1 **Population-specific responses to an invasive species**

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3 Martin Reichard[1]*, Karel Douda[2], Mirosław Przybyłski[3], Oana P. Popa[4], Eva

4 Karbanová[2], Klára Matasová[2], Kateřina Rylková[2], Matej Polačik[1], Radim Blažek[1] and

5 Carl Smith[1,5]

6

7 [1]Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná

8 8, 603 65 Brno, Czech Republic

9 [2]Department of Zoology and Fisheries, Czech University of Life Sciences Prague,

10 Kamýcká 129, 165 00 Prague, Czech Republic

11 [3]Department of Ecology & Vertebrate Zoology, University of Łodz, Banacha 12/16,

12 90-237 Łodz, Poland

13 [4]Grigore Antipa National Museum of Natural History, Kiseleff Street, No 1, Bucharest

14 011341, Romania

15 [5]School of Biology, University of St Andrews, St Andrews, Fife KY16 8LB, United

16 Kingdom

17

18 *****Author for correspondence**: Dr Martin Reichard, Institute of Vertebrate Biology,

19 Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic

20 (email: reichard@ivb.cz; tel: 00420 543 422 522)

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22   **Summary**

23   Predicting the impacts of non-native species remains a challenge. As populations of a species

24   are genetically and phenotypically variable, the impact of non-native species on local taxa

25   could crucially depend on population-specific traits and adaptations of both native and non-

26   native species. Bitterling fishes are brood parasites of unionid mussels and unionid mussels

27   produce larvae that parasitize fish. We used common garden experiments to measure three

28   key elements in the bitterling-mussel association among two populations of an invasive

29   mussel (*Anodonta woodiana*) and four populations of European bitterling (*Rhodeus amarus*).

30   The impact of the invasive mussel varied between geographically distinct *R. amarus* lineages

31   and between local populations within lineages. The capacity of parasitic larvae of the invasive

32   mussel to exploit *R. amarus* was higher in a Danubian than in a Baltic *R. amarus* lineage and

33   in allopatric than in sympatric *R. amarus* populations. Maladaptive oviposition by *R. amarus*

34   into *A. woodiana* varied among populations, with significant population-specific

35   consequences for *R. amarus* recruitment. We suggest that variation in coevolutionary states

36   may predispose different populations to divergent responses. Given that coevolutionary

37   relationships are ubiquitous, population-specific attributes of invasive and native populations

38   may play a critical role in the outcome of invasion. We argue for a shift from a species-

39   centred to population-centred perspective of the impacts of invasions.

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43   parasite dynamics, symbiosis

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45   **Running title**: Population-specific responses to an invader

1. Introduction

Cases of biological invasions, where species are translocated to new geographic areas where they establish and spread, raises concerns for their potentially negative ecological and economic consequences [1]. A substantial research effort has focused on understanding the mechanisms of dispersal and establishment of non-native species, and the ecological traits that predispose them to invasiveness [2]. While this approach has significantly improved predictability of the risk that invasion can occur, the predictive power of the impacts of invaders on native species and communities remains limited [3-5]. The most visible invasions, and those with the most damaging consequences for ecosystem services, tend to receive the greatest attention. These cases strongly bias our understanding of the impacts of invasions [6, 7], because impacts of invasive species can often, at least initially, be subtle and affect local processes and species interactions [8-10].

The conventional approach to invasion ecology has been to concentrate at the species level, but a conceptual shift to consider particular populations of an invasive species can provide deeper insights [3, 11]. This view recognizes that populations of a species are genetically and phenotypically variable across their range, with potentially different capacities for establishing and impacting local communities. Similarly, different populations of native species can vary in their susceptibility to the impact of invasions. From this perspective the impact of non-native species on local taxa will crucially depend on population-specific traits and adaptations, with potentially variable outcomes for different populations. The impact of invasive species is defined here in its broadest sense as any change to the recipient ecosystem [2], but primarily as any qualitative or quantitative change to the ecological or evolutionary characteristics of existing native populations and inter-specific relationships [5].

Here we use the association between bitterling fishes and unionid mussels to test whether interactions between native and invasive species vary in a population-specific

71   context. Bitterling (Acheilognathinae, Cyprinidae) are freshwater fishes that originate, and

72   show their greatest diversity and abundance, in East Asia [12]. All bitterling species lay their

73   eggs in the gills of living unionid mussels via their exhalant siphons and their embryos

74   complete development inside the mussel gill cavity, typically in one month. Most bitterling

75   fishes use several mussel species as host, but often express a preference for particular species

76   [13]. Host mussel preference may vary among bitterling populations [14, 15]. Hosting

77   bitterling embryos is costly to mussels and they have evolved adaptations to eject bitterling

78   eggs and embryos, mirrored by counter-adaptations in bitterling embryos to avoid ejection

79   [14, 16-18]. In addition, and independently of the bitterling utilization of mussel hosts,

80   unionid mussels possess a larval stage (glochidium) that must attach to a fish host (bitterling

81   or other species) to complete development. Female mussels discharge ripe larvae into the

82   water column where they attach to a host fish, remain encysted for several days and finally

83   metamorphose into juvenile mussels. Hosting mussel larvae is costly to fish [19], leading to

84   fish adaptations to reject them [20] and population-specific compatibility between native

85   mussels and their fish hosts [21]. The adaptations of mussels to eject bitterling eggs and

86   adaptations of fish to reject mussel larvae are independent, and different physiological

87   mechanisms are involved.

88   *Rhodeus amarus* is the only bitterling species in Europe, where its distribution is

89   natural and where it has been present for at least 2 millions years [22-24]. All other bitterling

90   species are restricted to East Asia where they are abundant [12]. *R. amarus* is a relatively

91   thermophilic species [25] and expanded across Europe from glacial refugia in the Pontic and

92   Mediterranean regions in warmer climatic periods of the Quaternary [22, 23, 26]. Two distinct

93   phylogeographic clades colonised much of continental Europe independently, each

94   originating from the same refugium. A Danubian clade colonized Central and Western Europe

95   via the Danube basin while a Baltic clade colonized Eastern and Northern Europe via the

96   Rivers Dnieper, Dniester, and Bug [22, 23]. Populations of *R. amarus* are generalists and use

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97   all native European unionid mussel species for oviposition but display preference for *Unio*

98   *tumidus*, *Unio pictorum*, and *Anodonta anatina* over *Anodonta cygnea* [15, 17, 27]. Native

99   unionid mussel populations across continental Europe express limited adaptations to eject or

100   avoid bitterling eggs compared to unionid populations in the Pontic region. This difference is

101   likely due to the shorter duration of their sympatry and lower encounter rate with *R. amarus*

102   in continental Europe than in the Pontic region where mussels routinely eject *R. amarus* eggs

103   [18]. *R. amarus* is not a suitable host of parasitic larvae of European mussels [28]; attached

104   larvae (both *Anodonta* and *Unio*) are typically rejected within the first day of infection and *R.*

105   *amarus* thereby avoid costs associated with mussel larvae infection [29].

106   *Anodonta woodiana* is a mussel native to a large region of East Asia where it is an

107   abundant and widely distributed species [30], commonly used for oviposition by several

108   bitterling species [31]. *A. woodiana* was introduced into European freshwaters in the 1970s,

109   with many new populations appeared during the 21st century [30, 32, 33]. The arrival of *A.*

110   *woodiana* in Europe has transformed the outcome of bitterling-mussel associations, with

111   indications of a potential disparity in the response of *R. amarus* to two isolated populations of

112   invasive *A. woodiana*. *R. amarus* readily used *A. woodiana* introduced to Poland (Baltic

113   region) for oviposition, while *A. woodiana* from this Polish population ejected bitterling eggs

114   before they completed development [34]. In contrast, another *A. woodiana* population

115   established in the Czech Republic (Danubian region) was avoided by sympatric *R. amarus* for

116   oviposition [35], which thereby escaped the negative impact of the egg ejection by the

117   invader. In addition, the Danubian population of *A. woodiana*, in contrast to all native

118   European mussel species, was readily able to use *R. amarus* as a host for its parasitic larvae,

119   effectively reversing the roles of host and parasite in the association [35]. These outcomes

120   suggest potential differences in the ecological impacts of the invasive mussel, depending on

121   the population-specific context.

Here we specifically tested population-specific impacts of *A. woodiana* on *R. amarus* populations by examining three key elements of the association. We used two genetically distinct invasive populations of *A. woodiana* (Baltic and Danubian) and measured their interactions with four *R. amarus* populations that varied in their prior exposure to *A. woodiana* (allopatric or recently sympatric to them), but which otherwise represented pairs of closely related populations from each of the two major phylogeographic clades of *R. amarus* (Baltic sympatric, Baltic allopatric, Danubian sympatric, Danubian allopatric) (figure 1, electronic supplementary material, table S1). To separate the role of population-specific traits from the effects of different environmental or community settings, we standardized test conditions for each combination of populations by using a common experimental environment.

With these populations we experimentally tested: 1. the capacity of *A. woodiana* larvae to develop on *R. amarus*; 2. the preference/avoidance response by *R. amarus* for oviposition in the gills of *A. woodiana*; 3. the impact on the reproductive success of *R. amarus* of the addition of *A. woodiana* to the mussel community. Given the high population-specificity of relationships with fish hosts in European unionids [21], we predicted a variable capacity of *A. woodiana* larvae to develop on *R. amarus* from different populations. We predicted significant avoidance of Danubian *A. woodiana*, but a maladaptive utilisation of Baltic *A. woodiana* mussels with ovipositions followed by egg ejection [34, 35]. Finally, we predicted that differences in the oviposition preferences for the two *A. woodiana* populations (avoidance vs. active use) would translate into population-specific impacts of the *A. woodiana* invasion in terms of bitterling recruitment. A decrease in reproductive success of *R. amarus* was predicted for the invasion of Baltic *A. woodiana*, but a limited impact by Danubian *A. woodiana*. The reason for the contrasting predictions was that Baltic *A. woodiana* was readily used by *R. amarus* for oviposition followed by complete egg ejection, while Danubian *A. woodiana* was avoided for oviposition, but still reduced the reproductive success of *R. amarus*

148   by increasing density-dependent mortality of eggs in more heavily parasitized native mussels

149   [36].

2. Materials and methods

(a) Study populations

152   Both allopatric populations of *R. amarus* were naive to *A. woodiana*, while sympatric

153   bitterling had been exposed to *A. woodiana* for several generations [32, 37]. Baltic and

154   Danubian *R. amarus* populations (belonging to distinct phylogeographic clades) were

155   predicted to have evolved different adaptations to use their native sympatric host mussels

156   [18]. The estimates of genetic divergence based on 9 nuclear microsatellite markers are F_{ST} =

157   0.321-0.494 ($p < 0.001$) for the difference between Baltic and Danubian populations [23]. The

158   Danubian pair of *R. amarus* populations originated from the adjacent Rivers Morava and

159   Kyjovka (Czech Republic) and were genetically similar ($F_{ST} = 0.006$, $p = 0.095$) [23], but

160   contemporary migration between them is prevented by regulation of the River Morava in the

161   1980s [38]. The Kyjovka *R. amarus* (i.e. Danubian sympatric) were exposed to *A. woodiana*

162   for at least 7 generations prior to their use in experiments. *Anodonta woodiana* was first

163   recorded in the River Kyjovka in 2005 and now comprises approximately 50% of the unionid

164   mussel community in the study stretch of the river [33]. In contrast, Morava *R. amarus* are

165   naive to *A. woodiana* (Danubian allopatric), with *A. woodiana* wholly absent from the River

166   Morava [37]. A Baltic population of *R. amarus* allopatric to *A. woodiana* was collected in the

167   Włocławek Reservoir on the River Vistula. A sympatric population was collected from Lake

168   Łichenskie, where *A. woodiana* was first recorded in the mid 1980s and is now abundant [32].

169   This makes sympatry between *A. woodiana* and *R. amarus* in the Baltic region about 30 *R.*

170   *amarus* generations and hence older than in the Danubian region. The two invasive *A.*

171   *woodiana* populations possess a moderate level of genetic differentiation ($F_{ST} = 0.074$, $p <$

172   0.001; supplementary electronic material).

(b) Experimental animals

Mussels were collected by hand from the River Kyjovka (Danubian *A. woodiana*, *Anodonta anatina*) and Lake Łichenskie (Baltic *A. woodiana*) (electronic supplementary material, table S1) and stored in large fibreglass containers before use in experiments. *Anodonta anatina* was used as the native mussel species in all experiments. This species was abundant at all the sites from which experimental *R. amarus* populations were collected and is typically used by *R. amarus* for oviposition [15, 27, 24]. Experimental *R. amarus* were collected by electrofishing (exact locations are given in electronic supplementary material, table S1).

(c) Exploitation of *R. amarus* by parasitic larvae of invasive *A. woodiana*

To test the capacity of *A. woodiana* to successfully metamorphose on *R. amarus*, mussels were collected from Lake Łichenskie and the River Kyjovka during July 2013. A mussel-opening device was used to non-destructively inspect the gills of mussels and females with ripening larvae were selected and transported to the lab in containers of aerated water. In the lab, mussels were separately held in 15 l containers until the spontaneous release of larvae [32]. The viability of larvae (a subset of 30 larvae for each mussel) was verified by evaluation of their snapping action in a sodium chloride solution prior to experimental infection.

Population level evaluation of *A. woodiana* larvae-*R. amarus* host compatibility was performed according to ref. [21]. We used larvae from one parent mussel to simultaneously infect 2-4 *R. amarus* from each source population. Altogether, we performed 14 experimental infections (seven female mussels per population) to infect a total of 120 *R. amarus* individuals (14-16 individuals for each *R. amarus*-*A. woodiana* population combination). Common infection tanks were used, which were subdivided into four sections using 3-mm plastic nets to prevent mixing of individuals from different source populations. The fish were infected in aerated suspensions (0.5 l per fish) with mean viable larvae densities of 2127 ± 1379 (mean ±

197   SD). After a 15 min exposure, fish were transferred into water without larvae for 30 min. to

198   rinse non-attached larvae. All fish were successfully infected.

199   Fish were subsequently placed individually into continuously aerated 18 l glass

200   aquaria (i.e. there was a single fish per aquarium, using a total of 120 aquaria), with the

201   bottom covered with a net (mesh size 3 mm) and monitored for larval development until the

202   end of their parasitic phase. Fish were fed daily with commercial flake fish food. Mean water

203   temperature was 22.2 °C (S.D. = 1.1) during the experiment. Parasitic larvae attached to fins

204   and gills of the fish and all completed their development (or were rejected) within two weeks.

205   Water was partially exchanged (approximately 80% of total water volume) and examined for

206   the presence of rejected larval mussel and metamorphosed juvenile mussels by siphoning the

207   tank daily for the period of two weeks. Rejected larvae and metamorphosed juvenile mussels

208   were collected from siphoned water using filters (mesh size 139 μm) and identified under a

209   microscope at 10-40x magnification. Mussels were scored as living juveniles if foot activity

210   or valve movement was observed. These methods enabled us to estimate both the absolute

211   number of juvenile mussels recovered from individual fish and the successful development of

212   initially attached *A. woodiana* larvae. The initial abundances were 44.4 ± 33.2 larvae (mean ±

213   s.d.) per gram of fish mass. The mortality of fish was less than 5 % during the experiment and

214   was not caused by *A. woodiana* larvae infestation.

215   Data on *A. woodiana* larvae transformation success were analysed using Generalized

216   Linear Model with a binomial error structure and log-link function. To account for infecting

217   several fish by larvae originating from the same mussel, we used Generalized Estimating

218   Equations (GEE) in the *geepack* package. The dependent variable was the ratio of

219   successfully transformed larvae to larvae rejected by host fish. The following factors and their

220   first-order interactions were included as explanatory variables: *Fish origin* (Danubian vs.

221   Baltic), *Mussel origin* (Danubian vs. Baltic), *Sympatry* (fish population sympatric or

222 allopatric to *A. woodiana*). Correlated observations from repeated use of the same parent

223 mussel as a source of larvae for experimental infection were accounted for by using an

224 'independence' correlation structure.

225 (d) Bitterling behavioural discrimination: mechanisms of *A. woodiana*

226 impact

227 The preference/avoidance of *R. amarus* to oviposit in the gills of *A. woodiana* and a native

228 mussel *A. anatina* were tested during May and June 2012, at the peak of the *R. amarus*

229 spawning season. The study with Danubian fish were conducted in the aquarium facility at the

230 Institute of Vertebrate Biology, Czech Republic, using aquaria measuring 750 x 400 x 400

231 mm. Experiments with Baltic fish were conducted at the University of Łodz, Poland, in

232 aquaria measuring 500 x 400 x 350 mm. Only an allopatric population of Baltic *R. amarus*

233 was tested for due to logistic reasons. Prior to their use in the experiment *R. amarus* were held

234 in large outdoor pools under natural light conditions and fed with a mixture of frozen

235 chironomid larvae and commercial fish food. During experiments water temperature varied

236 between 17 and 21 °C. The tanks contained a sand substrate and artificial plants as refuges

237 and were isolated by opaque barriers. For each replicate, two mussels (one native *A. anatina*,

238 one invasive *A. woodiana*) in separate sand-filled pots were placed at the centre of each tank,

239 0.35 m apart. Male *R. amarus* were introduced to the pools to examine the mussels and

240 initiate territorial behaviour. After at least 2 h, a female *R. amarus* in reproductive condition

241 (her readiness to oviposit indicated by an extended ovipositor) was introduced. Behavioural

242 recording commenced once the female had first approached and inspected a mussel.

243 Recording continued for 10 min. but was terminated at oviposition, if it occurred, since fish

244 behaviour changes post-oviposition and is not related to mussel preference [16]. Five

245 reproductive behaviours (male leading, sperm release, male inspection, female inspection,

246 female skimming) (detailed definitions in supplementary electronic material, table S2) were

247 recorded during observations, each clearly directed towards one of the two test mussels and

248 was interpreted as preference for that mussel [16]. Ovipositions, when they occurred, were

249 also recorded. A total of 102 paired replicates were completed; all subjects used only once,

250 giving a total of 102 unique pairs of *R. amarus* and 102 unique pairs of *A. woodiana* and *A.*

251 *anatina* mussels used in tests.

252   We tested whether fish from different *R. amarus* populations discriminated against *A.*

253 *woodiana* (i.e. behavioural avoidance). For behaviours directed towards non-native *A.*

254 *woodiana* and native control mussels (*A. anatina*), pair-wise differences in the rate of each

255 reproductive behaviour within a replicate were calculated. This approach generated a paired

256 design where one *A. woodiana* and one *A. anatina* were simultaneously presented to a pair of

257 *R. amarus*. Given strong collinearity between all 5 behavioural preference measures

258 (Spearman correlation, all $r_S > 0.36$, all $p < 0.001$, $n = 102$), the data matrix (i.e. pair-wise

259 differences for each reproductive behaviour) was simplified using Principal Component

260 Analysis (PCA). The first Principal Component (PC1) explained 60.8 % of variation

261 (eigenvalue = 3.04) and was the single best predictor of host mussel preference (electronic

262 supplementary material, table S3). All analyses were conducted using PC1 (named Preference

263 in the results) but the use of individual behaviours produced qualitatively identical results

264 (electronic supplementary material, figure S1). Least-squared means were calculated for PC1

265 for each *Fish origin* by *Mussel origin* combination. Negative mean values with 95%

266 confidence intervals that were non-overlapping with zero were interpreted as significant

267 avoidance of *A. woodiana* (and hence significant preference for *A. anatina* control),

268 confidence intervals overlapping zero indicated a lack of significant discrimination between

269 *A. woodiana* and *A. anatina*, and positive values with 95% confidence intervals non-

270 overlapping with zero denoted a preference for *A. woodiana* (and avoidance of *A. anatina*).

271 (e) Bitterling reproductive success: impact of *A. woodiana* invasion

272   The impact of the addition of *A. woodiana* to a unionid mussel community on the

273   reproductive success of *R. amarus* was tested in experimental ponds. The ponds comprised

274   large fibreglass outdoor pools (1.3 x 1.3 x 1.0 m) situated at the garden of the Institute of

275   Vertebrate Biology, Czech Republic. Each pond had a gravel substrate and was filled to a

276   depth of 0.6 m with water and furnished with artificial plants as refuges. Four sand-filled

277   plastic pots, each containing a mussel, were placed in the corner of each pond; pots kept

278   mussels in fixed positions but permitted them to adopt a natural position and to filter

279   normally. Under natural conditions at our study sites, *A. woodiana* comprise approximately

280   50% individuals in the unionid mussel community [32, 33]; therefore we experimentally

281   evaluated scenarios when 50% of native mussels were replaced by non-native *A. woodiana*.

282   This provided three levels of mussel community treatment; native community (4 individuals

283   of *A. anatina*), community invaded by Baltic *A. woodiana* (2 *A. anatina* and 2 Baltic *A.*

284   *woodiana*) and community invaded by Danubian *A. woodiana* (2 *A. anatina* and 2 Danubian

285   *A. woodiana*). Each mussel community treatment was replicated with both *R. amarus*

286   populations; sympatric and allopatric to *A. woodiana*. For logistical reasons Danubian and

287   Baltic *R. amarus* were tested in separate years. There were 7 replicates of each treatment

288   combination, resulting in 84 experimental populations tested over two spawning seasons.

289   Experimental *R. amarus* populations consisted of 5 males and 6 females. Experimental

290   fish foraged on natural food (algae, detritus, invertebrates) that established in experimental

291   ponds and were additionally fed daily with a mixture of frozen chironomid larvae and cyclops

292   nauplii, with an equal amount provided to each population. *Rhodeus amarus* were stocked on

293   11 May 2012 (Danubian fish) and 25 April 2014 (Baltic fish). A total of 420 male and 504

294   female *R. amarus*, 224 *A. anatina* and 56 Baltic and 56 Danubian *A. woodiana* were used.

295   Fish started to spawn approximately 2 (Danubian) and 4 (Baltic) weeks after stocking.

296   Experimental mussels were recovered from ponds on 8-12 June 2012 and 12-13 June 2014,

297   before *R. amarus* embryos had completed development. Mussel gills were dissected and all *R.*

298   *amarus* embryos were counted. A small number of juvenile *R. amarus* emerged from their

299   host mussels prior to mussel dissections (in a total of 6 pools in Baltic *R. amarus*). These were

300   collected from ponds and added to the sum of *R. amarus* embryos from their respective

301   populations.

302   To measure the impact of mussel community composition on *R. amarus* reproductive

303   success, the number of *R. amarus* embryos recovered from each experimental population was

304   tested with *Mussel community* (3 levels; native, invaded by Danubian *A. woodiana*, invaded

305   by Baltic *A. woodiana*) and *Fish population* (2 levels: sympatric, allopatric) as fixed factors.

306   For Danubian *R. amarus*, embryo abundance followed a normal distribution and a General

307   Linear Model (LM) was used. Data for Baltic *R. amarus* were initially tested using a Poisson

308   distribution but were found to be overdispersed and a quasi-Poisson distribution was used

309   (Generalized Linear Model with log-link function, GLM). There were some mortalities of

310   experimental *A. anatina* mussels during the experiment with Baltic *R. amarus*, distributed

311   randomly across treatments. Therefore, mussel mortality (the number of *A. anatina* mussels

312   that died before mussel dissection) was included as an additional covariate in the analysis.

313 2. Results

314 (a) Population-specific exploitation of *R. amarus* by invasive *A. woodiana*

315   The capacity of *A. woodiana* to successfully metamorphose on *R. amarus* differed among *R.*

316   *amarus* populations but not between *A. woodiana* populations (figure 2). Specifically,

317   Danubian *R. amarus* were considerably better hosts of *A. woodiana* than Baltic *R. amarus*

318   (GEE: Wald $\chi^2 = 46.7$, $p = 0.001$, $n_{balt} = 63$, $n_{dan} = 57$). Further, *A. woodiana* were more

319   successful in developing on allopatric *R. amarus* than sympatric *R. amarus* ($\chi^2 = 6.5$, $p =$

320   0.011, $n_{symp} = 60$, $n_{alop} = 60$), though the effect was smaller than for the effect of *R. amarus*

321   geographic origin. The two *A. woodiana* populations did not vary in their capacity to infect *R.*

322   *amarus* (χ^2 = 1.3, *p* = 0.263) and there was no significant interaction between the origin of *R.*

323   *amarus* and *A. woodiana* (χ^2 = 3.0, *p* = 0.085) (electronic supplementary material, figure S2).

324

325   (b) Population-specific impacts on native *R. amarus*: mechanisms and

326   consequences

327   *Rhodeus amarus* preference for host mussels revealed contrasting population-specific

328   responses (figure 3). *R. amarus* from the sympatric Danubian population showed different

329   responses to the two *A. woodiana* populations. They avoided locally sympatric Danubian *A.*

330   *woodiana* (*t*-test: t_{15} = 3.35, *p* = 0.005), but did not discriminate against unfamiliar Baltic *A.*

331   *woodiana* (t_{15} = 0.64, *p* = 0.502). The two allopatric *R. amarus* populations differed in their

332   response to the two *A. woodiana* populations. Danubian *R. amarus* avoided *A. woodiana*

333   (Danubian mussels: t_{16} = 2.47, *p* = 0.026; Baltic mussels: t_{16} = 2.67, *p* = 0.018), while Baltic

334   *R. amarus* did not discriminate against any *A. woodiana* population (Baltic *A. woodiana*: t_{22} =

335   1.34, *p* = 0.196; Danubian *A. woodiana*: t_{18} = 0.02, *p* = 0.981). Ovipositions were rare

336   (electronic supplementary material, table S4), but their distribution was congruent with the

337   behavioural preference score.

338   The presence of *A. woodiana* in the mussel community significantly decreased *R.*

339   *amarus* reproductive success (Danubian *R. amarus* populations, LM: $F_{2,36}$ = 3.34, *p* = 0.047;

340   Baltic *R. amarus* populations, GLM: $F_{2,39}$ = 3.98, *p* = 0.028). The fewest offspring were

341   recovered from the treatments with Baltic *A. woodiana* (figure 4, electronic supplementary

342   material, table S5). No significant effect of *R. amarus* sympatry with *A. woodiana* was

343   detected (sympatry: $F_{1,36}$ = 0.82, *p* = 0.372 and $F_{1,38}$ = 1.41, *p* = 0.243 for Danubian and Baltic

344   fish; interaction between mussel community and sympatry: $F_{2,36}$ = 1.80, *p* = 0.181 and $F_{2,35}$ =

345   1.39, *p* = 0.267, respectively). Mortality of native mussels had no effect on the outcome of

346   tests ($F_{2,35}$ = 0.23, *p* = 0.632).

347

4. Discussion

We demonstrated that interactions between native and invasive species can vary considerably among populations, yielding divergent outcomes and consequences of the interaction for both native and invasive species. The impact of the invasive species varied at two levels; both between geographically distinct lineages of the native species and, within these lineages, between local populations with contrasting histories of sympatry with the invader. At a geographic scale, the *R. amarus* - *A. woodiana* relationship in the Baltic region was more costly to both partners. Larvae of *A. woodiana* that parasitized Baltic *R. amarus* were less likely to successfully metamorphose into juvenile mussels, and Baltic *A. woodiana* imposed a greater reproductive cost on *R. amarus*. In contrast, the relationship proved relatively more benign in the Danubian region, where Danubian *R. amarus* were suitable hosts of *A. woodiana* larvae and with the fish avoiding *A. woodiana* as a host and thereby escaping the cost of egg ejections. At a local scale, behavioural discrimination against oviposition in an unsuitable non-native host, combined with higher resistance against parasitic larvae were detected in *R. amarus* sympatric with *A. woodiana*, implying a potential for rapid evolutionary response to the invader [8, 10] and partly mitigating its negative impacts. Given that our data come from a common garden experiment, the source of inter-population variation was attributable to the experimental populations and did not result from natural variation in environmental conditions or community structure. One caveat to this conclusion is that the use of wild-caught individuals did not permit us to separate genetic and maternal effects.

The impacts of invasive species may be strongly context-dependent and highly variable, both in the magnitude and direction of response [39]. It is generally assumed that context-dependency arises from climatic, environmental and community settings that naturally vary among regions where a species has invaded. However, we demonstrated that

373   variation in the impact of an invasion can derive from innate characteristics of populations.

374   The impacts of invasive species on local communities can often be precipitated via subtle

375   processes between intimately interacting species [8, 40, 41]. These relationships are often

376   characterized by coevolution, when an adaptation of one partner is matched by adaptation in

377   the second. Coevolutionary associations are inherently dynamic and, across species ranges,

378   they proceed at varying rates, generating a diverse geographic mosaic of variable states [42].

379   Species translocations can disrupt coevolved adaptations, exposing both native and non-native

380   species to novel interactions [9, 40].

381   We propose that coevolutionary dynamics within native communities may predispose

382   different populations to divergent responses to an invading species, with variation in

383   consequences. Coevolutionary dynamics have rarely been considered as modulating impacts

384   in invasion biology [43], but may present an important source of variation in outcomes. Our

385   experimental system was not suited to replication across a higher number of population

386   combinations and it may be argued that stochastic processes unrelated to coevolutionary

387   dynamics may have produced the observed pattern. Other systems with fine-scale

388   coevolutionary dynamics, such as plant-insect interactions (e.g. pollination, seed dispersal),

389   are also prone to perturbations from invasions of non-native species and may be easier to

390   replicate across more populations with a more reasonable cost and effort.

391   Several other examples indicate, at least indirectly, the potential importance of

392   coevolutionary dynamic states on the impact of invasions. In an example conceptually

393   matching the *R. amarus-A. woodiana* scenario, *Anguillicoloides crassus,* a nematode parasite

394   of East Asian eels (*Anguilla japonica*), caused massive mortalities of the European eel

395   *Anguilla anguilla* when *A. crassus* was introduced to Europe. As a parasite that apparently

396   coevolved to an equilibrium with a local population of *A. japonica* in its native range, its

397   virulence is lethal for evolutionarily naive *A. anguilla* hosts [43]. The introduction of *A.*

398   *crassus* to North America resulted in infections of the American eel, *Anguilla rostrata*, but

399   the impact of *A. crassus* on *A. rostrata*, while less understood, appears more limited than the

400   impact on *A. anguilla* [44]. Similarly, a monogenean parasite *Gyrodactylus salaris* is not

401   lethal to Baltic populations of the Atlantic salmon, *Salmo salar*, but caused substantial

402   mortalities once introduced into East Atlantic populations of *S. salar* [45].

403   Except for parasites invading new ranges (i.e. emerging infectious diseases), where the

404   impacts are apparent and often have acute consequences, such cases have rarely been

405   documented. We propose that coevolutionarily dynamic states between mutually interacting

406   species may actually play an important role in influencing the magnitude and direction of the

407   impacts of invasions. This perspective also recognizes the invasion of non-native genotypes

408   within an established species range [11], which may often go undetected but could have

409   important consequences for community structure and interspecific associations [46].

410   Impacts of invasive species also vary in time [40], and ecological and evolutionary

411   processes have been implicated as the source of this variation [47]. We have shown that *R.*

412   *amarus* populations that were sympatric with the invasive mussel were more efficient in

413   rejecting their parasitic larvae than evolutionary and ecologically naive allopatric *R. amarus*

414   populations. An initial rapid establishment and strong negative impact on native species can

415   stabilize after the initial population expansion by the invader [e.g. 48], though chronic effects

416   can persist and many impacts can be irreversible [41, 47]. Adaptive responses to invasive

417   populations can evolve relatively rapidly. For example, native anole lizards, *Anolis*

418   *carolinensis*, have adapted to a niche shift following invasion of a competitor, *Anolis sagrei*,

419   to Florida with significant changes in ecology and morphology over less than 20 generations

420   [10]. Likewise, evolutionary change in invasive populations that resulted in a decrease in their

421   impact on native species have been reported [49]. In the case of *R. amarus*, a sympatric

422   population appeared capable of discriminating against the invasive host mussel and avoided it

423   for oviposition, despite not being able to discriminate against the other, unfamiliar population

424   of the same invasive species.

425   An understanding of alternative effects of invasive species across space and time still

426   represents a major challenge for invasion science. Our results illustrate the inherent difficulty

427   in predicting the impact of a non-native species by demonstrating that fine-scale population-

428   specific attributes arising from local adaptation and fine-scale coevolutionary dynamics can

429   play a major role in driving outcomes. While an ability to predict the impact of non-native

430   species is a prerequisite for the successful management of biological invasions [1], achieving

431   this goal is not straightforward [4]. We argue here that a shift from a species-centred to a

432   more population-centred perspective of invasion may provide deeper insights into the success

433   and impacts of biological invasions.

434

451   **Conflict of interest.** We have no competing interests.

452

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Figure Legends

Figure 1. Native range of Asian *Anodonta woodiana* and European *Rhodeus amarus*, with an inset illustrating location of Baltic and Danubian populations used in the study and the distribution of two major *R. amarus* clades in Europe.

Figure 2. The success rate (mean and 95% confidence intervals) of parasitic larvae metamorphosis on each *R. amarus* population. Significant differences are denoted by asterisks. Note that there was no difference between the two *A. woodiana* populations and data for Danubian and Baltic *A. woodiana* populations were pooled. Results across the full population-specific design are shown in the electronic supplementary material.

Figure 3. Population specificity in behavioural discrimination of *A. woodiana* by *R. amarus* prior to oviposition. Paired contrasts in preference tests between *A. woodiana* and native *A. anatina*. Mean and 95% confidence intervals of preference score (significant avoidance denoted by asterisk) are shown, significant avoidance of *A. woodiana* is indicated by an asterisk.

Figure 4. Impact of mussel community on the reproductive success of *R. amarus* populations. Mean (+ 1 s.e.) number of *R. amarus* recovered from experimental ponds. Baltic and Danubian *R. amarus* were tested separately in different years.
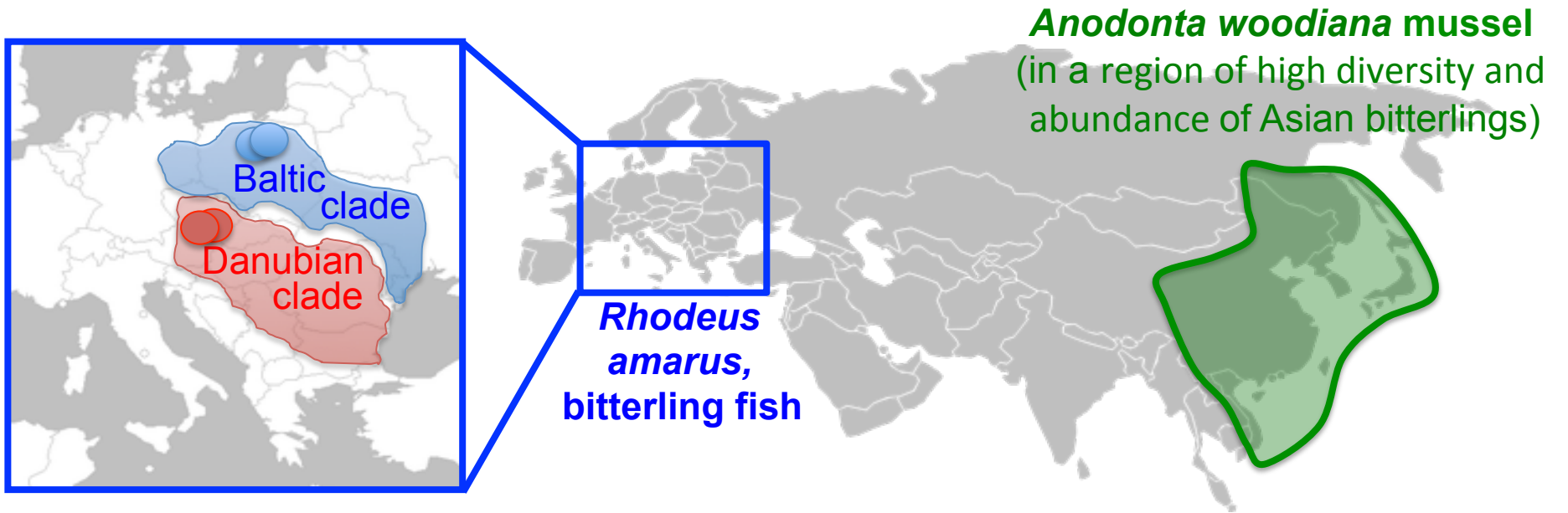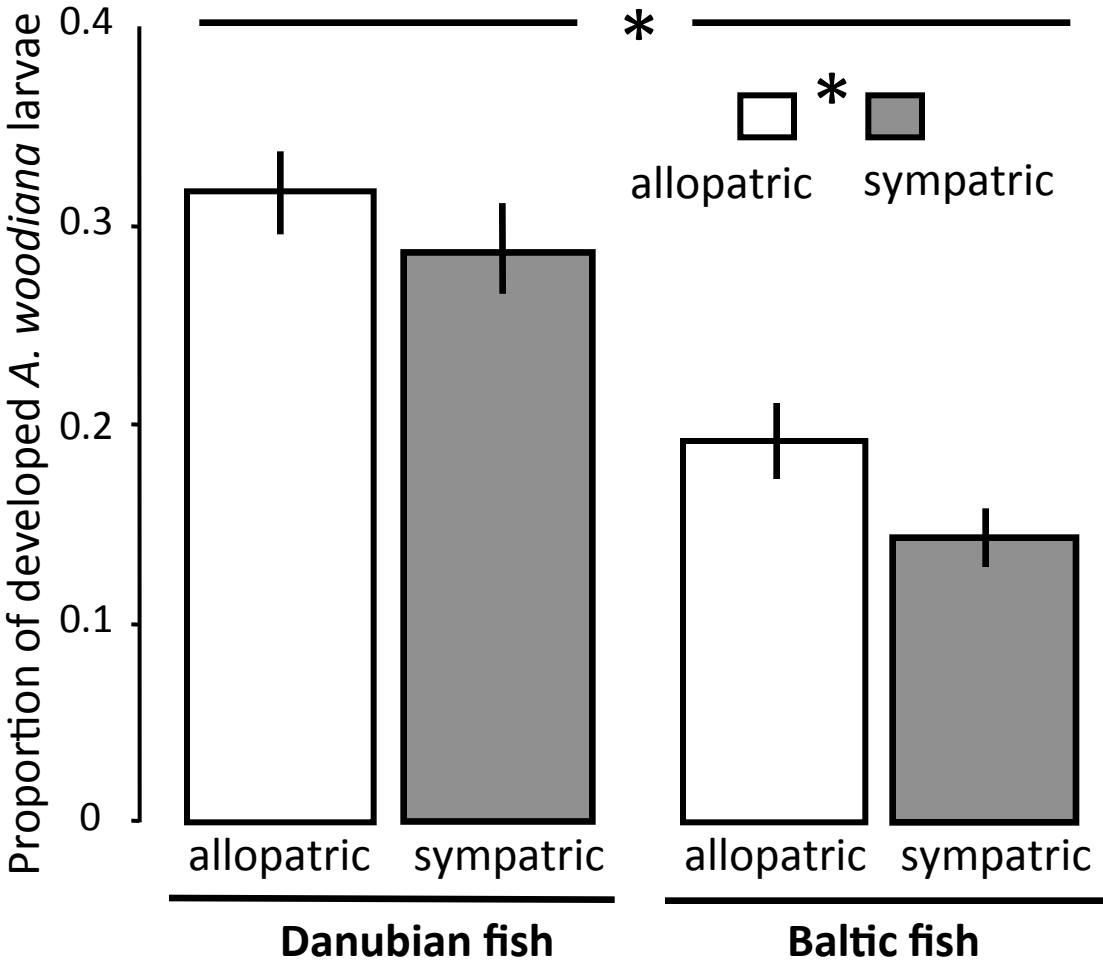
Fig. 1

Fig. 2

Fig. 3

Fig. 4

Danubian fish

Number of offspring

Danubian Baltic Native only

Baltic fish

Number of offspring

Danubian Baltic Native only

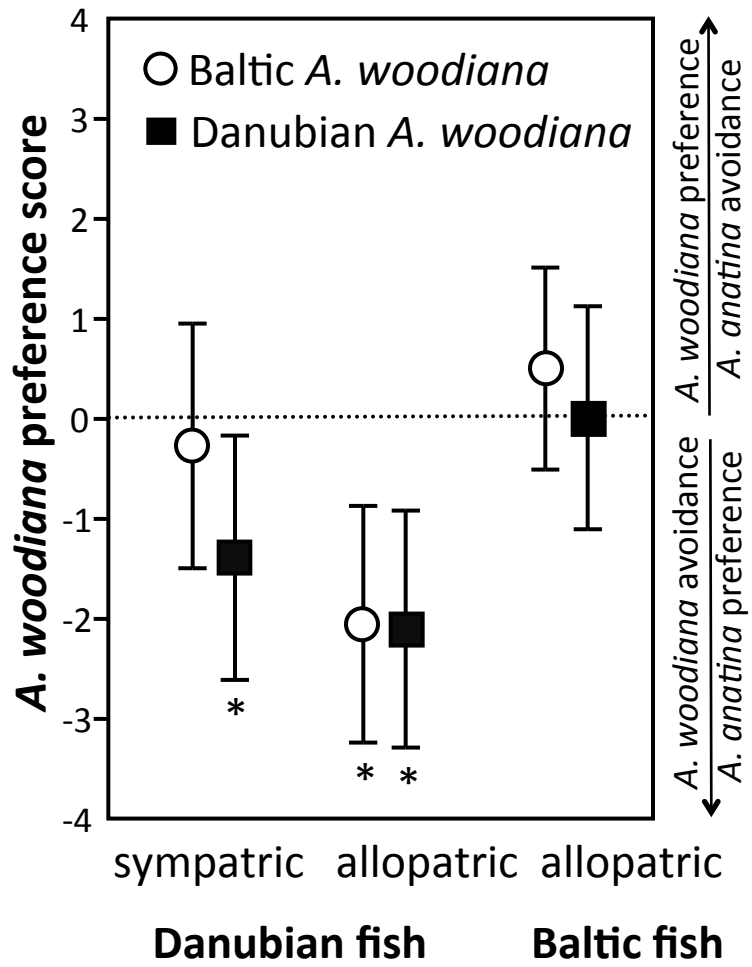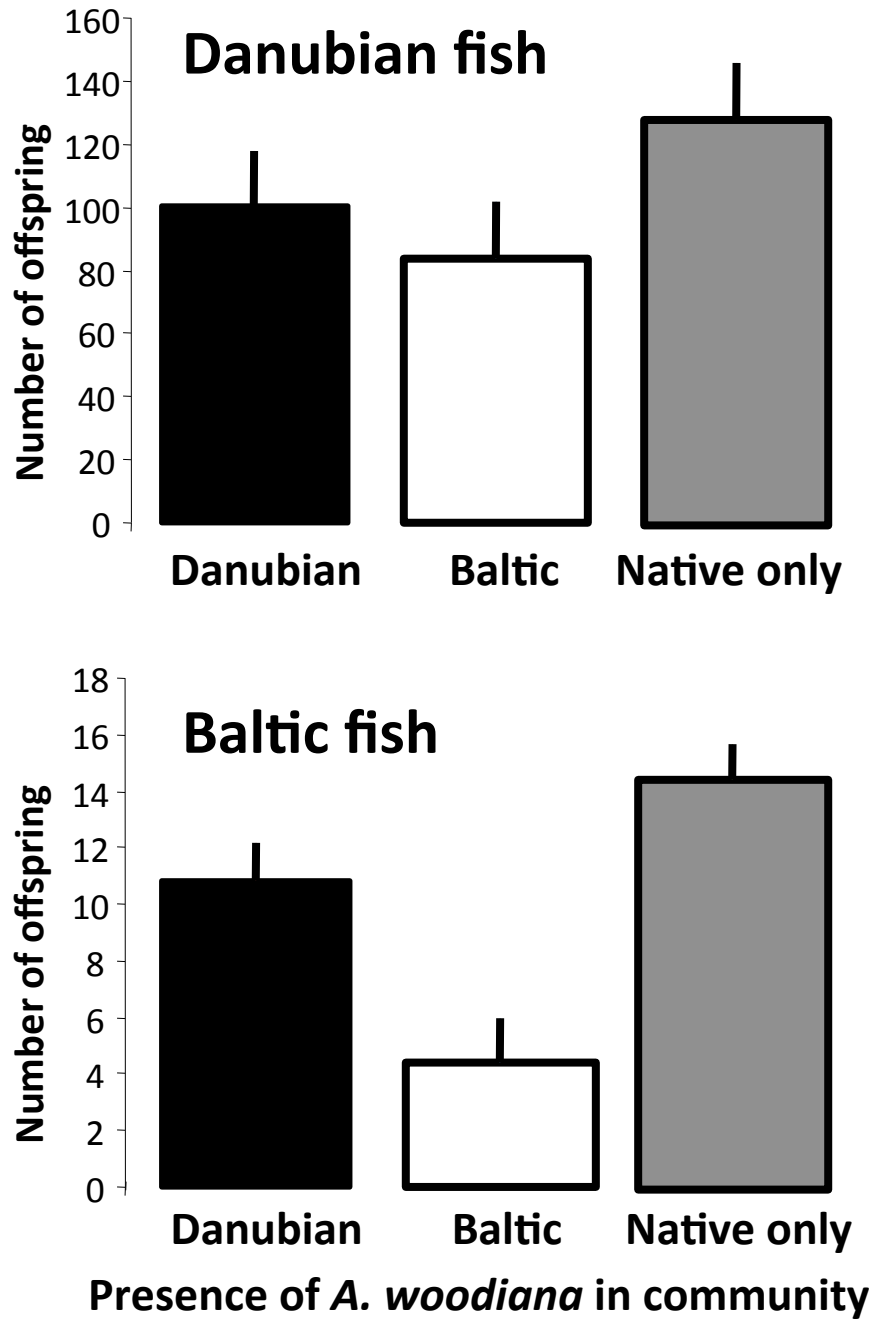Presence of *A. woodiana* in community

1   **<u>Electronic Supplementary Material</u>**

2

3   **Table S1.** Collection sites for all *Rhodeus amarus* and unionid mussel populations

4   used in the study.

Collection site	*A. woodiana* presence	GPS coordinates	
Czech Republic (Danubian region)		Latitude	Longitude
River Kyjovka	Yes (Sympatric)	N 48° 46' 45''	E 17° 01' 00''
River Morava	No (Allopatric)	N 48° 48' 43''	E 17° 00' 07''
Poland (Baltic region)			
Lake Łichenskie	Yes (Sympatric)	N 52° 20' 22''	E 18° 21' 28''
River Vistula	No (Allopatric)	N 52° 37' 38''	E 19° 18' 47''

5

6

Estimates of genetic differentiation in *R. amarus* and *A. woodiana*

Genetic differentiation was calculated using a standard estimation method of Weir & Cockerham [50] of the F_{ST} index as implemented in the GenAlEx software [51]. All details are fully described in [23]. Reported estimates are based on 9 nuclear microsatellite markers [23]. For *R. amarus*, pairwise differences among three populations are published [23]. *Rhodeus amarus* from Lake Łichenskie (Baltic sympatric) were not included in [23] at all and *R. amarus* from the River Vistula (Baltic allopatric) were not genotyped at cytochrome *B* in [23]. We genotyped 3 individuals for each Baltic population from samples we collected during this study at cytochrome *B*. The sequences from the two populations were identical, suggesting very close genetic relatedness of Baltic sympatric and Baltic allopatric *R. amarus* populations.

For *A. woodiana*, genetic differentiation was calculated using the same method [50, 51] as for *R. amarus*, based on the same number (9) of microsatellite markers. All microsatellite loci used were polymorphic, with 3-10 alleles per locus, observed (expected) heterozygosity was 0.629 (0.610) and 0.773 (0.683) for Danubian and Baltic populations respectively. There was no departure from the Hardy–Weinberg equilibrium.

27   **Table S2.** Reproductive behaviours recorded during observations, each demonstrating

28   a preference for a particular mussel.

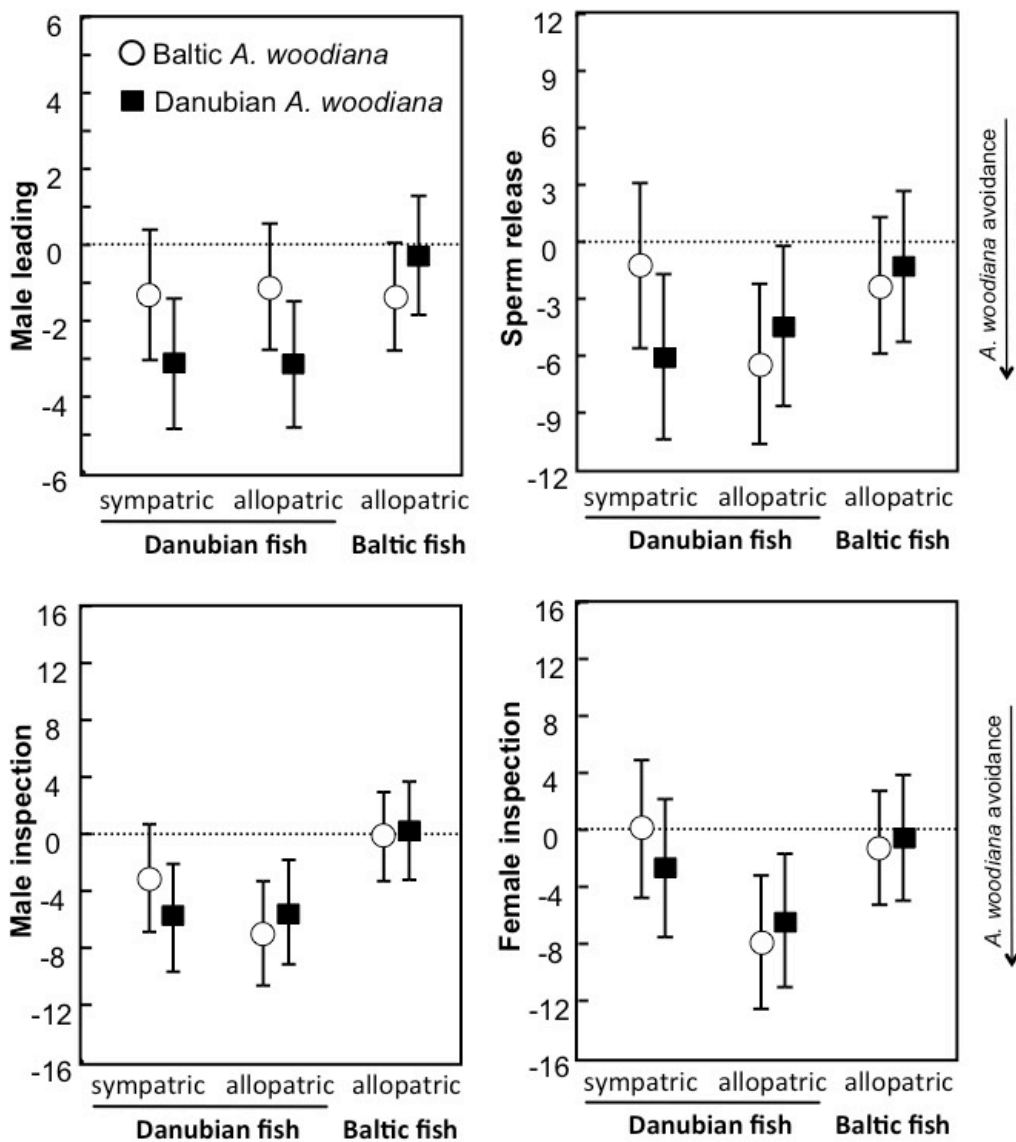| Behaviour	Description
Male leading	Males leads a female toward a mussel as a part of male courtship behaviour to which females respond by either following the male or leaving his territory.
Sperm release	A common reproductive behaviour in *R. amarus*, both before and after oviposition. It indicates direct male investment in a particular mussel. The male sweeps forward and down over mussel inhalant siphon and releases sperm.
Male inspection	Male inspects a mussel siphon by placing its snout close to the exhalant siphon of the mussel, apparently sampling water leaving the mussel, which may provide information relevant to oviposition decisions.
Female inspection	Female inspects a mussel siphon identically to male inspection. Female inspection is a typical prelude to oviposition
Skimming	Female performs a trial oviposition act but without inserting her ovipositor into the mussel siphon and with no eggs released.

29

30

31   **Table S3.** Principal Component Analysis (PCA) for behavioural preference of

32   oviposition site.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.04	0.91	0.57	0.35	0.13
Proportion of variance explained	60.79	18.20	11.37	6.98	2.66
Male leading	-0.570	0.708	0.380	0.171	-0.024
Male sperm release	-0.855	0.018	0.105	-0.507	-0.024
Male inspection	-0.694	0.338	-0.633	0.060	-0.010
Female inspection	-0.894	-0.316	0.082	0.146	0.272
Female skimming	-0.839	-0.442	0.071	0.195	-0.241

33

34   Factorial loading, eigenvalue and proportion of variance explained for each Principal

35   Component (PC) and correlation loading between each PC and individual behaviours.

36   PC1 has been used as a measure of mussel preference in the main analysis.
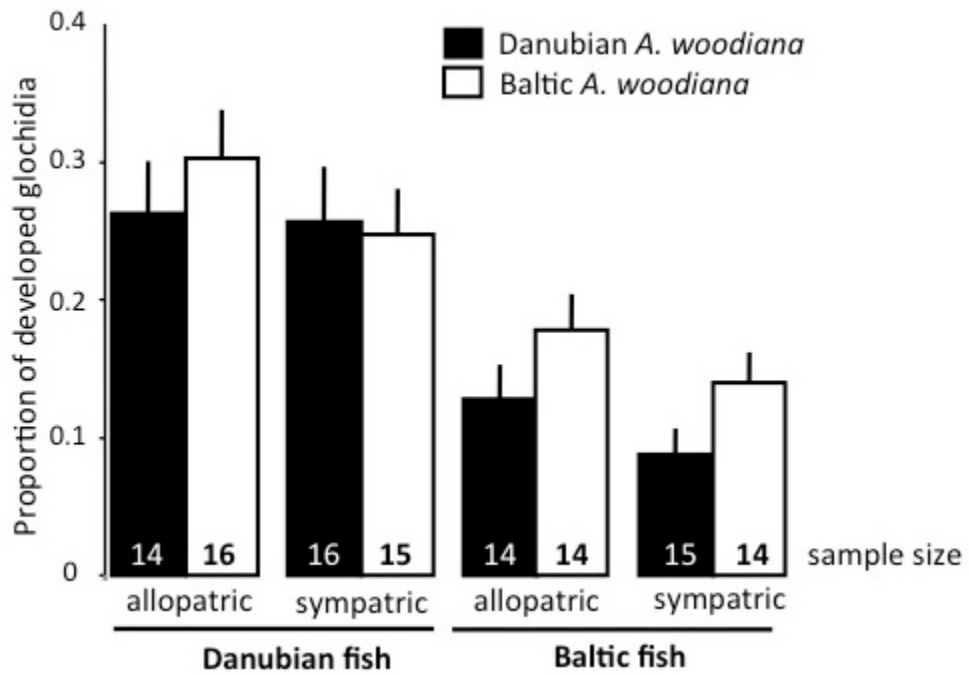
37 **Figure S1.** Population specificity in behavioural discrimination of *A. woodiana* by *R.*

38 *amarus* prior to oviposition analysed individually for four reproductive behaviours.

39 Skimming behaviour was too rare to analyse separately. Paired contrasts in preference

40 tests between *A. woodiana* and native *A. anatina* are shown. Mean and 95%

41 confidence intervals of preference scores (significant avoidance is denoted by no

42 overlap of the confidence interval with the dotted line).

43

44

45 **Figure S2.** The success rate of glochidia metamorphosis for each *A. woodiana* by *R.*

46 *amarus* population combination. Note that there was no difference between source *A.*

47 *woodiana* populations within each *R. amarus* populations.

48

49

50 **Table S4.** The number of ovipositions in native *A. anatina* and non-native *A.*

51 *woodiana* from Poland (Baltic *A. woodiana*) or the Czech Republic (Danubian *A.*

52 *woodiana*).

Rhodeus amarus population	*Anodonta anatina*	Baltic *A. woodiana*	$n^{\#}$	*Anodonta anatina*	Danubian *A. woodiana*	$n^{\#}$
Baltic allopatric	8	5	(22)	6	4	(18)
Danubian allopatric	6	2	(15)	5	0	(15)
Danubian sympatric	2	1	(16)	3	1	(16)

53
54 [#]number of behavioural observations for each combination of *R. amarus* and *A.*

55 *woodiana*. Note that ovipositions were observed only in a subset of replicates.

56

57

58 **Table S5.** Estimates of reproductive success of *R*. *amarus* coexisting with three

59 mussel communities.

Mussel community	Estimate	S.E.	*t*-value	P*
(a) Danubian fish (normal distribution)				
Native only	128.8	12.63	N.A.	N.A.
Invaded by Danubian *A. woodiana*	-28.4	17.87	-1.59	0.121
Invaded by Baltic *A. woodiana*	-44.9	17.87	-2.51	0.016
(b) Baltic fish (quasi-Poisson distribution)				
Native only	2.892	0.283	N.A.	N.A.
Invaded by Danubian *A. woodiana*	-0.375	0.354	-1.06	0.297
Invaded by Baltic *A. woodiana*	-1.269	0.476	-2.67	0.011
Anodonta anatina mortality	-0.203	0.176	-1.15	0.256

60

61 *Helmert contrasts with native community.

62 Native only: four *A. anatina*; Danubian: two *A. anatina* with two Danubian *A.*

63 *woodiana*; Baltic: two *A. anatina* with two Baltic *A. woodiana*. Sympatric and

64 allopatric fish populations were pooled given their response to mussel communities

65 was not statistically different.

66

67   **Supplementary references**

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