

1 **Bayesian inference supports the host selection hypothesis in explaining**  
2 **adaptive host specificity by European bitterling**

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13 **Abstract**

14 **Introduction** Generalist parasites have the capacity to infect multiple hosts. The temporal  
15 pattern of host specificity by generalist parasites is rarely studied, but is critical to  
16 understanding what variables underpin infection and thereby the impact of parasites on host  
17 species and the way they impose selection on hosts.

18 **Objective** Here, the temporal dynamics of infection of four species of freshwater mussel by  
19 European bitterling fish (*Rhodeus amarus*) was investigated over three spawning seasons.  
20 Bitterling lay their eggs in the gills of freshwater mussels, which suffer reduced growth,  
21 oxygen stress, gill damage and elevated mortality as a result of parasitism.

22 **Methods/Results** The temporal pattern of infection of mussels by European bitterling in  
23 multiple populations was examined. Using a Bernoulli Generalized Additive Mixed Model  
24 with Bayesian inference it was demonstrated that one mussel species, *Unio pictorum*, was  
25 exploited over the entire bitterling spawning season. As the season progressed, bitterling  
26 showed a preference for other mussel species, which were inferior hosts.

27 **Conclusions and significance** Temporal changes in host use reflected elevated density-  
28 dependent mortality in preferred hosts that were already infected. Plasticity in host specificity  
29 by bitterling conformed with the predictions of the *host selection hypothesis*. The relationship  
30 between bitterling and their host mussels differs qualitatively from that of avian brood  
31 parasites.

32 **Keywords** Brood parasite · Host-parasite co-evolution · Oviposition · Spawning site ·  
33 Superparasitism

34

## 35 **Introduction**

36 The extent to which a parasite exploits different host species, termed host specificity, can vary  
37 at a number of levels. Host specificity may vary spatially and temporally, corresponding with  
38 morphological, physiological or ecological differences in host and parasite traits or the  
39 environments they occupy (Payne 1997; Poulin 2011). Specificity might also reflect previous  
40 exposure of hosts to parasitism, parasite prevalence, the availability of intermediate hosts, or  
41 the phylogenetic relationships among hosts (Kaltz and Shykoff 1998; Detwiler and Minchella  
42 2009; Poulin 2011; Feeney et al. 2014). Understanding host specificity, both at a proximate  
43 and evolutionary scale, is a key question in ecological and evolutionary parasitology and  
44 represents a fundamental step in understanding the distribution and spread of parasites in  
45 response to ecological change (Poulin et al. 2011). Despite its significance, the factors that  
46 shape host specificity are poorly understood, even in well-studied host-parasite systems (e.g.  
47 Smith and Myers-Smith 1998; Giorgi et al. 2004; Feeney et al. 2014; Mendlová and Šimková  
48 2014).

49 In avian brood parasites, host specificity has been the focus of much research. Host  
50 preferences have been attributed to variables such as host population size, duration of nesting  
51 period, nest type, host aggression, 'superparasitism' (repeated parasitism of a host by one  
52 species of parasite), and host habitat use (Brooker and Brooker 1990; Payne 1997; Soler et al.  
53 1999; Honza et al. 2002; Patten et al. 2011; Feeney et al. 2014; Soler 2014). Several  
54 hypotheses have been invoked to explain host specificity in avian brood parasites (Smith and  
55 Myers-Smith 1998). The *parasite density hypothesis* is a null hypothesis that predicts that  
56 host use is simply a function of parasite abundance, irrespective of host species or habitat  
57 features (Hoover and Bittingham 1993). The *parasite habitat preference hypothesis* predicts  
58 that the frequency and intensity of parasitism depends primarily on the habitat occupied by  
59 hosts (Briskie et al. 1990; Ward and Smith 2000). In contrast, under the *host selection*

60 *hypothesis* different host species vary in quality to the parasite. An additional feature of this  
61 hypothesis is that high levels of superparasitism could erode host quality, resulting in  
62 switches from preferred host species to previously non-preferred hosts. Finally, the *host*  
63 *defence hypothesis* predicts that parasites avoid hosts that are effective in defending  
64 themselves against parasitism. Host defence may vary interspecifically, but also intra-  
65 specifically over the host range through geographic variation in evolved responses to  
66 parasitism (Briskie et al. 1992). These explanations for host specificity are not mutually  
67 exclusive. Thus, a parasite may express both host and habitat preferences simultaneously and  
68 be sensitive to variation in host defences.

69 Bitterling are freshwater fishes that parasitize freshwater mussels as oviposition sites and  
70 share many attributes of avian brood parasites (Karplus 2014; Davies 2015; Wootton and  
71 Smith 2015). Like avian brood parasites they offer a tractable system for studying coevolution  
72 in nature, and have the additional advantage of being amenable to laboratory experiments  
73 (Smith et al. 2004). Female bitterling use a long ovipositor to place their eggs in the gill  
74 chamber of unionid and margaritiferid mussels (Wiepkema 1961; Smith et al. 2004), and  
75 bitterling embryos show a range of highly derived adaptations to enable them to develop in  
76 mussel gill chambers (Smith et al. 2004). Female European bitterling (*Rhodeus amarus*) lay  
77 multiple small clutches of 2-6 eggs, repeatedly visiting the same or different mussels to  
78 oviposit (Reichard et al. 2008; Pateman-Jones et al. 2011). Bitterling embryos remain in their  
79 host mussel for approximately one month and impose significant costs on the host by  
80 competing for oxygen and nutrients (Spence and Smith 2013), limiting growth and fecundity  
81 (Reichard et al. 2006, 2007a) and potentially damaging host gills (Stadnichenko and  
82 Stadnichenko 1980). Mussels have evolved defences against bitterling parasitism, primarily  
83 by ejecting their eggs and developing embryos (Reichard et al. 2006, 2009, 2012, 2015), but  
84 also by rapidly closing their siphons to prevent bitterling oviposition and by diverting the

85 bitterling ovipositor into their mantle cavity rather than their gills (Reichard et al. 2010).  
86 Mussel body size appears not affect bitterling oviposition preference or quality as a host  
87 (Smith et al. 2004), though in brood parasites this trait is a strong predictor of host specificity  
88 (Medina and Langmore 2016). There is good evidence for coevolution between bitterling and  
89 host mussels across their respective distributions. For example, host mussels have evolved  
90 counteradaptations that enable them to avoid bitterling oviposition, or eject developing  
91 bitterling eggs and embryos, while bitterling almost entirely avoid infection by the parasitic  
92 glochidial larvae of mussels (Reichard et al. 2006, 2007a, 2010). Notably these relationships  
93 are stronger in regions of ancient bitterling-mussel sympatry compared with regions where  
94 the association is more recent (Reichard et al. 2010, 2012, 2015).

95 The aims of the present study were to investigate temporal changes in host specificity over  
96 the course of a spawning season in the European bitterling, a generalist bitterling capable of  
97 exploiting a range of hosts, with the goal of establishing which hypothesis for host specificity  
98 best fits observed data across years and among populations.

99

## 100 **Materials and methods**

### 101 **Study sites**

102 Fieldwork was conducted in the southeast of the Czech Republic, at the centre of the natural  
103 range of European bitterling in Europe (Van Damme et al. 2007; Zaki et al. 2008; Bryja et al.  
104 2010). Field sites comprised 13 oxbow lakes created during the 1980s situated along a 40-km  
105 stretch of the Rivers Morava and Dyje, tributaries of the River Danube. European bitterling  
106 and four species of unionid mussel (*Anodonta anatina*, *A. cygnea*, *Unio pictorum* and *U.*  
107 *tumidus*) occur in the region. Oxbow lakes varied in surface area from 6.8 to 22.7 ha. The  
108 onset of spawning in bitterling is cued by photoperiod (Shimizu et al. 1994). The bitterling  
109 spawning season in these oxbow lakes begins in late April and continues until mid to late

110 June, with a peak in spawning in early May (Smith et al. 2000a). Consequently, for the  
111 purposes of this study, a putative start date to the spawning season was designated as the 1<sup>st</sup>  
112 May.

### 113 **Embryo releases**

114 The presence of early life stages (hereafter termed embryos for simplicity, but including egg,  
115 embryo, larval and juvenile stages) of European bitterling in mussels was estimated in the  
116 years 1995–1997 from May to August, with day of sampling scored from the 01-May. Rather  
117 than dissecting mussels to detect the presence of bitterling embryos, mussels were enclosed in  
118 fine mesh bags (mesh size 0.5 x 0.5 mm) measuring approximately 150 x 200 mm and sealed  
119 with a Velcro strip. The mesh bags permitted mussels to filter water normally but retained any  
120 bitterling embryos that were released. After sealing in a bag, mussels were placed back in the  
121 substrate in the exact location from which they had been taken. The water depth at which the  
122 mussel was collected was measured to the nearest 10 mm. Bags were checked after 24 h and  
123 the number of bitterling embryos that had been released was recorded. Mussels were collected  
124 by hand by a diver and were selected as they were encountered. Sampling mussels by hand is  
125 an efficient method of collection that provides an accurate picture of the mussel assemblage  
126 (Miller and Payne 1993; Hornbach and Deneka 1996; Smith et al. 2000a). To avoid sampling  
127 the same mussel repeatedly within lakes within years, mussels were collected from different  
128 areas of each lake on each sampling occasion within years. Mussel distribution within lakes  
129 shows a marked depth distribution pattern, but no horizontal pattern (Smith et al. 2000a; C.  
130 Smith, unpublished data). Over the 3-year survey 1,889 mussels from 13 populations were  
131 scored for the presence of bitterling embryos (Table 1). Sampling took place on 54 occasions  
132 on 27 discrete dates after 01-May. The distribution of mussels among species reflected the  
133 relative frequency of mussels among the oxbow lakes in the study, with 773 *A. anatina*, 430  
134 *A. cygnea*, 371 *U. pictorum* and 315 *U. tumidus* recorded. These data were poorly balanced.

135 Thus, there were no releases of embryos from mussels in August, and data from this month  
136 were excluded from subsequent analysis (Table 1). Not all mussel species were encountered  
137 in two oxbow lakes, which were also not sampled in every month, and data for these sites  
138 were also excluded from the analysis (Table 1). In the resulting subset of data, there were 723  
139 *A. anatina* of which 202 released embryos, 385 *A. cygnea* with 20 showing embryo releases,  
140 339 *U. pictorum* of which 233 released embryos and 285 *U. tumidus* with 82 releasing  
141 embryos.

142 Handling of the mussels within the mesh bags likely led to the emergence of early stages  
143 that would have otherwise remained within the host except in the case of well-developed  
144 juveniles that would be expected to depart from the mussel gill once they completed  
145 absorption of their yolk-sac. Removing and replacing mussels inevitably resulted in the  
146 animal attempting to rebury itself and these movements and contractions of the valves can  
147 result in the premature ejection of bitterling embryos.

148 A subset of data from this survey was previously published in a study by Smith et al.  
149 (2000b). In the study by Smith et al. (2000b), only data from 9 sites in a single year were  
150 considered and only for fully developed juvenile bitterling, not all early life stages, which was  
151 the case here. No hypotheses, analyses or findings from Smith et al. (2000b) are repeated in  
152 the current study.

### 153 **Mussel dissection**

154 Releases of bitterling embryos from mussels served as an indication of the occurrence of  
155 spawning by bitterling in a particular mussel. An alternative approach would have been to  
156 dissect all mussels collected, but this was considered an unethical approach. However, to  
157 establish whether releases reliably reflected the number of developing bitterling in the gills of  
158 a mussel, a subsample of 54 mussels was first placed in mesh bags for 24 h in the way

159 described above, then dissected and the number of bitterling embryos counted. Two models  
160 were fitted to these data. A Poisson GLM was fitted as:

$$161 \quad PReleased_i \sim Poisson(\mu_i)$$

$$162 \quad E(Released_i) = \mu_i$$

$$163 \quad \log(\mu_i) = 1.05 + 0.01 \times Dissected_i$$

164 Where  $PReleased_i$  is the number of bitterling embryos released from mussel  $i$  assuming a  
165 Poisson distribution with mean  $\mu_i$ .  $Dissected_i$  is the number of embryos dissected from  
166 mussel  $i$ . In addition, a Bernoulli distribution was fitted to the same data as:

$$167 \quad BReleased_i \sim Binomial(\pi_i)$$

$$168 \quad E(Released_i) = \pi_i$$

$$169 \quad var(Released_i) = \pi_i \times (1 - \pi_i)$$

$$170 \quad \text{logit}(\pi_i) = -2.38 + 0.26 \times Dissected_i$$

171  $BReleased_i$  is the number of bitterling embryos released from mussel  $i$  assuming a Bernoulli  
172 distribution with mean  $\pi_i$  and variance  $\pi_i \times (1 - \pi_i)$ .

173 While the presence of embryos in mesh bags was accurately predicted by the number of  
174 bitterling early life stages in mussels (Binomial GLM, generalised  $R^2 = 0.58$ ), the number of  
175 bitterling released was not (Poisson GLM, generalised  $R^2 = 0.17$ ). Consequently, embryo  
176 releases from mussels were analysed as binomial data, which best reflected the presence or  
177 absence of embryos in a mussel gill.

## 178 **Statistical analysis**

179 Before applying statistical models a data exploration was undertaken following the protocol  
180 described in Ieno and Zuur (2015). The data were examined for outliers in the response and  
181 explanatory variables, homogeneity and zero inflation in the response variable, collinearity

182 between explanatory variables and the nature of relationships between the response and  
183 explanatory variables. Mussel total length and the depth at which mussels were found were  
184 collinear with species and were subsequently dropped from the analysis.

185 The temporal patterns of infection of mussels by bitterling embryos was modelled using a  
186 Bernoulli Generalized Additive Mixed Model (GAMM), which took the form:

$$187 \quad \text{Bitterling}_{ijk} \sim \text{Binomial}(\pi_{ijk})$$

$$188 \quad E(\text{Bitterling}_{ijk}) = \pi_{ijk}$$

$$189 \quad \eta_{ijk} = \beta + \text{Species}_{ijk} + f_s(\text{Day}_{ijk}) + \text{Oxbow}_j + \text{Year}_k$$

$$190 \quad \text{logit}(\pi_{ijk}) = \eta_{ijk}$$

$$191 \quad \text{Oxbow}_j = N(0, \sigma_{\text{Oxbow}}^2)$$

$$192 \quad \text{Year}_k = N(0, \sigma_{\text{Year}}^2)$$

193  $\text{Bitterling}_{ijk}$  is the presence or absence of bitterling parasitism in mussel  $i$  in oxbow lake  $j$  in  
194 year  $k$ .  $\text{Species}_{ijk}$  is a categorical covariate with four levels, corresponding with the four  
195 species of mussel, while  $f_s(\text{Day}_{ijk})$  is a smooth function to model non-linear changes in host  
196 mussel infection by bitterling over the course of a spawning season. Data exploration showed  
197 differences in the temporal pattern of parasitism among mussel species, so a separate  
198 smoother was fitted for each species. Model fit with four smoothers, one for each mussel  
199 species, gave a better fit than with a single smoother for all species, and so a model with a  
200 separate smoother for each mussel species was used. Smoothers were estimated using  
201 O'Sullivan splines (Wand and Ormerod 2008). The number of knots per smoother was fixed  
202 at 5, with knot position permitted to vary. The random intercepts  $\text{Oxbow}_j$  and  $\text{Year}_k$  were  
203 included to introduce a correlation structure between observations for the same oxbow lake  
204 and year respectively.

205 To make inferences about the parameters in the model a Bayesian approach was used. A  
206 Bayesian GAMM is robust in dealing with relatively complex datasets like the one in the  
207 present study, specifically unbalanced nested data, dependency due to repeated measures at  
208 sampling sites, and a highly varied non-normal response variable (embryo presence).  
209 Bayesian models are flexible in allowing the estimation of a posterior distribution of  
210 differences between parameters and across levels of factors. These are relatively  
211 straightforward procedures using Bayesian inference, but extremely problematic in a  
212 frequentist framework (Zuur et al. 2014; Kruschke 2015). Notwithstanding more general  
213 reservations in using frequentist analyses (Burnham and Anderson 2014), the probabilities for  
214 null hypothesis significance testing are particularly unreliable with mixed models that use  
215 smoothing functions (Zuur et al. 2014; Kruschke 2015). In addition, fitting the model in a  
216 Bayesian context permitting flexibility in assessing temporal differences in smoothers, and  
217 particularly enabled statistically important differences in the incidence of parasitism among  
218 host species to be identified across the spawning season, which would be unfeasible in a  
219 frequentist setting.

220 Diffuse or non-informative univariate priors were put on all parameters. The model was  
221 fitted in a Bayesian framework using Markov Chain Monte Carlo (MCMC) with the *R2jags*  
222 package (Su and Yajima 2012) in the R statistical environment (R Development Core Team  
223 2016) and mirrored the modelling approach outlined for cowbird brood parasitism by Zuur et  
224 al. (2014). Three independent Markov chains were run simultaneously with a burn-in of  
225 50,000 iterations and then 500,000 iterations for estimates of parameter and 95% credibility  
226 intervals. Chains were thinned every 10<sup>th</sup> iteration, resulting in 50,000 Markov Chain samples  
227 for each estimated parameter. Mixing and autocorrelation of chains were checked visually  
228 using trace plots and the Gelman-Rubin statistic (Kruschke 2015). Autocorrelation was low  
229 and good mixing was achieved in each case. The Gelman-Rubin statistic was estimated to be

230 less than 1.004 in all cases, indicating good convergence. Model validation showed no  
231 evidence of overdispersion, heterogeneity or non-linear patterns in the model residuals (Zuur  
232 et al. 2009). As part of the model-fitting process, the model was used to simulate an  
233 alternative dataset. This procedure allowed the fitted values to be compared with the  
234 simulated data, with probability values for each data point used to assess model fit. A  
235 probability of 0.49 indicated the model complied closely with the data (Zuur et al. 2014).

236 To examine whether there were temporal changes in the relative abundance of host  
237 mussels among lakes among years a binomial GLM was fitted to data for the abundance of  
238 each host species. The model was fitted as:

$$239 \quad \text{Number}_i \sim \text{Binomial}(\pi_i, \text{Total}_i)$$

$$240 \quad E(\text{Number}_i) = \text{Total}_i \times \pi_i$$

$$241 \quad \text{var}(\text{Number}_i) = \text{Total}_i \times \pi_i \times (1 - \pi_i)$$

$$242 \quad \eta_i = \beta + \text{Oxbow}_i + \text{Year}_i$$

$$243 \quad \text{logit}(\pi_i) = \eta_i$$

244 Where  $\text{Number}_i$  is the abundance of host species  $i$  and  $\text{Total}_i$  is the total abundance of all other  
245 host species. The model was fitted to a subset of lakes for which there were data for all  
246 mussel species in all years, which comprised a dataset from four lakes (lakes 2, 6, 8 and 13)  
247 over three years (1995-1997).

248

## 249 **Results**

250 Parasitism of mussel hosts by bitterling varied across the spawning season, showing a peak at  
251 the end of May. Posterior mean smoothers for all species showed non-linear effects with day  
252 that deviated from zero (Fig. 1). Prevalence of bitterling early life stages in all host mussel  
253 species was greatest between days 35-45 (04-Jun to 14-Jun), though the period over which

254 bitterling embryos were encountered varied among species, with embryos recovered from *A.*  
255 *cygnea* between days 18 and 52 (18-May to 21-Jun), while bitterling were recovered from *U.*  
256 *pictorum* from day 18 to 92 (18-May to 31-Jul). Early life stages of bitterling were recovered  
257 from *A. anatina* and *U. tumidus* from day 18 (18-May) to 74 (13-Jul).

258 The temporal pattern of parasitism varied among host mussel species (Table 2). *U.*  
259 *pictorum* was consistently the most parasitized mussel species, followed by *A. anatina* and *U.*  
260 *tumidus*, while *A. cygnea* experienced the lowest probability of parasitism (Fig. 2). Posterior  
261 mean probabilities of bitterling parasitism for *U. pictorum* were significantly higher than for  
262 *A. anatina* (Table 2). In contrast, the posterior mean probability of parasitism for *A. cygnea*  
263 was significantly lower than for *A. anatina* (Table 2), while there was no evidence for a  
264 difference in bitterling prevalence between *U. tumidus* and *A. anatina* (Table 2).

265 Mean differences between the posterior mean smoothers for host mussels identified the  
266 periods of the bitterling spawning season when there were changes in the utilisation of host  
267 species. *U. pictorum* was significantly preferred over *A. anatina* at the start of the season, but  
268 *A. anatina* subsequently showed an increase in infection prevalence during the middle part of  
269 the spawning season (days 34-65) (Fig. 3a). At the end of the spawning period, *A. anatina*  
270 declined in infection frequency faster than *U. pictorum*. This pattern was repeated between *U.*  
271 *pictorum* and *U. tumidus*, with *U. pictorum* showing a greater increase in bitterling prevalence  
272 up until day 34 and with *U. tumidus* subsequently showing a greater increase in infection  
273 prevalence between days 53-63. (Fig. 3b). In contrast, *U. pictorum* showed a greater increase  
274 in infection frequency compared with *A. cygnea* from the onset of data collection until day 38  
275 (Fig. 3c). From day 43 to 52 *U. pictorum* showed a greater decline in bitterling infection than  
276 *A. cygnea*. A comparable, but less pronounced, pattern was shown between *A. cygnea* and *A.*  
277 *anatina* (Fig. 3d) and *U. tumidus* (Fig. 3e). *A. anatina* and *U. tumidus* showed no significant

278 difference in the posterior mean of the smoothers (Fig. 3f), indicating no difference in the  
279 pattern of preference by bitterling for the two species over the spawning season.

280 The relative temporal abundance of host mussels was consistent within lakes among years  
281 for all mussel species (Table 3).

282

## 283 **Discussion**

284 The distribution of early life stages of bitterling recovered from four host species of mussel  
285 revealed a clear-cut pattern. One species, *U. pictorum*, showed the highest prevalence of  
286 infection over the course of the bitterling spawning season while *U. tumidus* and *A. anatina*  
287 showed a lower level of infection by bitterling, though they did not differ from each other. A  
288 fourth host species, *A. cygnea*, was utilized least. This is the first replicated population-level  
289 study to demonstrate this pattern of host specificity by European bitterling.

290 These changes in host utilization by bitterling reflect adaptive behavioural preferences  
291 observed in lab and field experiments. In a previous study (Smith et al. 2000b) it was  
292 established that the mortality rate of the early life stages of bitterling during incubation varied  
293 among host species. *U. pictorum* proved the best host and *A. cygnea* the worst, with *U.*  
294 *tumidus* and *A. anatina* intermediate between these two. Bitterling spawning preferences  
295 reflected this variation in host quality, with female bitterling preferentially ovipositing in *U.*  
296 *pictorum* and avoiding *A. cygnea*. Why these mussels vary in quality as hosts is not wholly  
297 clear, but may reflect differences in oxygen conditions inside the mussel gill chamber which  
298 favour embryo development (Spence and Smith 2013). Alternatively, or additionally, the gill  
299 structure of different mussel species may better suit embryo development (Liu et al. 2006).

300 While there are clear differences in host quality, which are mirrored by variation in host  
301 preferences by bitterling, host quality declines with the density of bitterling embryos in the  
302 mussel gill chamber; bitterling embryo survival is negatively density-dependent (Smith et al.

303 2000b). An outcome is that bitterling reduce the frequency of oviposition in preferred hosts in  
304 favour of non-preferred hosts as the former decline in quality through 'superparasitism' (*sensu*  
305 van Dijken and Waage 1987). Because increasing embryo density erodes mussel quality, a  
306 point can be reached when the preferred host species is comparable in quality to unparasitised  
307 individuals of the next preferred species, at which point a shift in host preference is predicted.  
308 Using Bayesian inference, plots of posterior mean smoothers in the present analysis permitted  
309 the timing of this temporal change in preference to be identified. Thus, *U. pictorum* is shown  
310 to be preferred to *A. anatina* and *U. tumidus* early in the spawning season (Fig. 3a, b), with a  
311 subsequent increase in the rate of parasitism of *A. anatina* and *U. tumidus* as bitterling began  
312 substituting already parasitized *U. pictorum* with the next best alternative hosts. Thus the  
313 pattern of posterior mean smoothers is a reflection of the dynamic temporal change in host  
314 quality as spawning occurs, but with the order of preferred hosts the same over the spawning  
315 season.

316 The findings of the present study provide support for the *host selection hypothesis*, with the  
317 preferred host the one that provides the highest quality oviposition site for bitterling. Erosion  
318 of preferred host quality through superparasitism generates temporal changes in host quality  
319 that result in temporal changes in host specificity. The primary determinant of host quality  
320 that explains the observed host specificity by bitterling has yet to be conclusively identified,  
321 but is probably the dissolved oxygen conditions inside the mussel gill (Smith et al. 2001;  
322 Spence and Smith 2013; Smith and Reichard 2013). Mussel species vary in their capacity to  
323 extract oxygen from water entering their gill cavity (Smith et al. 2001). Notably several cues  
324 are used by bitterling in making oviposition decisions, including ophthalmoception,  
325 chemoreception and tactioception (Smith et al. 2001, 2004, 2014). However, female bitterling  
326 in particular show a strong response to the dissolved oxygen concentration of water emerging  
327 from the exhalant siphon of a mussel in making oviposition-site decisions (Smith et al. 2001).

328 The analysis failed to demonstrate support for the *parasite habitat preference hypothesis*.  
329 A habitat variable, water depth, was measured in the study but was collinear with species and  
330 was subsequently dropped from the analysis to (Zuur et al. 2010). If collinearity was ignored,  
331 and depth included in the model as a covariate, a significant effect was detected, with greater  
332 prevalence of parasitism at shallow depths. However, caution is needed in the interpretation  
333 of this result because *A. cygnea* occurred at a greater mean depth than the other three host  
334 species. Given that *A. cygnea* was also the overall least preferred host species, this depth  
335 effect is most likely driven by the vertical distribution of hosts. If *A. cygnea* was excluded  
336 from the analysis the depth effect was not statistically important.

337 Native unionid mussel populations across much of continental Europe express a limited  
338 capacity to eject or avoid bitterling eggs (Reichard et al. 2010, 2012). This situation contrasts  
339 with mussel populations in the Pontic region, which show several adaptations to avoid  
340 European bitterling parasitism (Reichard et al. 2010, 2015). This difference in response is  
341 likely due to the shorter duration of sympatry and lower encounter rate with *R. amarus* in  
342 west and central Europe compared with mussels in the Pontic region, where the length of the  
343 association may be as much as two million years (Bryja et al. 2010; Reichard et al. 2015).  
344 Consequently, the *host defence hypothesis* does not explain the observed host specificity,  
345 though this explanation may apply to host specificity elsewhere in the range of the European  
346 bitterling where the bitterling-mussel association is longer established. In Asia, where there  
347 are somewhere in the region of 70 bitterling species (Chang et al. 2014; Kawamura et al.  
348 2014), with long historical associations with freshwater mussels there is good evidence for  
349 coevolutionary responses between bitterling and host mussels (Liu et al. 2006; Reichard et al.  
350 2007b, 2015; Kitamura et al. 2012), and host responses may play a role in shaping host  
351 specificity.

352 In the case of avian brood parasites, in contrast to the present findings, it is typically the  
353 *host defence hypothesis* that best describes patterns of host specificity (Feeney et al. 2014).  
354 There is some evidence for the *host selection hypothesis* for host specificity in cowbirds  
355 (Mason 1986; Smith and Myers-Smith 1998), though this may be an exception (Briskie et al.  
356 1990). However, attempts to quantify host 'quality' characteristics, measured in fitness terms,  
357 have not been systematically conducted in avian brood parasites. In addition, in cases where  
358 selection for improved host defences means that formerly profitable hosts acquire efficient  
359 defences, resulting in reduced parasite fitness, formerly unprofitable hosts effectively become  
360 profitable, with a predicted switch in parasite host preference (Soler 2014). Thus the unstable  
361 dynamics of avian brood parasites, driven by evolving host defences, means that a form of  
362 host selection must operate, with the implication that host quality is not a fixed property of a  
363 host species. Given the known variation in host responses in the bitterling-mussel system  
364 (Reichard et al. 2010, 2012, 2015), the same dynamic process may operate and warrants  
365 investigation.

366 The present data support the observation that the European bitterling is a generalist  
367 parasite, able to exploit a range of host mussel species (Smith et al. 2004). A possible  
368 explanation for its low host specificity is that host taxa are closely related, with specialisation  
369 to exploit one species also permitting exploitation of other species in the same lineage (Poulin  
370 2011). However, evidence from a recent comprehensive phylogenetic analysis of the unionid  
371 mussels demonstrated an ancient divergence of the Unioninae (including *U. pictorum* and *U.*  
372 *tumidus*) and Anodontinae (*A. anatina* and *A. cygnea*) (Lopes-Lima et al. 2017). Thus, while  
373 European bitterling readily exploit both *U. pictorum* and *U. tumidus*, they also use *A. anatina*  
374 but avoid *A. cygnea*, a situation that fails to support a macroevolutionary explanation for  
375 observed host preferences. A future approach might examine the 'functional diversity' of

376 hosts, based on host species traits (*sensu* Medina and Langmore 2016), perhaps focusing on  
377 the internal environment of the mussel gill as a site of incubation or host habitat preferences.

378 A caveat to the findings of the study is that vulnerability to premature ejection of bitterling  
379 embryos might be host species specific, thereby influencing intra-specific differences in  
380 patterns of ejection. However, if the case, a predicted outcome would be a difference in  
381 ejection rates between *Anodonta* spp. and *Unio* spp. which differ in gill anatomy (Liu et al.  
382 2006). In reality the ejection of embryos varied as much within genera as between genera.  
383 Thus *A. anatina* differed from *A. cygnea*, and *U. pictorum* differed from *U. tumidus*, while *A.*  
384 *anatina* did not differ from *U. tumidus*. The risk of bias from mussel-specific ejection rates  
385 was also mitigated by the dissection data, which demonstrated that while ejections did not  
386 reliably reflect the number of embryos on the gills of each species, it did reflect presence of  
387 embryos, irrespective of mussel species.

388 The impact of bitterling on host mussels at the population level has yet to be investigated.  
389 Inhibiting oviposition by bitterling significantly enhances mussel growth (Reichard et al.  
390 2006). There is strong evidence across several unionid mussel species that mussel size is  
391 positively correlated with fecundity (Bauer 1994), thus any reduction in mussel growth will  
392 potentially translate into a fitness cost. Further experimental and modelling studies might  
393 address the extent to which bitterling regulate unionid mussel populations, a group that is  
394 threatened globally (Lopes-Lima et al. 2014).

395 In conclusion, this study demonstrates a clear temporal shift in host specificity by a  
396 generalist parasite of its host species. Changes in host specificity reflect temporal changes in  
397 host quality as a result of superparasitism and provides support for the *host selection*  
398 *hypothesis* in the host preferences of European bitterling.

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553 **Table 1** Number of mussel samples collected during the study from each study lake and in  
 554 each month. Data for lakes 3 and 11 and for the month of August (indicated with asterisks)  
 555 were excluded from the analysis.

556

| Lake  | Month |      |      |         | Total |
|-------|-------|------|------|---------|-------|
|       | May   | June | July | August* |       |
| 2     | 36    | 59   | 25   | 0       | 120   |
| 3*    | 0     | 0    | 19   | 0       | 19    |
| 4     | 53    | 63   | 28   | 0       | 144   |
| 5     | 41    | 22   | 32   | 0       | 95    |
| 6     | 136   | 82   | 176  | 72      | 466   |
| 7     | 26    | 54   | 98   | 0       | 178   |
| 8     | 50    | 37   | 144  | 33      | 264   |
| 9     | 13    | 58   | 6    | 0       | 77    |
| 10    | 50    | 20   | 39   | 0       | 109   |
| 11*   | 0     | 0    | 33   | 0       | 33    |
| 12    | 30    | 59   | 65   | 0       | 154   |
| 13    | 28    | 31   | 33   | 0       | 92    |
| 14    | 38    | 47   | 53   | 0       | 138   |
| Total | 501   | 532  | 751  | 105     | 1889  |

557

558 **Table 2** Parameter estimates of mussel infection by European bitterling modelled using a  
559 Bernoulli GAMM. CrI is the 95% Bayesian credible interval. Credible intervals that do not  
560 contain zero in bold to indicate statistical importance. Parameter estimates are presented for  
561 each host species as the baseline category.  
562

| Model parameter                       | Posterior mean | Lower CrI     | Upper CrI    |
|---------------------------------------|----------------|---------------|--------------|
| Fixed intercept <sub>(anatina)</sub>  | -1.65          | -4.82         | 1.49         |
| <i>Species</i> <sub>(cygnea)</sub>    | -5.01          | <b>-11.96</b> | <b>-2.09</b> |
| <i>Species</i> <sub>(pictorum)</sub>  | 2.41           | <b>1.91</b>   | <b>3.02</b>  |
| <i>Species</i> <sub>(tumidus)</sub>   | -0.01          | -0.95         | 0.75         |
| Fixed intercept <sub>(cygnea)</sub>   | -6.58          | <b>-11.11</b> | <b>-2.46</b> |
| <i>Species</i> <sub>(anatina)</sub>   | 4.95           | <b>2.32</b>   | <b>8.44</b>  |
| <i>Species</i> <sub>(pictorum)</sub>  | 7.34           | <b>4.74</b>   | <b>10.83</b> |
| <i>Species</i> <sub>(tumidus)</sub>   | 4.89           | <b>2.10</b>   | <b>8.51</b>  |
| Fixed intercept <sub>(pictorum)</sub> | 0.75           | -2.24         | 3.68         |
| <i>Species</i> <sub>(anatina)</sub>   | -2.37          | <b>-2.96</b>  | <b>-1.91</b> |
| <i>Species</i> <sub>(cygnea)</sub>    | -7.65          | <b>-11.47</b> | <b>-4.74</b> |
| <i>Species</i> <sub>(tumidus)</sub>   | -2.44          | <b>-3.46</b>  | <b>-1.74</b> |
| Fixed intercept <sub>(tumidus)</sub>  | -1.69          | <b>-5.01</b>  | <b>-1.68</b> |
| <i>Species</i> <sub>(anatina)</sub>   | 0.04           | -0.70         | 1.02         |
| <i>Species</i> <sub>(cygnea)</sub>    | -5.47          | <b>-12.65</b> | <b>-2.12</b> |
| <i>Species</i> <sub>(pictorum)</sub>  | 2.43           | <b>1.74</b>   | <b>3.39</b>  |
| Random intercept <sub>(oxbow)</sub>   | 0.18           | <b>0.01</b>   | <b>0.46</b>  |
| Randon intercept <sub>(year)</sub>    | 1.89           | -0.24         | 0.97         |

563

564 **Table 3** Summary of binomial GLM to examine the relative temporal abundance of host  
 565 mussels within lakes among years for each host species.

566

| Parameter          | Estimate | s.e.  | <i>z</i> | <i>P</i> |
|--------------------|----------|-------|----------|----------|
| <i>A. anatina</i>  |          |       |          |          |
| Intercept          | -0.316   | 0.183 | -1.73    | 0.084    |
| lake x year        | -0.001   | 0.001 | -0.58    | 0.565    |
| <i>A. cygnea</i>   |          |       |          |          |
| Intercept          | -0.447   | 0.189 | -2.36    | 0.018    |
| lake x year        | -0.001   | 0.001 | -1.29    | 0.196    |
| <i>U. pictorum</i> |          |       |          |          |
| Intercept          | -2.202   | 0.271 | -8.13    | <0.001   |
| lake x year        | -0.001   | 0.001 | 1.19     | 0.233    |
| <i>U. tumidus</i>  |          |       |          |          |
| Intercept          | -2.204   | 0.267 | -8.27    | <0.001   |
| lake x year        | -0.001   | 0.001 | 1.44     | 0.149    |

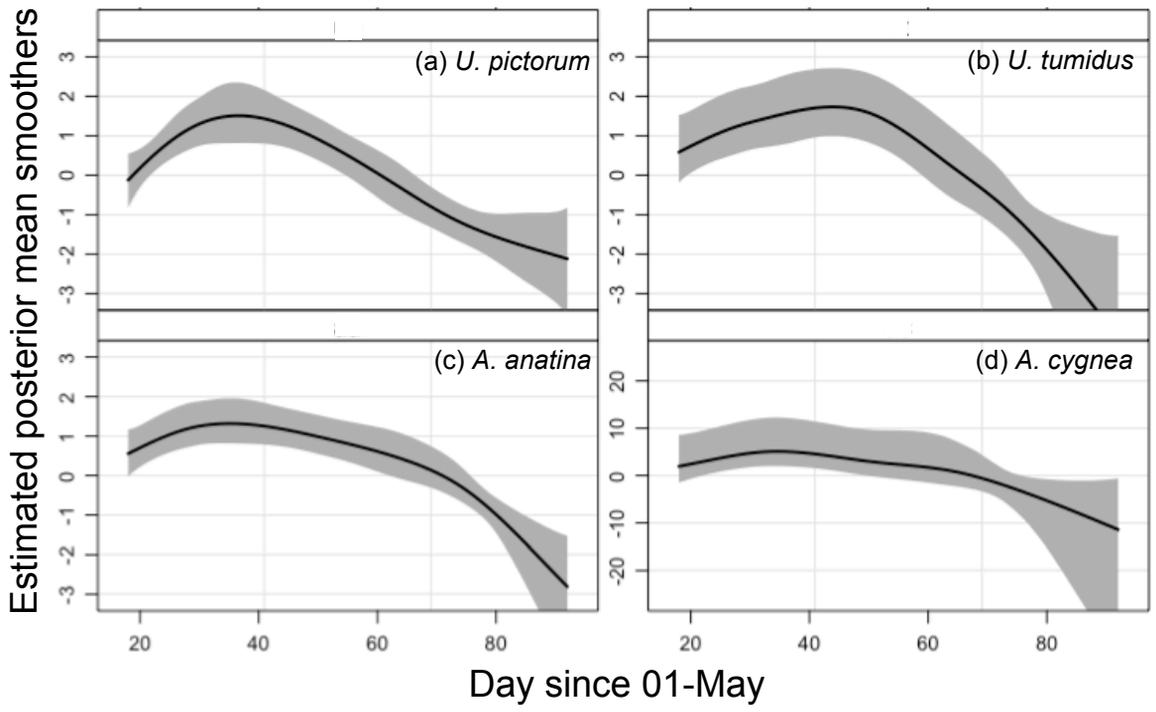
567

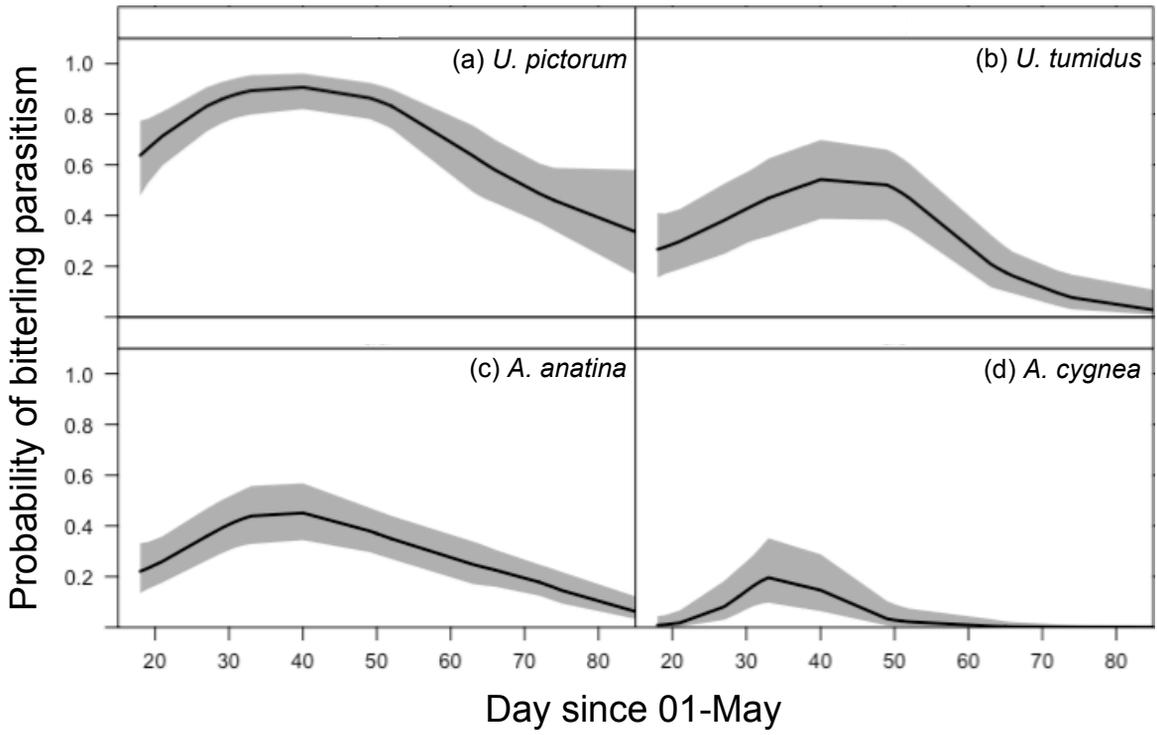
568 **Figure legends**

569 **Fig. 1** Posterior mean smoothers (solid line) and 95% credible intervals (shaded area) of  
570 European bitterling (*Rhodeus amarus*) parasitism of different host mussel species **a** *Anodonta*  
571 *anatina*; **b** *A. cygnea*; **c** *Unio pictorum*; **d** *U. tumidus*, for day since 1<sup>st</sup> May (the putative start  
572 of the bitterling spawning season) for a Bernoulli GAMM estimated by MCMC and  
573 comprising 50,000 Markov Chain samples for each estimated parameter

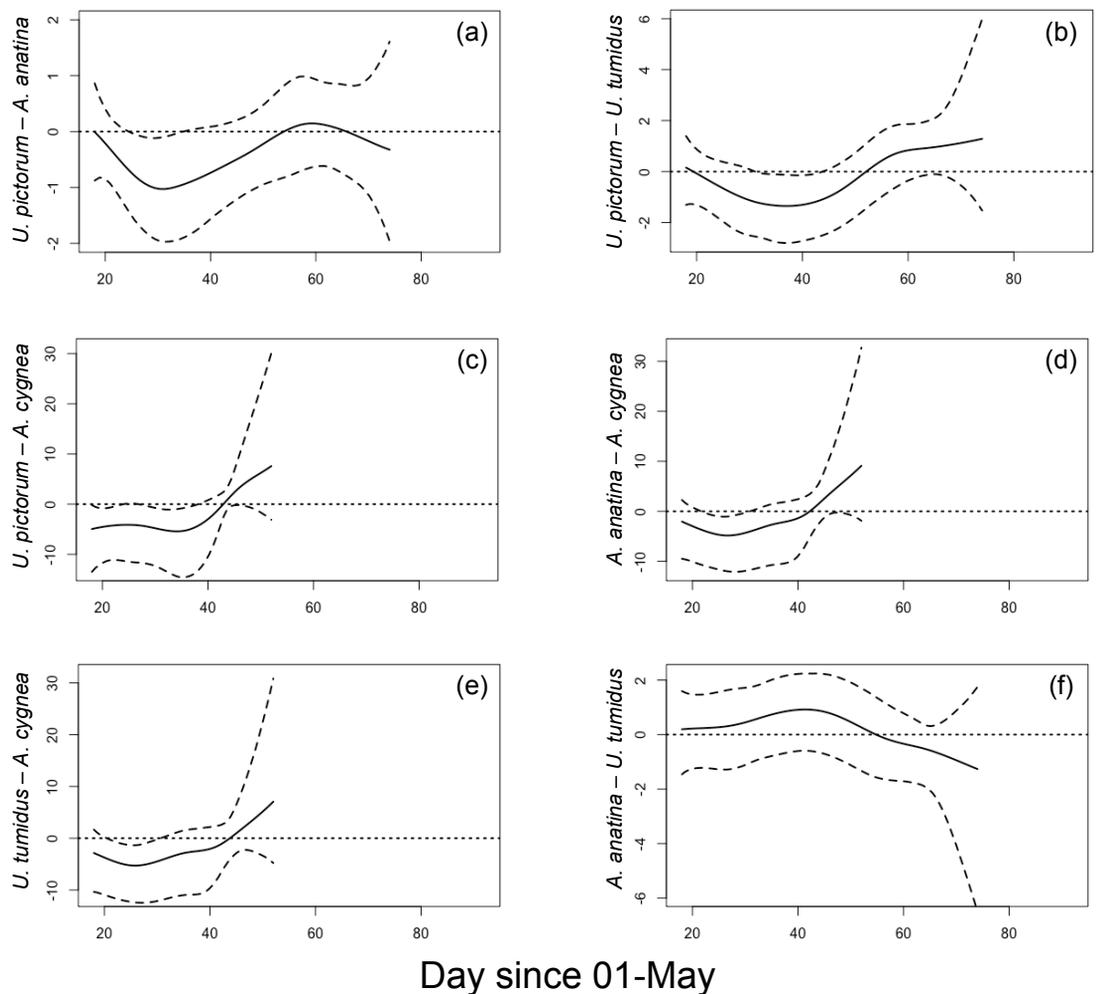
574 **Fig. 2** Mean fitted probability (solid line) of parasitism by European bitterling (*Rhodeus*  
575 *amarus*) and 95% credible intervals (shaded area) for different host mussel species **a**  
576 *Anodonta anatina*; **b** *A. cygnea*; **c** *Unio pictorum*; **d** *U. tumidus*, for day since 1<sup>st</sup> May (the  
577 putative start of the bitterling spawning season) for a Bernoulli GAMM estimated by MCMC  
578 comprising 50,000 Markov Chain samples for each estimated parameter. Probabilities were  
579 derived by adding the intercept, species effect, smoother and covariate, and applying the  
580 inverse logistic link function for each MCMC iteration

581 **Fig. 3** Pairwise differences in parasitism by European bitterling (*Rhodeus amarus*) between  
582 50,000 estimated smoothers for mussel hosts. The solid line is the posterior mean of the  
583 differences and the dashed lines the 95% credible intervals for: **a** *U. pictorum* vs. *A. anatina*;  
584 **b** *U. pictorum* vs. *U. tumidus*; **c** *U. pictorum* vs. *A. cygnea*; **d** *A. anatina* vs. *A. cygnea*; **e** *U.*  
585 *tumidus* vs. *A. cygnea*; **f** *A. anatina* vs. *U. tumidus*





Species differences between posterior mean smoothers



Day since 01-May