



Research

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Population-specific responses to an invasive species

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Predicting the impacts of non-native species remains a challenge. As populations of a species are genetically and phenotypically variable, the impact of non-native species on local taxa could crucially depend on population-specific traits and adaptations of both native and non-native species. Bitterling fishes are brood parasites of unionid mussels and unionid mussels produce larvae that parasitize fishes. We used common garden experiments to measure three key elements in the bitterling–mussel association among two populations of an invasive mussel (*Anodonta woodiana*) and four populations of European bitterling (*Rhodeus amarus*). The impact of the invasive mussel varied between geographically distinct *R. amarus* lineages and between local populations within lineages. The capacity of parasitic larvae of the invasive mussel to exploit *R. amarus* was higher in a Danubian than in a Baltic *R. amarus* lineage and in allopatric than in sympatric *R. amarus* populations. Maladaptive oviposition by *R. amarus* into *A. woodiana* varied among populations, with significant population-specific consequences for *R. amarus* recruitment. We suggest that variation in coevolutionary states may predispose different populations to divergent responses. Given that coevolutionary relationships are ubiquitous, population-specific attributes of invasive and native populations may play a critical role in the outcome of invasion. We argue for a shift from a species-centred to population-centred perspective of the impacts of invasions.

1. Introduction

Cases of biological invasions, where species are translocated to new geographical 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 interspecific 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 context. Bitterling (Acheilognathinae, Cyprinidae) are freshwater fishes that originate, and show their greatest diversity and abundance, in East Asia [12]. All bitterling species lay their eggs in the gills of living unionid mussels via their exhalant siphons and their embryos complete development inside the mussel gill cavity, typically in one month. Most bitterling fishes use several mussel species as hosts, but often express a preference for particular species [13]. Host mussel preference may vary among bitterling populations [14,15]. Hosting bitterling embryos is costly to mussels and they have evolved adaptations to eject bitterling eggs and embryos, mirrored by counter-adaptations in bitterling embryos to avoid ejection [14,16–18]. In addition, and independently of the bitterling utilization of mussel hosts, unionid mussels possess a larval stage (glochidium) that must attach to a fish host (bitterling or other species) to complete development. Female mussels discharge ripe larvae into the water column where they attach to a host fish, remain encysted for several days and finally metamorphose into juvenile mussels. Hosting mussel larvae is costly to fish [19], leading to fish adaptations to reject them [20] and population-specific compatibility between native mussels and their fish hosts [21]. The adaptations of mussels to eject bitterling eggs and adaptations of fish to reject mussel larvae are independent, and different physiological mechanisms are involved.

Rhodeus amarus is the only bitterling species in Europe, where its distribution is natural and where it has been present for at least 2 Myr [22–24]. All other bitterling species are restricted to East Asia where they are abundant [12]. *Rhodeus amarus* is a relatively thermophilic species [25] and expanded across Europe from glacial refugia in the Pontic and Mediterranean regions in warmer climatic periods of the Quaternary [22,23,26]. Two distinct phylogeographic clades colonized much of continental Europe independently, each originating from the same refugium. A Danubian clade colonized central and western Europe via the Danube basin, whereas a Baltic clade colonized eastern and northern Europe via the Rivers Dnieper, Dniester and Bug [22,23]. Populations of *R. amarus* are generalists and use all native European unionid mussel species for oviposition but display preference for *Unio tumidus*, *Unio pictorum* and *Anodonta anatina* over *Anodonta cygnea* [15,17,27]. Native unionid mussel populations across continental Europe express limited adaptations to eject or avoid bitterling eggs compared with unionid populations in the Pontic region. This difference is probably owing to the shorter duration of their sympatry and lower encounter rate with *R. amarus* in continental Europe than in the Pontic region where mussels routinely eject *R. amarus* eggs [18].

Rhodeus amarus is not a suitable host of parasitic larvae of European mussels [28]; attached larvae (both *Anodonta* and *Unio*) are typically rejected within the first day of infection and *R. amarus* thereby avoid costs associated with mussel larvae infection [29].

Anodonta woodiana is a mussel native to a large region of East Asia where it is an abundant and widely distributed species [30], commonly used for oviposition by several bitterling species [31]. *Anodonta woodiana* was introduced into European freshwaters in the 1970s, with many new populations appearing during the twenty-first century [30,32,33]. The arrival of *A. woodiana* in Europe has transformed the outcome of bitterling–mussel associations, with indications of a potential disparity in the response of *R. amarus* to two isolated populations of invasive *A. woodiana*. *Rhodeus amarus* readily used *A. woodiana* introduced to Poland (Baltic region) for oviposition, while *A. woodiana* from this Polish population ejected bitterling eggs before they completed development [34]. By contrast, another *A. woodiana* population established in the Czech Republic (Danubian region) was avoided by sympatric *R. amarus* for oviposition [35], which thereby escaped the negative impact of the egg ejection by the invader. In addition, the Danubian population of *A. woodiana*, in contrast to all native European mussel species, was readily able to use *R. amarus* as a host for its parasitic larvae, effectively reversing the roles of host and parasite in the association [35]. These outcomes suggest potential differences in the ecological impacts of the invasive mussel, depending on 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 and 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: (i) the capacity of *A. woodiana* larvae to develop on *R. amarus*; (ii) the preference/avoidance response by *R. amarus* for oviposition in the gills of *A. woodiana*; and (iii) 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 utilization 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 versus 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

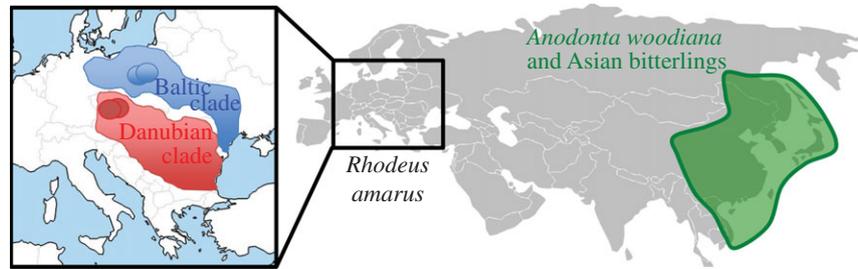


Figure 1. Native range of Asian *A. woodiana* and European *R. amarus*, with an inset illustrating location of Baltic and Danubian populations used in the study and the distribution of two major *R. amarus* clades in Europe. (Online version in colour.)

followed by complete egg ejection, whereas Danubian *A. woodiana* was avoided for oviposition, but still reduced the reproductive success of *R. amarus* by increasing density-dependent mortality of eggs in more heavily parasitized native mussels [36].

2. Material and methods

(a) Study populations

Both allopatric populations of *R. amarus* were naive to *A. woodiana*, while sympatric bitterling had been exposed to *A. woodiana* for several generations [32,37]. Baltic and Danubian *R. amarus* populations (belonging to distinct phylogeographic clades) were predicted to have evolved different adaptations to use their native sympatric host mussels [18]. The estimates of genetic divergence based on nine nuclear microsatellite markers are $F_{ST} = 0.321\text{--}0.494$ ($p < 0.001$) for the difference between Baltic and Danubian populations [23]. The Danubian pair of *R. amarus* populations originated from the adjacent Rivers Morava and Kyjovka (Czech Republic) and were genetically similar ($F_{ST} = 0.006$, $p = 0.095$) [23], but contemporary migration between them is prevented by regulation of the River Morava in the 1980s [38]. The Kyjovka *R. amarus* (i.e. Danubian sympatric) were exposed to *A. woodiana* for at least seven generations prior to their use in experiments. *Anodonta woodiana* was first recorded in the River Kyjovka in 2005 and now comprises approximately 50% of the unionid mussel community in the study stretch of the river [33]. By contrast, Morava *R. amarus* are naive to *A. woodiana* (Danubian allopatric), with *A. woodiana* wholly absent from the River Morava [37]. A Baltic population of *R. amarus* allopatric to *A. woodiana* was collected in the Włocławek Reservoir on the River Vistula. A sympatric population was collected from Lake Lichenskie, where *A. woodiana* was first recorded in the mid-1980s and is now abundant [32]. This makes sympatry between *A. woodiana* and *R. amarus* in the Baltic region about 30 *R. amarus* generations and hence older than in the Danubian region. The two invasive *A. woodiana* populations possess a moderate level of genetic differentiation ($F_{ST} = 0.074$, $p < 0.001$; electronic supplementary material).

(b) Experimental animals

Mussels were collected by hand from the River Kyjovka (Danubian *A. woodiana*, *A. anatina*) and Lake Lichenskie (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 the electronic supplementary material, table S1).

(c) Exploitation of *Rhodeus amarus* by parasitic larvae of invasive *Anodonta woodiana*

To test the capacity of *A. woodiana* to successfully metamorphose on *R. amarus*, mussels were collected from Lake Lichenskie 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 laboratory in containers of aerated water. In the laboratory, 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 Douđa *et al.* [21]. We used larvae from one parent mussel to simultaneously infect two to four *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 \pm s.d.). After a 15 min exposure, fish were transferred into water without larvae for 30 min to rinse non-attached larvae. All fish were successfully infected.

Fish were subsequently placed individually into continuously aerated 18 l glass aquaria (i.e. there was a single fish per aquarium, using a total of 120 aquaria), with the bottom covered with a net (mesh size 3 mm) and monitored for larval development until the end of their parasitic phase. Fish were fed daily with commercial flake fish food. Mean water temperature was 22.2°C (s.d. = 1.1) during the experiment. Parasitic larvae attached to fins and gills of the fish and all completed their development (or were rejected) within two weeks. Water was partially exchanged (approx. 80% of total water volume) and examined for the presence of rejected larval mussel and metamorphosed juvenile mussels by siphoning the tank daily for the period of two weeks. Rejected larvae and metamorphosed juvenile mussels were collected from siphoned water using filters (mesh size 139 μm) and identified under a microscope at 10–40 \times magnification. Mussels were scored as living juveniles if foot activity or valve movement was observed. These methods enabled us to estimate both the absolute number of juvenile mussels recovered from individual fish and the successful development of initially attached *A. woodiana* larvae. The initial abundances were 44.4 ± 33.2 larvae (mean \pm s.d.) per gram of fish mass. The mortality of fish was less than 5% during the experiment and was not caused by *A. woodiana* larvae infestation.

Data on *A. woodiana* larvae transformation success were analysed using generalized linear model with a binomial error structure and log-link function. To account for infecting several fish by larvae originating from the same mussel, we used generalized estimating equations (GEE) in the *geepack* package.

The dependent variable was the ratio of successfully transformed larvae to larvae rejected by host fish. The following factors and their first-order interactions were included as explanatory variables: *fish origin* (Danubian versus Baltic), *mussel origin* (Danubian versus Baltic), *sympatry* (fish population sympatric or allopatric to *A. woodiana*). Correlated observations from repeated use of the same parent mussel as a source of larvae for experimental infection were accounted for by using an 'independence' correlation structure.

(d) Bitterling behavioural discrimination: mechanisms of *Anodonta woodiana* impact

The preference/avoidance of *R. amarus* to oviposit in the gills of *A. woodiana* and a native mussel *A. anatina* were tested during May and June 2012, at the peak of the *R. amarus* spawning season. The study with Danubian fish were conducted in the aquarium facility at the Institute of Vertebrate Biology, Czech Republic, using aquaria measuring 750 × 400 × 400 mm. Experiments with Baltic fish were conducted at the University of Lodz, Poland, in aquaria measuring 500 × 400 × 350 mm. Only an allopatric population of Baltic *R. amarus* was tested owing to logistic reasons. Prior to their use in the experiment, *R. amarus* were held in large outdoor pools under natural light conditions and fed with a mixture of frozen chironomid larvae and commercial fish food. During experiments, water temperature varied between 17 and 21°C. The tanks contained a sand substrate and artificial plants as refuges and were isolated by opaque barriers. For each replicate, two mussels (one native *A. anatina* and one invasive *A. woodiana*) in separate sand-filled pots were placed at the centre of each tank, 0.35 m apart. Male *R. amarus* were introduced to the pools to examine the mussels and initiate territorial behaviour. After at least 2 h, a female *R. amarus* in reproductive condition (her readiness to oviposit indicated by an extended ovipositor) was introduced. Behavioural recording commenced once the female had first approached and inspected a mussel. Recording continued for 10 min but was terminated at oviposition, if it occurred, as fish behaviour changes post-oviposition and is not related to mussel preference [16]. Five reproductive behaviours (male leading, sperm release, male inspection, female inspection and female skimming) (detailed definitions in the electronic supplementary material, table S2) were recorded during observations, each clearly directed towards one of the two test mussels and was interpreted as preference for that mussel [16]. Ovipositions, when they occurred, were also recorded. A total of 102 paired replicates were completed; all subjects used only once, giving a total of 102 unique pairs of *R. amarus* and 102 unique pairs of *A. woodiana* and *A. anatina* mussels used in tests.

We tested whether fish from different *R. amarus* populations discriminated against *A. woodiana* (i.e. behavioural avoidance). For behaviours directed towards non-native *A. woodiana* and native control mussels (*A. anatina*), pairwise differences in the rate of each reproductive behaviour within a replicate were calculated. This approach generated a paired design where one *A. woodiana* and one *A. anatina* were simultaneously presented to a pair of *R. amarus*. Given strong collinearity between all five behavioural preference measures (Spearman's correlation, all $r_s > 0.36$, all $p < 0.001$, $n = 102$), the data matrix (i.e. pairwise differences for each reproductive behaviour) was simplified using principal component (PC) analysis. The first principal component (PC1) explained 60.8% of variation (eigenvalue = 3.04) and was the single best predictor of host mussel preference (electronic supplementary material, table S3). All analyses were conducted using PC1 (named Preference in the results) but the use of individual behaviours produced qualitatively identical results (electronic supplementary material, figure S1). Least-squared means were calculated for PC1 for each *fish origin* by

mussel origin combination. Negative mean values with 95% CIs that were non-overlapping with zero were interpreted as significant avoidance of *A. woodiana* (and hence significant preference for *A. anatina* control), confidence intervals overlapping zero indicated a lack of significant discrimination between *A. woodiana* and *A. anatina*, and positive values with 95% CIs non-overlapping with zero denoted a preference for *A. woodiana* (and avoidance of *A. anatina*).

(e) Bitterling reproductive success: impact of *Anodonta woodiana* invasion

The impact of the addition of *A. woodiana* to a unionid mussel community on the reproductive success of *R. amarus* was tested in experimental ponds. The ponds comprised large fibreglass outdoor pools (1.3 × 1.3 × 1.0 m) situated at the garden of the Institute of Vertebrate Biology, Czech Republic. Each pond had a gravel substrate and was filled to a depth of 0.6 m with water and furnished with artificial plants as refuges. Four sand-filled plastic pots, each containing a mussel, were placed in the corner of each pond; pots kept mussels in fixed positions but permitted them to adopt a natural position and to filter normally. Under natural conditions at our study sites, *A. woodiana* comprise approximately 50% individuals in the unionid mussel community [32,33]; therefore, we experimentally evaluated scenarios when 50% of native mussels were replaced by non-native *A. woodiana*. This provided three levels of mussel community treatment; native community (four individuals of *A. anatina*), community invaded by Baltic *A. woodiana* (two *A. anatina* and two Baltic *A. woodiana*), and community invaded by Danubian *A. woodiana* (two *A. anatina* and two Danubian *A. woodiana*). Each mussel community treatment was replicated with both *R. amarus* populations; sympatric and allopatric to *A. woodiana*. For logistical reasons, Danubian and Baltic *R. amarus* were tested in separate years. There were seven replicates of each treatment combination, resulting in 84 experimental populations tested over two spawning seasons.

Experimental *R. amarus* populations consisted of five males and six females. Experimental fish foraged on natural food (algae, detritus and invertebrates) that established in experimental ponds and were additionally fed daily with a mixture of frozen chironomid larvae and cyclops nauplii, with an equal amount provided to each population. *Rhodeus amarus* were stocked on 11 May 2012 (Danubian fish) and 25 April 2014 (Baltic fish). A total of 420 male and 504 female *R. amarus*, 224 *A. anatina* and 56 Baltic and 56 Danubian *A. woodiana* were used. Fish started to spawn approximately two (Danubian) and four (Baltic) weeks after stocking. Experimental mussels were recovered from ponds on 8–12 June 2012 and 12–13 June 2014, before *R. amarus* embryos had completed development. Mussel gills were dissected and all *R. amarus* embryos were counted. A small number of juvenile *R. amarus* emerged from their host mussels prior to mussel dissections (in a total of six pools in Baltic *R. amarus*). These were collected from ponds and added to the sum of *R. amarus* embryos from their respective populations.

To measure the impact of mussel community composition on *R. amarus* reproductive success, the number of *R. amarus* embryos recovered from each experimental population was tested with *mussel community* (three levels; native, invaded by Danubian *A. woodiana*, invaded by Baltic *A. woodiana*) and *fish population* (two levels: sympatric, allopatric) as fixed factors. For Danubian *R. amarus*, embryo abundance followed a normal distribution and a general linear model (LM) was used. Data for Baltic *R. amarus* were initially tested using a Poisson distribution but were found to be overdispersed and a quasi-Poisson distribution was used (generalized linear model with log-link function, GLM). There were some mortalities of experimental *A. anatina* mussels during the experiment with Baltic *R. amarus*, distributed

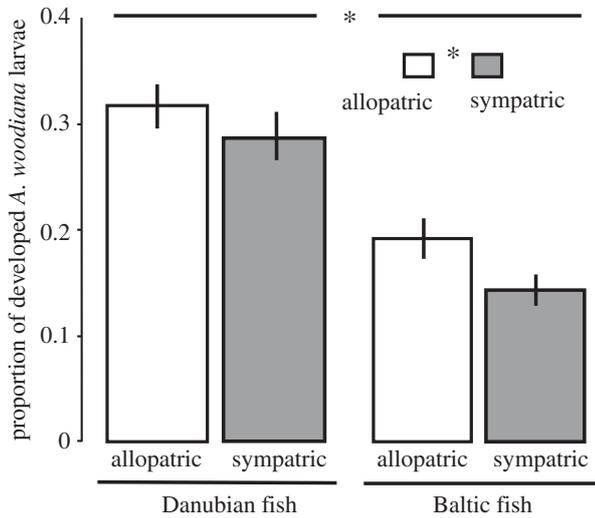


Figure 2. The success rate (mean and 95% CIs) of parasitic larvae metamorphosis on each *R. amarus* population. Significant differences are denoted by asterisks. Note that there was no difference between the two *A. woodiana* populations and data for Danubian and Baltic *A. woodiana* populations were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

randomly across treatments. Therefore, mussel mortality (the number of *A. anatina* mussels that died before mussel dissection) was included as an additional covariate in the analysis.

3. Results

(a) Population-specific exploitation of *Rhodeus amarus* by invasive *Anodonta woodiana*

The capacity of *A. woodiana* to successfully metamorphose on *R. amarus* differed among *R. amarus* populations but not between *A. woodiana* populations (figure 2). Specifically, Danubian *R. amarus* were considerably better hosts of *A. woodiana* than Baltic *R. amarus* (GEE: Wald $\chi^2 = 46.7$, $p = 0.001$, $n_{\text{balt}} = 63$, $n_{\text{dan}} = 57$). Further, *A. woodiana* were more successful in developing on allopatric *R. amarus* than sympatric *R. amarus* ($\chi^2 = 6.5$, $p = 0.011$, $n_{\text{symp}} = 60$, $n_{\text{alop}} = 60$), though the effect was smaller than for the effect of *R. amarus* geographical origin. The two *A. woodiana* populations did not vary in their capacity to infect *R. amarus* ($\chi^2 = 1.3$, $p = 0.263$) and there was no significant interaction between the origin of *R. amarus* and *A. woodiana* ($\chi^2 = 3.0$, $p = 0.085$) (electronic supplementary material, figure S2).

(b) Population-specific impacts on native *Rhodeus amarus*: mechanisms and consequences

Rhodeus amarus preference for host mussels revealed contrasting population-specific responses (figure 3). *Rhodeus amarus* from the sympatric Danubian population showed different responses to the two *A. woodiana* populations. They avoided locally sympatric Danubian *A. woodiana* (t -test: $t_{15} = 3.35$, $p = 0.005$), but did not discriminate against unfamiliar Baltic *A. woodiana* ($t_{15} = 0.64$, $p = 0.502$). The two allopatric *R. amarus* populations differed in their response to the two *A. woodiana* populations. Danubian *R. amarus* avoided *A. woodiana* (Danubian mussels: $t_{16} = 2.47$, $p = 0.026$; Baltic mussels: $t_{16} = 2.67$, $p = 0.018$), while Baltic *R. amarus* did not

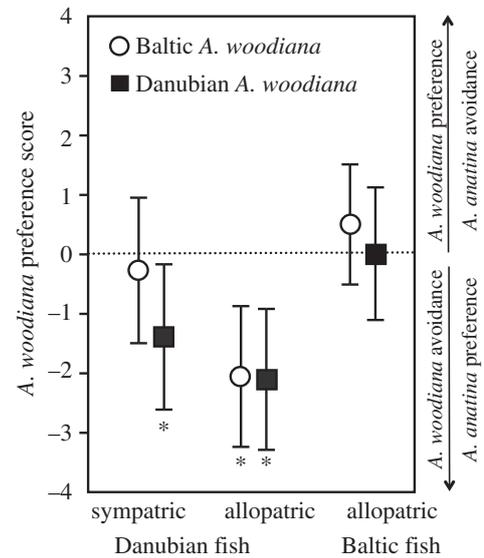


Figure 3. Population specificity in behavioural discrimination of *A. woodiana* by *R. amarus* prior to oviposition. Paired contrasts in preference tests between *A. woodiana* and native *A. anatina*. Mean and 95% CIs of preference score (significant avoidance denoted by asterisk) are shown, significant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

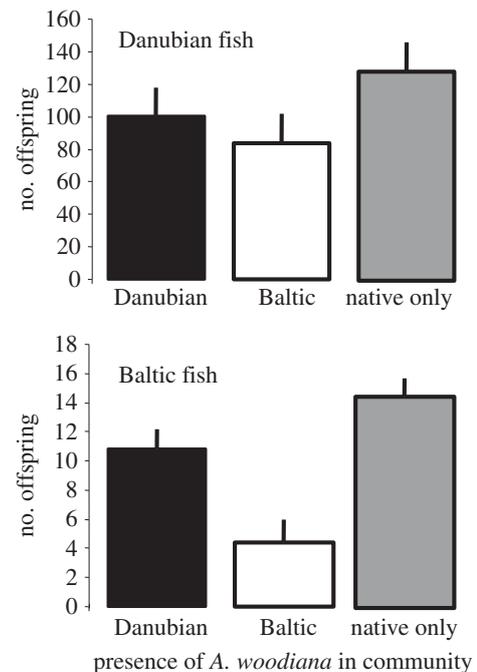


Figure 4. Impact of mussel community on the reproductive success of *R. amarus* populations. Mean (+1 s.e.) number of *R. amarus* recovered from experimental ponds. Baltic and Danubian *R. amarus* were tested separately in different years.

discriminate against any *A. woodiana* population (Baltic *A. woodiana*: $t_{22} = 1.34$, $p = 0.196$; Danubian *A. woodiana*: $t_{18} = 0.02$, $p = 0.981$). Ovipositions were rare (electronic supplementary material, table S4), but their distribution was congruent with the behavioural preference score.

The presence of *A. woodiana* in the mussel community significantly decreased *R. amarus* reproductive success (Danubian *R. amarus* populations, LM: $F_{2,36} = 3.34$, $p = 0.047$; Baltic *R. amarus* populations, GLM: $F_{2,39} = 3.98$, $p = 0.028$). The fewest offspring were recovered from the treatments with Baltic *A. woodiana* (figure 4 and electronic supplementary

material, table S5). No significant effect of *R. amarus* sympatry with *A. woodiana* was detected (sympatry: $F_{1,36} = 0.82$, $p = 0.372$ and $F_{1,38} = 1.41$, $p = 0.243$ for Danubian and Baltic fish; interaction between mussel community and sympatry: $F_{2,36} = 1.80$, $p = 0.181$ and $F_{2,35} = 1.39$, $p = 0.267$, respectively). Mortality of native mussels had no effect on the outcome of tests ($F_{2,35} = 0.23$, $p = 0.632$).

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 geographical 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*. By 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 variation in the impact of an invasion can derive from innate characteristics of populations. The impacts of invasive species on local communities can often be precipitated via subtle processes between intimately interacting species [8,40,41]. These relationships are often characterized by coevolution, when an adaptation of one partner is matched by adaptation in the second. Coevolutionary associations are inherently dynamic and, across species ranges, they proceed at varying rates, generating a diverse geographical mosaic of variable states [42]. Species translocations can disrupt coevolved adaptations, exposing both native and non-native species to novel interactions [9,40].

We propose that coevolutionary dynamics within native communities may predispose different populations to divergent responses to an invading species, with variation in consequences. Coevolutionary dynamics have rarely been considered as modulating impacts in invasion biology [43], but

may present an important source of variation in outcomes. Our experimental system was not suited to replication across a higher number of population combinations and it may be argued that stochastic processes unrelated to coevolutionary dynamics may have produced the observed pattern. Other systems with fine-scale coevolutionary dynamics, such as plant–insect interactions (e.g. pollination, seed dispersal), are also prone to perturbations from invasions of non-native species and may be easier to replicate across more populations with a more reasonable cost and effort.

Several other examples indicate, at least indirectly, the potential importance of coevolutionary dynamic states on the impact of invasions. In an example conceptually matching the *R. amarus*–*A. woodiana* scenario, *Anguillicoloides crassus*, a nematode parasite of East Asian eels (*Anguilla japonica*), caused massive mortalities of the European eel *Anguilla anguilla* when *A. crassus* was introduced to Europe. As a parasite that apparently coevolved to an equilibrium with a local population of *A. japonica* in its native range, its virulence is lethal for evolutionarily naive *A. anguilla* hosts [43]. The introduction of *A. crassus* to North America resulted in infections of the American eel, *Anguilla rostrata*, but the impact of *A. crassus* on *A. rostrata*, while less understood, appears more limited than the impact on *A. anguilla* [44]. Similarly, a monogenean parasite *Gyrodactylus salaris* is not lethal to Baltic populations of the Atlantic salmon, *Salmo salar*, but caused substantial mortalities once introduced into East Atlantic populations of *S. salar* [45].

Except for parasites invading new ranges (i.e. emerging infectious diseases), where the impacts are apparent and often have acute consequences, such cases have rarely been documented. We propose that coevolutionarily dynamic states between mutually interacting species may actually play an important role in influencing the magnitude and direction of the impacts of invasions. This perspective also recognizes the invasion of non-native genotypes within an established species range [11], which may often go undetected but could have important consequences for community structure and interspecific associations [46].

Impacts of invasive species also vary in time [40], and ecological and evolutionary processes have been implicated as the source of this variation [47]. We have shown that *R. amarus* populations which were sympatric with the invasive mussel were more efficient in rejecting their parasitic larvae than evolutionary and ecologically naive allopatric *R. amarus* populations. An initial rapid establishment and strong negative impact on native species can stabilize after the initial population expansion by the invader (e.g. [48]), though chronic effects can persist and many impacts can be irreversible [41,47]. Adaptive responses to invasive populations can evolve relatively rapidly. For example, native anole lizards, *Anolis carolinensis*, have adapted to a niche shift following invasion of a competitor, *Anolis sagrei*, to Florida with significant changes in ecology and morphology over less than 20 generations [10]. Likewise, evolutionary change in invasive populations that resulted in a decrease in their impact on native species has been reported [49]. In the case of *R. amarus*, a sympatric population appeared capable of discriminating against the invasive host mussel and avoided it for oviposition, despite not being able to discriminate against the other, unfamiliar population of the same invasive species.

An understanding of alternative effects of invasive species across space and time still represents a major challenge for invasion science. Our results illustrate the inherent difficulty in

predicting the impact of a non-native species by demonstrating that fine-scale population-specific attributes arising from local adaptation and fine-scale coevolutionary dynamics can play a major role in driving outcomes. While an ability to predict the impact of non-native species is a prerequisite for the successful management of biological invasions [1], achieving this goal is not straightforward [4]. We argue here that a shift from a species-centred to a more population-centred perspective of invasion may provide deeper insights into the success and impacts of biological invasions.

Ethics. All work was approved by the ethical committees of the IVB (no. 163-12) and the Ministry of Agriculture (CZ 62760203) and complies with the legal regulations of the Czech Republic and Poland.

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