

1 **RESEARCH ARTICLE**

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

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25 **The call of the squeak beetle: bioacoustics of *Hygrobia hermanni***  
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27 **Astract**

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

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

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## 52 Introduction

53 Sound production is widespread in insects, being found across almost all orders (Alexander  
54 1963; Bailey 1991) and frequently resulting from stridulation. This typically involves one  
55 structure (the scraper or plectrum) being moved across a finely-ridged surface (the file or *pars*  
56 *stridens*) or vice versa, resulting in sound output as it does so (Arrow 1942). Structures involved  
57 in sound production clearly relate to the characteristics of the sound produced (Endler 1992;  
58 Casaretto, Picciulin, and Hawkins 2016), although not all studies of insect bioacoustics link  
59 these two aspects. Stridulatory files are present on a wide range of body surfaces, including  
60 wings, legs and elytra, and stridulation is used by insects in a variety of contexts including  
61 defence, competition and reproduction (Alexander 1967; Masters 1980; Lyal and King 1996;  
62 Mason 1996; Smith and Harper 2003). In some cases, it may serve to repel predators by  
63 initiating a startle response – so called disturbance stridulation. Masters (1979) noted that wolf  
64 spiders showed greater attack persistence on silenced compared to phonic individuals of  
65 *Tropisternus* Solier, 1834 (Hydrophilidae). Masters (1979) also compared mutillid wasps that  
66 were allowed to stridulate with muted individuals, noting that predators persisted for longer  
67 and killed more wasps when stridulation was prevented, all suggesting that sound production  
68 acts as a deterrent (Haskell 1961). Similarly, Bauer (1976) found that the carabid *Elaphrus*  
69 *cupreus* Duftschmidt, 1812 was more persistently attacked and frequently eaten by predators  
70 if its stridulatory apparatus had been removed (see also Thiele 1977).

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

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

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

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

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

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

## 128 **Material and methods**

### 129 *Specimen collection and maintenance*

130 Specimens of *Hygrobia hermanni* were collected using a D-framed pond net with 1 mm mesh  
131 in November 2016. Post-teneral adults were netted from a muddy, semi-permanent pond  
132 frequented by livestock near Yelverton, Devon, UK (50°31'04.42"N, 4°02'12.37"W, 369 m).  
133 Beetles used in bioacoustic studies were free from visible peritrich ciliate infection (which has  
134 the potential to affect stridulatory behaviour) and were maintained in six litre tanks of artificial  
135 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*  
136 *libitum* on a diet of chironomid larvae (Cuppen 2000). Sexes were distinguished by eye on the  
137 basis of fore-tarsal morphology (Dettner 1997).

### 138 *Morphology of sound producing structures*

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

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

### 156 ***Bioacoustic recording and set up***

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

### 176 ***Bioacoustic analyses***

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

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

185 Statistical analyses were carried out in R studio version 0.99.491 (R Core Team 2014).  
186 *lme4* (Bates, Mächler, Bolker, and Walker 2015) was used to generate linear mixed effect  
187 models of the effect of sex and time of recording on a given chirp component (see Figure 2b).  
188 In the model, sex and time of recording (and their interaction) were the fixed effects, with the  
189 intercept as the random effect. Where visual inspections of residual plots were unsatisfactory  
190 for normality and homoscedasticity, data were Log<sub>10</sub> transformed. Spectral parameters  
191 however were unable to satisfy these assumptions despite transformation. *lmerTest*  
192 (Kuznetsova, Brockhoff, and Bojesen Christensen 2015) was then used to run an Analysis of  
193 Variance (with Kenward-Roger approximation for degrees of freedom) on the linear mixed  
194 effects models.

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## 197 **Results**

### 198 ***Morphology of sound producing structures***

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

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

210 ( $t = 2.8174_{7.884} P = 0.023$ ); mean width  $8.43 (\pm 0.26 \text{ SE}) \mu\text{m}$  compared to  $7.34 (\pm 0.29 \text{ SE}) \mu\text{m}$   
211 in males. The mean width of individual teeth was  $29.93 (\pm 0.47 \text{ SE}) \mu\text{m}$  and  $28.49 (\pm 1.06 \text{ SE})$   
212  $\mu\text{m}$  in males and females respectively, but these did not differ significantly. The mean length  
213 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  
214 females, but again did not differ significantly between sexes. The mean widths of furrows  
215 between the two ridges of individual teeth were  $14.69 (\pm 0.48 \text{ SE}) \mu\text{m}$  in males and  $13.78 (\pm 0.72$   
216  $\text{SE}) \mu\text{m}$  in females, again not significantly different between the sexes.

## 217 **Bioacoustics**

218 The call of *Hygrobia hermanni* is biphasic, consisting of two chirps each made up of a series  
219 of pulses (Figure 2). These chirps correspond with the forward and backward stroke of the 7<sup>th</sup>  
220 abdominal ventrite against the pars stridens, respectively (Dettner 1997). Chirp 1 in both sexes  
221 shows amplitude modulation which appeared slightly stronger in females. The waveform also  
222 reveals that chirp 1 has a higher amplitude and is more distinctly pulsed than chirp 2 (Figure  
223 2). *Hygrobia hermanni* calls show a broad frequency spectrum, but with some harmonious  
224 structure; containing a peak frequency of just over 6 kHz (Figure 2a) in both chirp 1 and chirp  
225 2, this not differing between sexes. A second dominant peak occurs in both sexes at  
226 approximately 10.9 kHz. Some signal was also visible at higher frequencies, particularly in the  
227 first chirp (up to ca. 45 kHz), outside the flat response range of the hydrophone.

228 The mean duration of a complete call in *H. hermanni* was  $0.746 (\pm 0.038 \text{ SE}) \text{ s}$  in males  
229 and  $0.656 (\pm 0.035 \text{ SE}) \text{ s}$  in females, these durations not differing significantly (Table 1).  
230 Temporal variability was observed across recordings, however, males having longer calls  
231 during their final recordings, whereas females were more consistent (Table 1; Figure 3). Across  
232 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  
233 males and females respectively, but these timings did not differ significantly (Table 1).  
234 However, within males, chirp duration did differ significantly across time of recording, being  
235 longer during the last recording interval (Table 1; Figure 3). Chirp 2 was shorter than chirp 1  
236 (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  
237 not significantly different between sexes or recordings.

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



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

## 243 Discussion

244 Our study details the bioacoustic apparatus and sound production of squeak beetles. We  
245 provide quantitative analyses of the *pars stridens* and the spectral and temporal characteristics  
246 of *H. hermanni* stridulation for the first time, including an explicit attempt to determine whether  
247 the sexes differ in their sound producing apparatus and behaviour. Interestingly, both spectral  
248 and temporal parameters reported here contrast with those described by Beutel (1986), which  
249 is the only other study of *Hygrobia* bioacoustics to date. Beutel (1986) stated that *H. hermanni*  
250 exhibited a peak frequency of 0.5 kHz in both chirps of a call, with a second peak at around  
251 1.5 kHz; some 12 times lower than the values obtained here. Furthermore, total call durations  
252 in our study were some 1.3–1.5 x longer than the *ca* 0.5 s of Beutel (1986). Accurate  
253 comparisons between these investigations are difficult, since Beutel (1986) provides few  
254 details of the experimental set-up, although the use of a ‘Brüel & Kjaer Hydrophone Type  
255 8101’ suggests that recordings were also undertaken in water. It is possible that the recording  
256 devices used by Beutel under sampled higher frequency parts of the call (see, e.g., Robillard,  
257 ter Hofstede, Olivé and Vicente 2015), or that the tank setup influenced results. Alternatively,  
258 it is not impossible that there are regional differences in the call of this species. Consistent in  
259 both studies is the occurrence of two peak frequencies within the call spectrum (see Figure 2).  
260 Our observations of the stridulatory apparatus may explain why these two peaks occur. The  
261 *pars stridens* of *H. hermanni* possesses teeth with two ridges (see Figure 1), which essentially  
262 double the number of effective teeth between a break. By doing so, these structures potentially  
263 act as a frequency multiplier, which could account for the high frequency peak (10.9 kHz) of  
264 the call spectrum. The lower dominant peak frequency (6.1 kHz) would in turn be produced  
265 from the striking of the teeth as a whole, analogous to the situation described in the cricket  
266 *Eneoptera guyanensis* Chopard, 1931, which also possesses dual peak frequencies and a double  
267 toothed *pars stridens* (Robillard and Desutters-Grandcolas 2011).

268 Our results are consistent with the possibility that stridulation in *H. hermanni* serves  
269 additional functions besides defence. The call spectrum, with strong peak frequencies, is  
270 consistent with a use in sexual advertisement (Gerhardt and Huber 2002; Guerra and Morris  
271 2002; Forrest, Lajoie and Cuswick 2006), although in many such cases peak frequency differs  
272 between sexes (e.g., Hyder and Oseto 1989; Gray 1997), which was not the case here. This is

273 surprising, since male and female *H. hermanni* do show differentiation in the width of ridges  
274 on the *pars stridens*, and in insects it is often the case that spectral characteristics are directly  
275 related to the morphology of the sound producing organs (Claridge 1974; Hyder and Oseto  
276 1989; Robillard and Desutters-Grandcolas 2011; Robillard et al. 2015). However, in the case  
277 of phaneropterid bush crickets, Heller and von Helverson (1986) found that despite dramatic  
278 differences in the type of apparatus ('pegs' vs 'teeth'), as well as the size of the *pars stridens*  
279 in conspecific males and females, frequency spectra were remarkably similar. Determining  
280 why peak frequencies are the same in both sexes of *H. hermanni*, if stridulation serves a sexual  
281 function, is difficult as there are no data on the reproductive behaviour of this species. Heller  
282 and von Helverson (1986) suggested that in the phaneropterid bush crickets, spectral  
283 parameters could serve as species identifiers, with the receptor organ being most sensitive to  
284 the frequencies of conspecific sounds. In males, coevolutionary matching of transmitter and  
285 receiver may be due to intrasexual rivalry. As a result, a responding female would then have to  
286 modify the spectrum of her calls in order to be heard by males, meaning that sexual selection  
287 would favour females producing similar sounds to males. Exploration of the auditory  
288 interneurone system in *H. hermanni* would be instructive here and would support an  
289 intraspecific function for stridulation if spectral tuning was found to match conspecific calls  
290 (see Dobler, Stumpner and Heller 1994; Stumper 1997).

291 The limited differences found here between male and female calls, particularly  
292 spectrally, could also partly result from the context under which stridulation occurred. In this  
293 study, beetles were grasped to induce stridulation. The sounds were therefore likely to mimic  
294 those used to repel predators, and it remains possible that other intraspecific calls exist in the  
295 species repertoire that could not be observed here (see Hall, Mason, Howard, Padhi and Smith  
296 2013). Furthermore, if both sexes are producing disturbance calls in response to the same  
297 predators, these calls would be selected to be the same. Claridge (1974), for example, found no  
298 differences between sexes in the defensive stridulations of the ground beetle *Cychrus*  
299 *caraboides* (Linnaeus, 1758), but noted that this did not rule out an intraspecific function of  
300 stridulation in this species due scant knowledge of its behaviour, something which also applies  
301 to *H. hermanni*. Calls may also be modulated if *Hygrobia* produces them whilst partially buried  
302 in the substrate, which has been observed (see above) (Roberts and Elliott 2017).

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

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

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

### 328 **Acknowledgements**

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

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479

#### 480 **Figure legends**

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

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

489 Figure 3. Call properties of male and female *Hygrobia hermanni* (Fabricius, 1775): (a) chirp  
490 1 duration; (b) interval duration; (c) total call duration, 1, 2 and 3 indicate recording number.  
491 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
<b>Complete Call</b>	♂ = 0.746 $\pm$ 0.038	Sex	0.02541	0.02541	1,60.352	1.2427	0.27
	♀ = 0.656 $\pm$ 0.035						
<b>Chirp 1</b>	♂ = 0.338 $\pm$ 0.014	Recording	0.20620	0.20620	1,42.000	10.0844	**
	♀ = 0.332 $\pm$ 0.015	Sex:Recording	0.12584	0.12584	1,42.000	6.1545	*
<b>Chirp 2</b>	♂ = 0.250 $\pm$ 0.018	Sex	0.001821	0.001821	1,57.394	0.11439	0.74
	♀ = 0.209 $\pm$ 0.018	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
<b>Interval</b>	♂ = 0.158 $\pm$ 0.017	Sex	0.15798	0.15798	1,56.285	4.1291	*
	♀ = 0.125 $\pm$ 0.011	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