwich 1994; Howard and Hill 2006), differences in call intervals between sexes are most likely driven by differences in the time taken for the abdomen to revert, something which may, therefore, provide some indication of individual fitness. Unexpectedly, the length of beetle calls in both sexes changed with time spent in the laboratory, this effect being most apparent with male call intervals, whose duration increased markedly with recording (see Figure 3). Balfour-Browne (1922) noted that males tend to have a shorter lifespans (*ca* 1 year) than females (*ca* 3 years) and died more frequently in captivity. Since sound production is likely to be energetically costly (Prestwich 1994), it is likely to change with individual condition. Whilst a temporal effect is seen in both sexes, male *H. hermanni* may lose condition more rapidly in the laboratory than females, leading to slower movements of the abdomen and thus longer pauses (i.e., intervals) during calls. If calls do function as intraspecific signals, this may therefore convey information about individual fitness. Differences in beetle age may also at least partly account for differences in call duration observed between this study and that of Beutel (1986).

In conclusion, our results provide new insights into the bioacoustics of *Hygrobia hermanni*. Structural differences in the *pars stridens* of males and females, coupled with subtle differences in call, particularly the more marked changes with time spent in the laboratory in males than females, are consistent with the hypothesis that the call of the squeak beetle may function in intraspecific communication, in addition to being an antipredator adaptation. Future studies of interactions between beetles would be illuminating, as would exploration of the bioacoustics of other extant species of Hygrobiidae.

Acknowledgements

This study was conducted as part of an undergraduate project by JB, supervised by DTB, in the School of Biological and Marine Sciences at the University of Plymouth. Thanks go to Ann Torr for assisting with sample collection and Jane Akerman for her help and advice throughout. Sarah Collins and Svenja Tidau kindly assisted with bioacoustics, and Mark Briffa with statistical analyses. The comments of three anonymous referees genuinely improved the manuscript.

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Figure legends

Figure 1. Morphology of *Hygrobia hermanni* (Fabricius, 1775): (a) male dorsal habitus; (b) underside of male elytron, arrow indicates location of *pars stridens*; (c) close-up of male *pars stridens*; (d) last abdominal ventrite of male; (e) last abdominal ventrite of female; (f–g) male *pars stridens*, scanning electron micrograph; *T* = tooth, *S* = spacing, *r* = ridge, *f* = furrow (scale bars a–b = 1 mm; c–e = 0.5 mm).

Figure 2. Bioacoustics of *Hygrobia hermanni* (Fabricius, 1775): (a) waveform (top), spectrogram (bottom) and power spectrum (left hand side) of male, dB scale shows dB re 1 μ Pa; (b) waveform of a single male call, showing temporal parameters analysed.

Figure 3. Call properties of male and female *Hygrobia hermanni* (Fabricius, 1775): (a) chirp 1 duration; (b) interval duration; (c) total call duration, 1, 2 and 3 indicate recording number. All plots show mean + standard error.

Table 1. Analysis of temporal parameters measured in *Hygrobia hermanni* (Fabricius, 1775) calls.

Parameter	Mean ($\pm$ SE) duration (s)	Fixed Factor	SS	MS	DF	F	P
Complete Call	♂ = 0.746 ± 0.038 ♀ = 0.656 ± 0.035	Sex	0.02541	0.02541	1,60.352	1.2427	0.27
Chirp 1	♂ = 0.338 ± 0.014 ♀ = 0.332 ± 0.015	Recording	0.20620	0.20620	1,42.000	10.0844	**
		Sex:Recording	0.12584	0.12584	1,42.000	6.1545	*
Chirp 2	♂ = 0.250 ± 0.018 ♀ = 0.209 ± 0.018	Sex	0.001821	0.001821	1,57.394	0.11439	0.74
		Recording	0.001267	0.001267	1,42.000	0.07961	0.78
		Sex:Recording	0.044644	0.044644	1,42.000	2.80442	0.10
Interval	♂ = 0.158 ± 0.017 ♀ = 0.125 ± 0.011	Sex	0.15798	0.15798	1,56.285	4.1291	*
		Recording	0.74908	0.74908	1,42.000	19.5784	***
		Sex:Recording	0.30568	0.30568	1,42.000	7.9894	**

*P<0.05, **P<0.01, ***P<0.001