Costs and benefits of larval jumping behaviour of Bathyplectes anurus

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Abstract

Bathyplectes anurus, a parasitoid of the alfalfa weevils, forms a cocoon in the late larval stage, and exhibits jumping behaviour. Adaptive significance and costs of the cocoon jumping have not been thoroughly studied. We hypothesized that jumping has the fitness benefits of enabling habitat selection by avoiding unfavourable environments. We conducted laboratory experiments, which demonstrated that jumping frequencies increased in the presence of light, with greater magnitudes of temperature increase, and at lower relative humidity. In addition, when *B. anurus* individuals were allowed to freely jump in an arena with a light gradient, more cocoons were found in the shady area, suggesting microhabitat selection. In a field experiment, mortality of cocoons placed in the sun was significantly higher than for cocoons placed in the shade. *B. anurus* cocoons respond to environmental stress by jumping, resulting in habitat selection. In the presence of potential predators (ants), jumping frequencies were higher than in the control (no ant) arenas, though jumping frequencies decreased after direct contact with the predators. Body mass of *B. anurus* cocoons induced to jump significantly decreased over time than cocoons that did not jump, suggesting a cost to jumping. We discuss the benefits and costs of jumping behaviour and potential evolutionary advantages of this peculiar trait, which is present in a limited number of species.

Key Words

Biological control, Cocoon, Habitat selection, Leaping behaviour, Locomotory mode, Parasitoid

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Introduction

Habitat selection is one of the fundamental activities organisms use to increase fitness (Alcock 1998). Habitat characteristics affecting fitness may be abiotic, such as light intensity and temperature, and biotic such as resource availability (Morris 2003; Krämer et al. 2012). These components also interact with each other, causing habitat quality to fluctuate temporally and spatially (Orians and Wittenberger 1991). Organisms may behaviourally avoid unfavourable habitats, leading them toward more suitable habitats.

Selection among habitats, however, may be a challenge for immobile or sessile organisms whose flexible movements are limited. To overcome the challenge, some species may relocate themselves with the aid of wind (i.e. seed dispersal; Bazzaz 1991) or with the aid of mobile parents (i.e. oviposition; Eilers et al. 2013); others may construct protective shelters (e.g. diatom; Hamm et al. 2003). In addition, some species may adopt various locomotory modes besides non-volant movements such as phoresy (Hagstrum and Subramanyam 2010; Reynolds et al. 2014). For insects, adults of many species possess wings and/or limbs, while immature insects are often limbless (e.g. maggots), small, or parasitic, and are immobile or restricted to short ranges of mobility. Locomotory modes of these species may include crawling and sinusoidal undulation using peristaltic contraction (Berrigan and Lighton 1993; Berrigan and Pepin 1995; Brackenbury 2000). The locomotory mode and distribution of preferred habitats are often closely related. For example, less mobile larvae may be found in unfavourable environments, developing various strategies to survive, such as protective outer layers or insulations, while mobile larvae may migrate to high quality habitats (Frouz et al. 2003; Hagstrum and Subramanyam 2010). Thus, it is important to understand mobility affecting the

73 distribution of organisms.

74 Jumping, a peculiar locomotory mode of immature insects, is observed in a few taxa (e.g.
75 Heckrotte 1983; Maitland 1992), such as *Bathyplectes*, *Spudastica* and *Phobocampe*
76 (Ichneumonidae: Campopleginae). They are parasitic wasp larvae in cocoons, and suggested to
77 break out from the host and/or host cocoon by jumping (Gauld and Bolton 1988). The jumping
78 may also function to avoid unfavourable abiotic environments and predators or parasitoids, and
79 may conserve energy relative to creeping locomotion (Day 1970; Maitland 1992; Bonduriansky
80 2002). Despite these potential benefits, jumping behaviour is limited to only a few species,
81 suggesting constraints to the evolution of this behaviour.

82 *Bathyplectes anurus* is one of main biological control agents of a serious pest of
83 Fabaceaeous crops, the alfalfa weevil, *Hypera postica* (Radcliffe and Flanders 1998; Iwase et al.
84 2015). The endoparasitoid (approximately 3 mm) lays eggs in alfalfa weevil larvae. The host
85 larva spins a cocoon at its last instar, and the parasitoid larva crawls out from the host larva and
86 forms a cocoon inside the host cocoon after feeding on the host larva. The parasitoid cocoons
87 are oval-shaped, approximately 3−4 mm in length, 2 mm in width, and brown in colour with a
88 white belt. The parasitoid larva in the cocoon twitches, making the whole cocoon jump
89 approximately 5 cm.

90 Here, we investigate benefits and costs associated with jumping behaviour of *B. anurus*,
91 to discuss possible grounds for the evolution of the behaviour. We hypothesize that jumping of
92 *B. anurus* functions as microhabitat selection, leading them to more favourable and
93 inconspicuous areas to avoid predation or parasitisation, and that the behaviour imposes
94 energetic costs. Specifically, we test whether the jumping of *B. anurus* cocoons is stimulated by

95 light, high temperature, humidity, and presence of predators, and whether jumping causes
96 excessive loss in the body mass.
97
98 **Methods**
99 We collected *Bathyplectes anurus* cocoons found on the narrow-leaved vetch, *Vicia sativa* ssp.
100 *nigra* and burclover, *Medicago polymorpha* in Fukuoka prefecture, Japan in May, 2013 and
101 2014. *B. anurus* cocoons were stored in gauze pouches (100 individuals/pouch) and placed in a
102 plastic container containing sphagnum moss soaked with water at 25 ± 0.5ºC until they were
103 used. We conducted all experiments from May through September of either 2013 or 2014, and
104 no experiment was replicated over the two years to avoid possible year effects. In addition, all
105 replications in each experiment were conducted within a week to minimize variation in age of
106 the samples.
107
108 *Effects of abiotic environment on the jump frequency*
109 Effects of light
110 To examine whether the light increases the jumping frequency of *B. anurus* cocoons, we
111 conducted a lab experiment with two treatments, dark and light. For the dark treatment, we
112 covered a clear plastic cup (diameter 10 cm) with the thick black paper to exclude the light. For
113 the light treatment, the cup was left uncovered. We placed a cocoon individually in each plastic
114 cup. We then placed these two treatment cups side-by-side at a distance of 15 cm under a
115 fluorescent lamp (FL15W, Toshiba, Tokyo, Japan; 162.5 lux). As *B. anurus* cocoons make a
116 clicking sound when jumping (Fig. 1), we recorded the jumping sound of the cocoons for 7 min

117 with a microphone equipped with an iPhone (Apple) inserted through a hole (diameter:
118 approximately 1 cm) on the side of the cup (10 replicates). It is presumed that the clicking
119 sound is the whipping movement of the larvae tapping the cocoon from inside. The recorded
120 sound data were uploaded to a computer. We allowed cocoons to settle 2 min after placing them
121 in the cups and counted the number of jumping events from 2 to 7 min. We measured the
122 temperature of plastic cups in both treatments and confirmed that the temperatures did not differ
123 between them ($p > 0.05$). Since homogeneity of variance of the data was violated (Levene's test:
124 $p = 0.01$), we conducted non-parametric test (Mann-Whitney U test) to examine the difference
125 between the numbers of jumping in the light and dark treatments.
126
127 Effects of temperature
128 For the effect of temperature on the jump frequency, we placed individual *B. anurus* cocoons in
129 clear plastic cups placed under a heat lamp (Exo Terra® Heat Glo Infrared Heat Lamp 100W)
130 on a height adjustable lamp stand. The plastic cup was covered with a black cloth to avoid the
131 effect of light on jumping behaviour. We counted the number of jumping events in a 5 minute
132 period, as per the methodology above. We varied the temperature by moving the height of the
133 lamp (30, 35 and 40 cm from the floor) or removing the lamp (10 replicates for each). Since
134 temperatures at each trial were constantly increased due to the heat from the lamp, we measured
135 the temperature in the plastic cup every minute (CTH-204: Digital Thermo Hygrometer,
136 Custom, Tokyo, Japan). We tested whether the number of jumping events was related to the
137 temperature and the change in temperature using single regressions (dependent variable: the
138 number of jumping events from 2 to 7 min; independent variables: the median temperatures of

the 2-7 min of testing period for each treatment and the change in temperature [i.e. difference in the temperature between the beginning and ending of the time interval]).

Effects of humidity

To test whether humidity affected the jumping of *B. anurus* cocoons, we prepared two treatment cups. One plastic cup contained calcium chloride to absorb humidity, and the other cup was humidified by spraying distilled water. We placed a *B. anurus* cocoon in a smaller cup and placed it in the treatment cup. We collected the data with the same manner as above. We measured the relative humidity every minute. The relative humidity in the moist treatment was significantly higher than that in the dry treatment (Mann-Whitney U test: $U_{15,15} = 225$, $p < 0.01$; high humidity treatment: 78.7 ± 0.87%; dry treatment: 33.0 ± 0.45%). The temperatures were maintained at 25 ± 0.5ºC and there was no difference in temperature between the two treatments. We tested whether the jumping frequency of *B. anurus* cocoons differed between the two treatments with an analysis of variances (ANOVA).

Effects of the light on the micro-habitat selection in B. anurus cocoons

To examine whether *B. anurus* cocoons avoid light and move toward shady area, we conducted a lab experiment. We filled clear plastic containers (22 × 16 × 3 cm LWD) with potting soil to 1 cm depth (flower, Sun and Hope, Fukuoka culture soil). We then illuminated 2 fluorescent lamps (15W) at a height of 15 cm above the container. Twenty cocoons were placed on the soil in the centre of the container. Half of the surface of the container lid was covered with black paper, such that only one half of soil was illuminated.

161 After 1 h, we measured distribution of cocoons by counting the number of cocoons in 4
162 areas, divided evenly in the container from the lighted side to the dark side (very dark area: 0
163 lux, dark area: 2.5 lux, bright area: 111.5 lux, very bright area: 130 lux). The cocoons were
164 counted area by area. Because some cocoons did not jump at all, we excluded individuals that
165 were in the range of 1 cm width each from the centre of the container where cocoons were
166 released. To eliminate confounding factors, the soil was replaced each time and the side of
167 lighting area was switched each time. We also ensured the temperatures did not differ between
168 dark and light sides ($p > 0.05$). We tested whether there was a gradient in the final distribution
169 of *B. anurus* among 4 areas with a chi square test after pooling the data of 14 replications. For
170 post hoc tests, we conducted pair-wise comparisons with chi square tests and evaluated
171 significances with Bonferroni correction ($p = 0.05/6 = 0.0083$).
172
173 *Costs of jumping behaviour*
174 We measured the change in the mass of *B. anurus* cocoons as a cost of jumping behaviour. We
175 first measured 60 individual cocoons with an electronic balance (Mettler Toledo, AT20) to the
176 nearest 0.1 mg. The samples were divided into two treatments: dark and light treatments ($n = 30$
177 for each treatment). Each sample was then individually placed in a centrifuge tube with a hole at
178 the tip of the bottom. These centrifuge tubes were placed in a plastic cup (diameter of 10 cm)
179 upside down. To estimate weight loss by jumping, we recorded jumping frequency in the dark
180 and light conditions. Since *B. anurus* cocoons do not jump in the dark as frequently as in the
181 light, we considered the weight loss in the dark as the metabolic weight loss. To create the dark
182 condition, we covered the plastic cup containing centrifuge tubes of cocoons with black paper,

183 and placed in the growth chamber at 25 ± 0.1ºC, 70 ± 5% r.h. and a L14:D10 cycle. The
184 condition was set based on the field environment at the same period of time not to interfere with
185 the circadian rhythm of the samples.
186 We measured the cocoon mass and the number of jumping events of each individual on
187 0^{th}, 14^{th}, 21^{st}, and 28^{th} day. For counting the jumping frequency, we used the same method as the
188 light experiment, but conducted in the growth chamber. We tested whether the decrease in the
189 cocoon mass through time differ between the light and dark treatment with repeated measures
190 ANOVA. We also regressed the weight loss over 4 weeks on the pooled number of jumping and
191 initial body mass.
192
193 *Effects of predators on B. anurus jumping*
194 To investigate effects of predators on the jumping behaviour of *B. anurus*, we examined the
195 number of jumping events when ants were present (ant treatment; $n = 15$) and absent (control; n
196 = 15). We placed a *B. anurus* cocoon in an arena (146 × 208 × 56 mm LWD), in which Japanese
197 giant ants (Formicidae: *Camponotus obscuripes*) were introduced as predators. To facilitate the
198 introduction, a plaster ant nest (62 × 87 × 20 mm LWD) was connected to the test arena through
199 the hole. We recorded the number of cocoon jumping events for 10 min and the number of
200 contacts to the cocoon by ants. We used two colonies of ants: one had 16 workers and the other
201 had 23 workers. We counted the number of cocoon jumping events with the same manner as
202 above and counted the number of contacts by ants.
203 To examine whether the frequency of *B. anurus* cocoon jumping behaviour increased with
204 the presence of ants, we compared the per-second frequency of jumping between the ant

205 treatment and control with ANOVA. We chose per-unit-time frequency, since the time of contact
206 by an ant varied among samples. The data used in the analysis were the frequency of jumping (s^{-1})
207 before contacts by ants for ant treatment and that during the first 120 s for control (equivalent
208 period to the ant treatment).
209 We also examined whether jumping frequency differed between before and after the
210 contact by an ant with a Wilcoxon signed rank test, using the data from the ant treatment.
211 Finally, we tested whether jumping frequency depended on the number of contacts by ants using
212 a generalized linear model with log-link and a Poisson distribution, after testing the effect of the
213 ant colony.
214
215 *Field experiment*
216 To examine whether the light decreased survivorship of *B. anurus* cocoons, we placed 10
217 individuals in a pot (25 × 25 × 20 cm LWD) containing soil (10 cm of depth; $n = 10$). All pots
218 were covered with a net to keep the cocoons in. Five pots were placed in the field exposed to the
219 sun all day, and the other five pots were placed in the shady area under the trees from August 1st
220 through September 25th, 2013 (mean ambient temperature through the testing period 28.0ºC
221 [range: 18.4−37.9ºC]; mean relative humidity 71.6% [min. 31%]). We recorded the
222 temperatures of the soil surface in the pots at 9:00, 12:00, and 16:00 on a day in August. We
223 checked the survival of the individuals by immediately dissecting non-jumping cocoons. We
224 tested whether mortality was lower in the shady area with a Mann-Whitney U test.
225 All analyses were conducted with SPSS ver. 15.0.
226

227 **Results**

228 *Effects of abiotic environment on the jump frequency (light, temperature, and humidity)*

229 *B. anurus* cocoons jumped approximately 2.85 times more often in the light (light: mean±SE =
230 62.1 ± 10.5; dark: mean±SE = 21.8 ± 4.0; $U_{10, 10} = 87.0$, $p < 0.01$). For the temperature
231 experiment, the median temperatures in the different treatments (no lamp: mean±SE = 23.9 ±
232 0.10; 40 cm: mean±SE = 31.7 ± 0.35; 35 cm: mean±SE = 35.7 ± 0.40; 30 cm: mean±SE = 41.0
233 ± 0.90) did not significantly affect the number of jumping events though we saw a marginal
234 trend toward increased jumping at higher temperatures ($F_{1,38} = 3.22$, $p = 0.08$, $R^2 = 0.08$).
235 However, the number of jumping events was significantly higher in cases where the change in
236 temperature was greater ($F_{1,38} = 6.13$, $p = 0.02$, $R^2 = 0.14$; Fig. 2), indicating that cocoons were
237 more responsive to rapid increases in temperature than to the temperature per se. Jumping
238 frequencies of *B. anurus* cocoons were approximately 60% higher at low relative humidity (low
239 humidity: mean±SE = 18.0 ± 4.5; high humidity: mean±SE = 12.7 ± 4.1; $F_{1,28} = 4.76$, $p = 0.04$).
240
241 *Effects of the light on the micro-habitat selection in B. anurus cocoons*
242 Individuals, which were found in the range of 1 cm width each from the centre, and were
243 removed from the analysis accounted for approximately 2.14 ± 0.57 (mean±SE) individuals per
244 trial (10.7%). The distribution of cocoons in the arena with the light gradient indicated that
245 significantly more *B. anurus* cocoons were found in the shady area after 1 h ($\chi^2 = 76.6$, $p < 0.01$;
246 Fig. 3). Post hoc tests showed that the all pair-wise combinations were significant after
247 Bonferroni correction ($p < 0.0083$), except for comparisons between very dark and dark ($p =$
248 0.04) and between bright and very bright areas ($p = 0.51$).

249

250 *Costs of jumping behaviour*

251 *B. anurus* in the dark treatment did not jump at all when we monitored their jumping frequency
252 on 0th, 14th, 21st, and 28th days after the experiment, while individuals in the light treatment
253 frequently jumped (mean±SE = 10.3 ± 2.1). There was a main effect of time on body mass ($F_{3,}$
254 $_{56}$ = 93.2, p < 0.01); but no significant effect of the treatment ($F_{1, 58}$ = 0.03, p = 0.87). In
255 addition, there was a significant interaction between time and light treatment ($F_{3, 56}$ = 60.1, p <
256 0.01; Fig. 4), because the decrease in body mass through the 4 weeks was greater in the light
257 treatment (light, 2.22%; dark, 0.38%).

258

259 *Effects of predators on B. anurus jumping*

260 In the ant treatment, 14 cocoons out of 15 replications were contacted by ants 124 ± 39 s
261 (mean±SE) after the introduction of the cocoon. The frequency of jumping was approximately
262 83% greater in the ant treatment (mean±SE = 0.28 ± 0.05 s^{-1}) than in the control (mean±SE =
263 0.15 ± 0.02 s^{-1}) (χ^2 = 4.68, p = 0.03). The colony used in the experiment did not affect jumping
264 frequency (χ^2 = 0.35, p = 0.55). Jumping frequency significantly decreased (approximately by
265 65%) after ants contacted the cocoon (from 0.28 ± 0.11 to 0.18 ± 0.07 s^{-1}; Z = −1.98, p = 0.048),
266 but there was no relationship between jumping frequency (s^{-1}) and the number of contacts by
267 ants ($F_{1,11}$ = 0.20, p = 0.66).

268

269 *Field experiment*

270 Mortality was lower in the shady area ($U_{5,5}$ = 25, p = 0.01 , sun: 82.1 ± 6.2%; shade: 4.5 ±

271 2.7%). The mean temperatures of the pots placed in the shady area were 28.4 ± 0.03ºC, 29.9 ±
272 0.07ºC, and 33.0 ± 0.42ºC at 9:00, 12:00, and 16:00 respectively, and those in the sunny area
273 were 32.4 ± 0.11ºC, 40.2 ± 1.02ºC, and 34.9 ± 0.27ºC.
274
275 **Discussion**
276 Our results showed increased jumping frequencies of *Bathyplectes anurus* under exposure to
277 light, rapid increases in ambient temperature, and low relative humidity. Furthermore, the
278 jumping behaviour of *B. anurus* resulted in the movement toward shady areas, and individuals
279 in the shady areas survived better. Since we have no evidence that cocoons display any
280 directional orientation in jumping behaviour (ST unpublished data, Jumping cocoons to bright
281 vs. dark areas: n = 29 vs. 31), we conclude that jumping patterns, which persisted in the light
282 and ceased in the dark, resulted in gathering in the shady area provided in our habitat selection
283 experiment. Our results indicate that *B. anurus* cocoons jump in response to light, temperature
284 change, and humidity, and effectively relocating themselves to areas that increase their
285 survivorship, at the cost of increased energy expenditure.
286 When *B. anurus* cocoons were placed in shady or sunny areas in the field, the mortality of
287 individuals in the sunny areas was significantly higher. Our result indicates that avoiding
288 sunny areas is critical in this species, and suggests the importance of microhabitat selection.
289 The higher mortality rate in the sunny habitat could be due to higher temperature, lower
290 humidity, and/or other biotic factors.
291 At a constant temperature of 25°C, the presence of light increased the frequency of
292 jumping in *B. anurus* cocoons, consistent with a previous study (Day 1970). In nature, the

293 presence of light may be linked to the risk of predation (Battisti et al. 2013). Particularly, for
294 organisms that are immobile or at resting stages (e.g. pupae), selection of microhabitat where
295 they are less vulnerable to predators may be critical for survivorship. For example, the stable
296 fly, *Stomoxys calcitrans*, preferentially pupates in the dark area, possibly to avoid predation
297 (McPheron and Broce 1996). Thus, the cocoons may jump to move toward the area with less
298 predation risk using light as a cue.

299 In ectotherms, solar radiation increases body temperature through increased ambient
300 temperatures, and affects performance (Heinrich 1981; McClure et al. 2011; Battisti et al.
301 2013). The jumping frequency of *B. anurus* cocoons, however, did not significantly increase in
302 our higher temperature treatments, inconsistent with Day (1970), but we did see a marginally
303 positive trend in the relationship between the jumping frequency and temperature and
304 increased temperature ($p = 0.08$). On the other hand, a rapid increase in temperature facilitated
305 the jumping of *B. anurus* cocoons, indicating high sensitivity to temperature change. Small
306 insects with high surface to volume ratio have higher heat transmission (Angilletta 2009;
307 Battisti et al. 2013).Therefore, cocoons may be able to quickly sense temperature change as the
308 environmental change, and jump to relocate themselves from possibly unfavourable
309 environments. High sensitivity to a change in temperature rather than to temperature itself may
310 be common. For example, patch exploitation in the hymenopteran parasitoid, *Venturia*
311 *canescens*, was increased, responding to a drop in temperature than to low temperature itself. It
312 is suggested that the parasitoid foresaw unfavourable environmental conditions through the
313 change in temperature and intensified the patch exploitation (Amat et al. 2006). The low R^2 in
314 our result (0.14); however, indicates high variability, implying low precision of the predictor

315 (temperature change) for the number of jumping events. Thus, other factors affecting the
316 jumping behaviour cannot be ruled out in the current study.
317 Many holometabolous insects wander to migrate toward more suitable environment before
318 pupation (i.e. wandering phase), as inhospitable environments can have a detrimental effect on
319 survival of the pupae (e.g. McPheron and Broce 1996). For example, the survival of fruit fly
320 pupae, which reside in the soil, significantly decreased at extremely high or low humidity,
321 indicating sensitivity to soil moisture. Accordingly, larvae at the prepupal stage prefer moist and
322 shady area to pupate (Alyokhin et al. 2001; Hulthen and Clarke 2006). Furthermore, selection of
323 the pupation site is a challenge for parasitoids that parasitize a host at the egg or larval stage and
324 pupate inside the host, because the pupal site suitable for the host larva and the parasitoid could
325 be different. Some parasitoids manipulate the host to pupate at a site favourable to the
326 parasitoids (Josso et al. 2011). *B. anurus* forms a cocoon in late spring and pupates within the
327 cocoon around October (Bartell and Pass 1978); emergence is at the end of March in Japan.
328 Consequently, *B. anurus* spends approximately 10 months within the cocoon. In spite of the
329 significant amount of time they spend in the cocoon, the cocoon is formed at the site where the
330 host decided to pupate. Thus, the initial site may not be suitable for the *B. anurus* pupae, and
331 their jumping behaviour may allow them to escape from the unsuitable environment prior to
332 pupation. In general, cocoons provide substantial benefits by preventing from mechanical
333 damage, desiccation, pathogen, and predation, and by regulating gas, temperature, and humidity
334 (Lyon and Cartar 1996; Tagawa 1996; Danks 2004; Roy et al. 2012; Chen et al. 2013). These
335 functions are especially beneficial to larvae/pupae in the resting stage going through winter
336 (Danks 2004). *B. anurus* cocoons, however, don't seem to have thermal insulation function

337 because our results showed instantaneous response to temperature changes, and thus jumping
338 behaviour may play an important role in harsh environments in this species.
339 *Bathyplectes curculionis*, a congenic species of *B. anurus*, parasitizes the same host, *H.*
340 *postica* and forms a similar cocoon. However, *B. curculionis* cocoons do not jump. In Iraq, Iran,
341 and Egypt, their distributions mostly overlap; however, *B. anurus* is relatively denser in areas
342 with moderate climate, while *B. curculionis* is found more often in hotter and drier areas
343 (Gonzalez et al. 1980). *B. curculionis* emerges during summer, spending the hottest time of the
344 year as the adult, more mobile stage than the larva or pupa in a cocoon (Cherry et al. 1976;
345 Bartell and Pass 1978; Berberet et al. 2002). Therefore, the two closely related species might
346 employ different strategies to overcome high temperature; behavioural adaptation in *B. anurus*
347 and phenological and life history adaptation in *B. curculionis.* Comparative studies between the
348 closely related species, focusing on the difference in life histories and cocoon materials, would
349 allow us further understanding of the adaptive significance of the jumping behaviour.
350 Our results showed that the frequency of jumping increased when ants were present,
351 suggesting that cocoons may respond to the presence of predators by jumping, in attempt to
352 escape from the given environment. *B. anurus* cocoons may be able to sense the risk of
353 predation through a cue, which is yet unknown in the current study, but possibly vibration
354 transmitted through the ground and/or chemical cues (Kats and Dill 1998). Interestingly, the
355 cocoons reduced the jumping frequencies after a physical contact by ants. Upon encountering
356 predators, organisms may either reduce their activities (e.g. death faint) or increasing activities
357 to escape from predators (Lima and Dill 1990; Bucher et al. 2014). The plastic anti-predatory
358 behaviour patterns may depend on locomotor levels (Miyatake et al. 2008), and may be under

359 selection (Juliano and Gravel 2002). The increase in jumping frequencies in the presence of
360 ants, but the reduced jumping behaviour after physical contact with ants, together may be a
361 flexible response to avoid predation.

362 Alternatively, previous studies indicated that the jumping of *B. anurus* cocoons was
363 effective in preventing hyperparasitism (Day 1970; Horn 1976). Organisms may change the
364 direction and magnitude of anti-predatory behaviour depending on predator species and body
365 size (Helfman 1989; Binz et al. 2014). Both large terrestrial crawling predatory insects, such as
366 ground beetles and crickets (Cherry and Armbrust 1977), and relatively small hyperparasitoid
367 wasps such as *Gelis* spp. (Ichneumonidae) are potential attackers of *B. anurus* cocoons (Horn
368 1976). Since possible predators and parasitoids for *B. anurus* cocoons could vary in taxa and
369 size, *B. anurus* cocoons may be able to alter jumping behaviour patterns depending on the
370 attacker.

371 The body mass of jumping individuals was reduced more than that of resting individuals,
372 indicating increased energy expenditure due to jumping behaviour. The jumping was induced by
373 the light in our experiment, and thus it is also possible that the presence of light increased the
374 metabolic rate facilitated by the coordinated circadian system and development (Fonken and
375 Nelson 2013). We consider, however, the light-induced metabolic rate would be relatively small
376 because the individuals in cocoons are not feeding, and are in the late larval stage where a
377 significant development is less likely. The reduction of body mass may affect adult body size,
378 which is strongly associated with fitness of insects (Kingsolver and Huey 2008), and could be
379 serious damage especially for the non-feeding individuals in the late larval stage. Although the
380 decrease in body mass was approximately 2.22% of initial body mass in our study, we suggest

381 that the weight loss may be critical for the jumping individuals and energy expenditure is one
382 cost of jumping behaviour.
383 When a *B.anurus* cocoon jumps, we observed the larva in the cocoon hitting the interior
384 side of the cocoon with whip-like movements using the whole body. The movement suggests
385 the use of hydrostatic skeleton utilized by many limbless invertebrates; therefore, high energy
386 expenditure may be incurred with the jumping behaviour (Casey 1991). Larval jumping is rarely
387 observed, but is found in multiple taxa: Lepidoptera (Heckrotte 1983; Humphreys and Darling
388 2013), Coleoptera (Ito 2012), Diptera (e.g. Maitland 1992), and Hymenoptera (Gauld and
389 Bolton 1988). There may be various factors limiting the jumping behaviour to a few species,
390 including morphological constraints, as well as energy expenditure. Yet, the costs of jumping
391 has received little attention to date. In addition to larval jumping, costs of jumping behaviour in
392 adult insects are discussed in a few studies. For example, jumping of fleas is suggested to be
393 energetically costly (reviewed in Krasnov 2012), and locusts develop and/or maintain muscles
394 for jumping at costs of other organs (Alexander 2000). At present, however, the costs of
395 jumping behaviour in insects don't seem to be thoroughly examined. We anticipate further
396 studies examining costs incurred with the behaviour to provide refined scenarios for evolution
397 of jumping behaviour.
398 Finally, the possibility that the behaviour is a by-product of other traits, such as induction
399 of heart activity by twitching (e.g. Kuusik et al. 2001) also remains. Studies on cocoon
400 structures and physical mechanisms of jumping behaviours, especially contrasting with the
401 congenic species, *B. curculionis*, would allow us to more fully understand the evolution of the
402 behaviour.

403

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Figure Legends

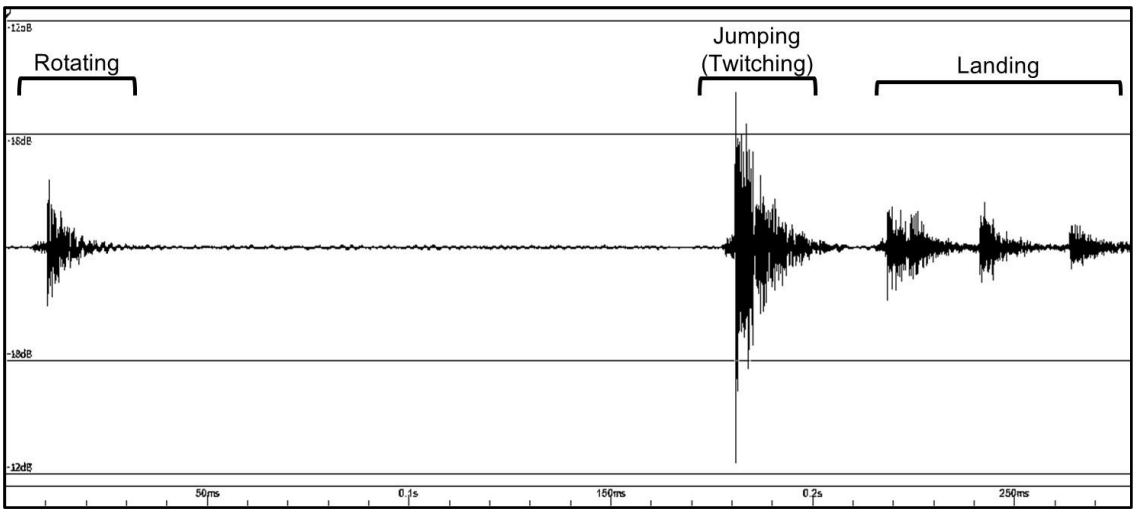

Fig. 1 Typical soundforms of a jump by *Bathyplectes anurus*, transcribed with WavePad® (NCH software v.5.71). The first small wave is the sound when the larva rotates to a twitching position. The second big wave is from the sound when the larva hits the interior of the cocoon resulting in the cocoon jumping. The waves after the second wave are associated with landing

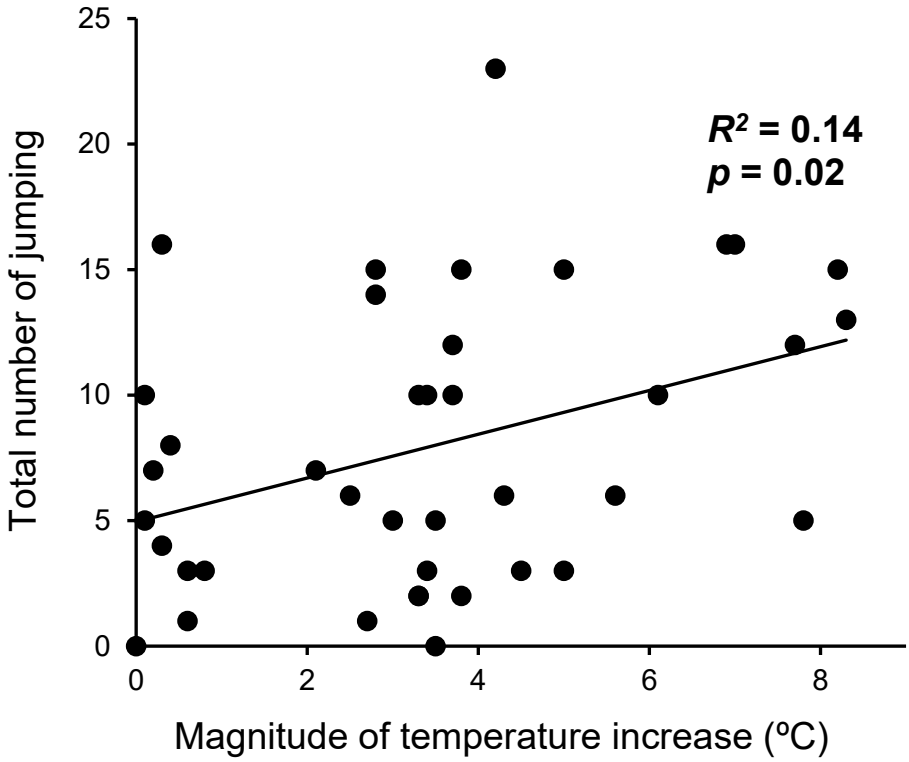

Fig. 2 The total number of jumping events by *Bathyplectes anurus* in a five minute interval at different magnitudes of temperature increases (°C). The line fit to the data is $Y = aX \pm b$, where $a = 0.87$ and $b = 4.95$

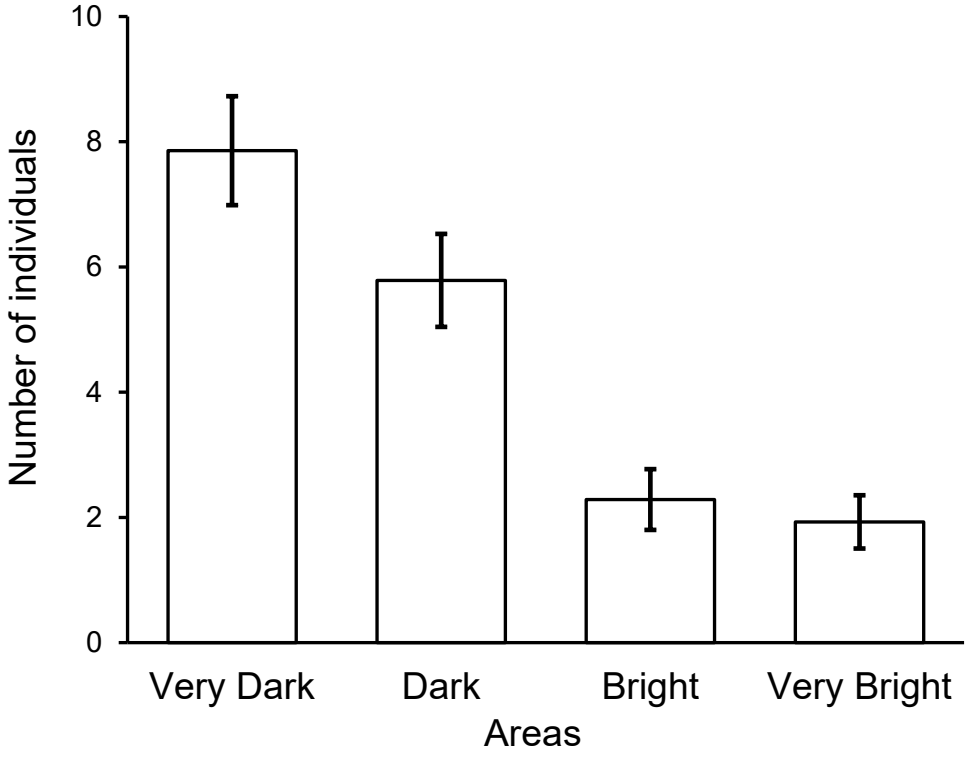

Fig. 3 The number of individuals (Mean±SE) found in four separate areas with light gradient (from dark to bright) one hour after we placed 20 cocoons in the arena. Individuals that did not jump were removed from the analyses (2.14 ± 0.57)

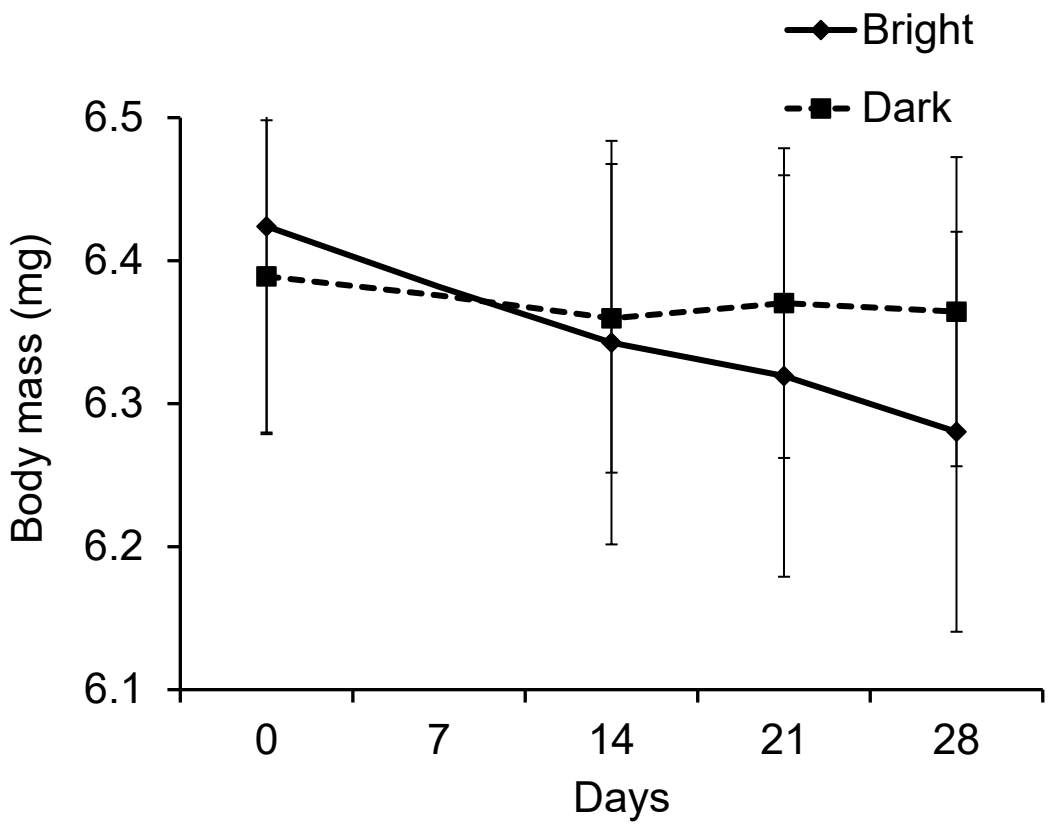

Fig. 4 Changes in *B. anurus* body mass in four weeks (Mean±SE). Individuals in the dark treatment were placed in the dark during the whole experimental period, and individuals in the light treatment were placed under the light to induce jumping