**BIODIVERSITY RESEARCH**

# Biotic homogenization as a threat to native affiliate species: fish introductions dilute freshwater mussel's host resources

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## ABSTRACT

**Aim** The indirect consequences of biotic homogenization, the process of a gradual increase in the similarity of regional biotas driven by the combined effects of species invasions and extinctions, are still poorly understood. In this study, we aimed to assess the ability of a native affiliate species to maintain its host resources under the condition of biotic homogenization of host communities.

**Location** Central (Vltava River Basin, Czech Republic) and western (Douro River Basin, Portugal) Europe.

**Methods** We tested the ability of non-native species to serve as an alternative partner in local host–affiliate relationships. We used a European freshwater mussel, *Anodonta anatina*, which is considered to be a host generalist of native fish species, and compared the compatibility of its glochidia with native versus non-native fishes in two distinct European regions. Subsequently, we projected the obtained host compatibility data into the recent progress of biotic homogenization and estimated the degree of host dilution.

**Results** We found significant differences in the ability of *A. anatina* glochidia to parasitize the native and non-native fish species in both the central and peripheral parts of the mussel's distribution range. As a result, the increasing presence of non-native species within fish communities across Europe likely significantly decreases the availability of the mussel's host. Biotic homogenization of host communities may interfere with general life history traits (host specificity) of their local affiliate species.

**Main conclusions** This study demonstrates that the mixing of regional biotas may lead to an excessive loss of host availability even for host generalists, such as the freshwater mussel *A. anatina*, with potentially broad consequences for their population dynamics. Conservation strategies of endangered affiliate species need to incorporate the biogeographical context of host–affiliate relationships and particularly the consequences of biotic homogenization.

**Keywords**
*Anodonta anatina*, biological invasions, co-extirpation, Czech Republic, freshwater ecosystems, glochidia, host specificity, host–parasite relationships, Portugal, Unionidae.

## INTRODUCTION

The gradual increase in biological similarity of regions (i.e. biotic homogenization) is a widespread process that shapes the composition and function of biotic communities and is mainly driven by the combined effects of species invasions and extinctions (Olden *et al.*, 2004; Olden, 2006; Winter *et al.*, 2009; Villeger *et al.*, 2011). As a result of biotic

homogenization, many species begin interacting with novel partners, and former co-evolutionarily balanced inter-specific relationships are lost. The outcomes of these novel interactions determine the conditions for the survival of a particular species and have become one of the critical issues in conservation biology (McKinney & Lockwood, 1999; Traveset & Richardson, 2006; Berg *et al.*, 2010).

Numerous studies have documented the direct negative effects of invading species on local biota via predation, competition or parasitism (Byers, 2000; Kats & Ferrer, 2003; Taraschewski, 2006; Ward & Ricciardi, 2007; Sousa *et al.*, 2011). These direct impacts have been used in numerous models that demonstrate the threat of species introductions to global biodiversity. Less obvious consequences of biotic homogenization remain poorly understood but may be even more detrimental to local biota (Koh *et al.*, 2004; Moir *et al.*, 2010). Specifically, the cascading effects of species decline or extinction on another species across trophic levels may multiply the impacts on local biodiversity (Petchey *et al.*, 2008). Indeed, the loss of one species as a result of the loss of another species (co-extinction) is one of the most common causes of biodiversity loss (Dunn *et al.*, 2009).

Affiliate species, which directly depend on the presence of another species, are particularly threatened by biotic homogenization. Their ability to survive and prosper within a rapidly changing host community depends mainly on the broadness of a suitable host spectrum (host specificity) or on the capacity of the affiliate species to substitute its former hosts with incomers (Moir *et al.*, 2010). Hence, the most threatened affiliates are considered to be the species that narrowly specialize on a few or even only one host species. Many examples from both the animal and plant kingdoms (e.g. insect parasites) document the decline or extinction of highly specialized species following the displacement of their exclusive hosts (e.g. Dunn, 2005). Furthermore, hosts associated with many obligate dependent affiliate species may be considered 'keystone mutualists' with large conservation importance (Koh *et al.*, 2004).

Although co-extinction rates and declines of highly specialized affiliate species have been well documented, the effects of biotic homogenization on affiliate species that are considered to be generalists remain understudied. It can be supposed that generalists may either use the remaining native species or the introduced species to compensate for the decline of their former hosts. Nevertheless, this assumes that the 'winners' and 'losers' of biotic homogenization are equally suitable hosts for an affiliate species. In contrast, recent evidence suggests that this assumption may not necessarily be true (or at least not very immediate). Successful invaders are less parasitized in their invaded range compared with native species (the Enemy Release Hypothesis; Torchin *et al.*, 2003). Expanding species are often liberated from their ancestral (native-range) affiliates and novel (invaded range) affiliates are not yet adapted to utilize the incomers (Taraschewski, 2006). The role of biotic homogenization on the generalist affiliate species thus remains unclear.

Freshwater mussels (Bivalvia, Unionoida) have a short-term larval stage (glochidium) that is obligatory parasitic on the gills or fins of fishes (Kat, 1984). The availability of experimental methods for studying host compatibilities has resulted in the use of unionid bivalves as a common model group for the study of host–affiliate relationships involving endangered species (Spooner *et al.*, 2011; Douda *et al.*, 2012a). From a conservation point of view, unionids are among the most critically threatened groups of animals world-wide (Lydeard *et al.*, 2004; Strayer *et al.*, 2004). The consequences of this catastrophic decline go far beyond the loss of species per se: freshwater mussels have critical trophic and non-trophic functional roles in aquatic environment (Strayer *et al.*, 1994; Vaughn *et al.*, 2004; Allen & Vaughn, 2011); thus, the decline of originally dense mussel populations can have interconnected implications for the functioning of aquatic ecosystems (Vaughn, 2010).

Unionid bivalves exhibit a varying degree of host specificity (Barnhart *et al.*, 2008). Several studies documented possible host limitations of specialized freshwater mussels following changes in fish host community composition (Strayer, 2008). Conversely, the limitations of host generalists remain unclear. This is the case for *Anodonta anatina* (Linnaeus 1758), a freshwater mussel that is widespread in Europe from the Iberian Peninsula and Northern Africa to Scandinavia in the north and to Russia (lake Baikal) in the east (Graf, 2007). Although *A. anatina* is widespread, little is known about its ecological status and population structure across Europe. Currently, there have been recorded several population declines around Europe, the species is listed as threatened and protected in Germany (Van Damme, 2011), and its population decrease can potentially impact also other species and functions of aquatic habitats. *Anodonta anatina* has large hooked glochidia, and like all anodontines, it is generally considered to have a wide host fish spectrum (Bauer, 2001a). This assumption is supported by studies carried out in central and northern European countries that tried to establish the host fish used by *A. anatina* (summarized in Weber, 2005). Nevertheless, relatively little is known about its compatibility with non-native fish species that have recently entered its natural range. There is a risk that *A. anatina* and other species considered to be host generalists may be unable to exploit the majority of individuals in novel host communities that are the result of human-mediated biotic homogenization. In this case, biotic homogenization would have manifold implications for the persistence and conservation of these affiliate species.

In this study, we examined the ability of a native affiliate species to exploit its host community (to maintain the generalism of host selection) despite the influx of non-native species. We tested the ability of non-native species to serve as alternative partners in local host–affiliate relationships. We used the European freshwater mussel *A. anatina*, which is considered to be broad host generalist of native fish species and compared the compatibility of its glochidia with native versus non-native fishes in two distinct European regions. The studied regions were located in the central and peripheral part of the mussel's

range to record the host–affiliate relationships within a broader biogeographical context and to investigate a possible variation in scenarios of host use throughout the species range. We then projected the obtained immunological host compatibility data into the recent progress of biotic homogenization and estimated the degree of host dilution. We discussed our outcomes in the context of strategies for conservation of endangered affiliate species.

## METHODS

### Study sites

We selected two geographically distant populations of *A. anatina* in areas differing in their native fish fauna and the composition of introduced fish species. One population was from Vltava River Basin (N 49°27′13″; E 14°39′26″) in the central part of the *A. anatina* range (Czech Republic – Central Europe), and the second population was from Douro River Basin (N 41°32′30″; W 7°47′14″) in the peripheral part of its range (Portugal – Iberian Peninsula). The Iberian freshwater fish fauna is distinct from the fish fauna in other parts of Palearctic region. In fact, isolation and the orographic and climatic peculiarities of the Iberian Peninsula have led to a high level of endemism (Almaça, 1995; Elvira, 1995). Freshwater biotopes in both regions have been subjected to numerous introductions of non-native freshwater fish. In many biotopes, the introduced fish became established and even predominant over the native species (Holcik, 1991; Clavero & Garcia-Berthou, 2006; Musil *et al.*, 2010).

### Studied species

We tested the compatibility of the local populations of *A. anatina* with native and non-native fish species specific for both regions. In total (224 individuals), we assessed 15 fish species from the Central region and 12 from the peripheral region. The list of species and the number of individuals are in Table 1. To prevent previous contact with glochidia, the fish used in the experiment originated from hatcheries or natural sites where unionid bivalves were absent. One-year-old (1+) fish were preferentially used.

### Evaluation of host compatibility

Experiments assessing the capability of *A. anatina* glochidia with native and non-native fish species were conducted between November and March from 2008 to 2011. Methods were adapted from Dodd *et al.* (2005) and Douda *et al.* (2012b). Gravid mussels identified according to the presence of swollen outer demibranches (marsupia) filled with ripe glochidia were transported to laboratory. Infective larvae were obtained by flushing a marsupium with water using a syringe, and their viability was verified by examining their snapping action after the addition of sodium chloride into a glochidia subsample. Glochidia from six gravid females with

a viability exceeding 90% were pooled and used for inoculation in each infestation trial.

Fish were infected in a strongly aerated (through airstones) glochidia suspension with a minimal bath volume of 0.5 l per fish individual containing a mean $\pm$ SD of 650 $\pm$ 333–4220 $\pm$ 1866 viable glochidia. The density of glochidia was assessed from ten 10 ml subsamples taken from the infestation bath of each trial during the course of inoculation. After 15–30 min of inoculation (time adjusted to reach visible inoculation of experimental fish by glochidia), the fish were removed from the glochidia suspension.

The developmental success of parasitizing glochidia was subsequently monitored in plastic tanks of dechlorinated tap water with a minimum volume of 5 l per individual fish, and a 3-mm net was used on the bottom of the tank to avoid juvenile predation. Both individual and common fish-holding systems were used during the study with regularly renewed water and continual temperature monitoring. The fish were fed daily with commercial flake fish food. Glochidia and juvenile mussels were collected at 1–2 day intervals by siphoning the water in tanks using filters (mesh size 139 and 180 μm). The living juvenile mussels were distinguished from untransformed glochidia or dead juveniles by foot movements and valve openings. The recorded time course of glochidia/juvenile shedding was used for back-calculating the initial number of attached glochidia and the proportion of successfully transformed juveniles (transformation rate). We also calculated the cumulative number of degree days as a sum of the mean daily temperatures during juvenile development on the host fish. The trials were terminated at least 4 days after the last juvenile was recovered from a tank, and the fish were checked for the presence of glochidia.

### Estimation of host resource loss

We compiled available data on fish introductions and extirpations from Czech and Portuguese inland free water bodies (Almaça, 1995; Almaça & Elvira, 2000; Lusk *et al.*, 2004; Ribeiro *et al.*, 2009; Musil *et al.*, 2010) to examine the historical and current numbers of native and non-native species. Subsequently, on the basis of the mean (and 95% confidence interval) proportion of suitable hosts across native and non-native fishes, we estimated the proportions of suitable hosts during the last 160 years, when there was a substantial influx of non-native species. The estimated share of suitable host species was calculated by subtracting the recorded proportions of non-host species (separately for native/non-native species and both regions) from the respective species pools in particular time points.

### Data analyses

Reproduction success of *A. anatina* glochidia on particular fish species was compared using generalized linear models with quasi-binomial error structure. We tested for differences in transformation rate (the proportion of successfully metamorphosed juvenile mussels and the number of all

**Table 1** Fish species studied and host compatibility tests results. 'Transformation rate' indicates the proportion of *Anodonta anatina* glochidia that successfully developed into juvenile mussels. 'Metamorphosis' indicates the range of days when living juveniles detached from fish.

| Fish Species | Number of fish (N) | Mean ± SD fish length (mm) | Mean ± SD temperature (°C) | Mean number of attached glochidia per fish | Mean number of juveniles per fish | Transformation rate (%) | Metamorphosis (days) |
|---|---|---|---|---|---|---|---|
| **Native** | | | | | | | |
| Portugal (peripheral) | | | | | | | |
| *Achondrostoma oligolepis* | 9 | 82.2 ± 40.1 | 19.0 ± 0.9 | 23.9 | 7.9 | 33.1 | 16–23 |
| *Cobitis paludica* | 6 | 78.8 ± 9.3 | 19.7 ± 1.7 | 4.9 | 0.5 | 10.2 | 16–20 |
| *Luciobarbus bocagei* | 8 | 140.3 ± 56.2 | 18.1 ± 1.5 | 19 | 4 | 21 | 13–17 |
| *Pseudochondrostoma duriense* | 9 | 110.8 ± 49.3 | 19.5 ± 5.1 | 18.3 | 10.3 | 56.4 | 7–20 |
| *Salmo trutta fario* | 6 | 170.1 ± 10.7 | 15.0 ± 3.5 | 39 | 16.5 | 42.3 | 22–24 |
| *Squalius alburnoides* | 16 | 86.9 ± 38.2 | 16.6 ± 1.6 | 27 | 12.8 | 47.4 | 16–23 |
| *Squalius carolitertii* | 7 | 100.1 ± 47.2 | 19.0 ± 1.2 | 37.2 | 16.4 | 44.1 | 12–23 |
| Czech Republic (central) | | | | | | | |
| *Abramis brama* | 6 | 69.3 ± 20.2 | 22.6 ± 0.4 | 142.5 | 33.8 | 22.2 | 6–14 |
| *Barbus barbus* | 8 | 90.4 ± 10.6 | 21.1 ± 0.4 | 729 | 413.3 | 56.7 | 6–26 |
| *Gobio gobio* | 5 | 51.8 ± 7.2 | 22.6 ± 0.4 | 53.8 | 12.8 | 25.3 | 6–10 |
| *Perca fluviatilis* | 10 | 54.3 ± 3.2 | 21.6 ± 0.6 | 220.9 | 126.2 | 57.1 | 6–17 |
| *Rutilus rutilus* | 5 | 100 ± 3.4 | 21.6 ± 0.6 | 566.6 | 17.8 | 3.1 | 6–12 |
| *Scardinius erythrophthalmus* | 5 | 67.2 ± 16.9 | 21.1 ± 0.4 | 211 | 154.9 | 73.4 | 8–20 |
| *Squalius cephalus* | 8 | 56.3 ± 7.9 | 21.1 ± 0.4 | 216 | 34.3 | 15.9 | 6–18 |
| *Squalius leuciscus* | 6 | 60.2 ± 2.8 | 22.6 ± 0.4 | 73 | 27.8 | 39.8 | 6–10 |
| *Tinca tinca* | 7 | 45 ± 5.4 | 21.6 ± 0.6 | 101.6 | 0.1 | 0.1 | 8 |
| *Vimba vimba* | 6 | 44.3 ± 4.4 | 21.1 ± 0.4 | 103 | 23 | 22.3 | 6–16 |
| **Non-native** | | | | | | | |
| Portugal (peripheral) | | | | | | | |
| *Cyprinus carpio* | 4 | 150.2 ± 58.1 | 16.6 ± 1.0 | 33.2 | 0 | 0 | – |
| *Gobio Lozanoi* | 16 | 100.2 ± 24.3 | 18.1 ± 1.6 | 47.1 | 0 | 0 | – |
| *Lepomis gibbosus* | 18 | 98.2 ± 45.9 | 19.5 ± 5.1 | 0 | 0 | 0 | – |
| *Micropterus salmoides* | 4 | 145.2 ± 66.7 | 15.0 ± 3.5 | 12.1 | 0 | 0 | – |
| *Oncorhynchus mykiss* | 6 | 165.2 ± 9.2 | 15.0 ± 3.5 | 24.2 | 12.5 | 51.7 | 23–24 |
| Czech Republic (central) | | | | | | | |
| *Carassius auratus* | 15 | 79.5 ± 6.4 | 22.6 ± 0.4 | 82.9 | 0.1 | 0.001 | 6 |
| *Carassius gibelio* | 8 | 82.3 ± 4.7 | 21.1 ± 0.4 | 463 | 0.5 | 0.1 | 6–10 |
| *Cyprinus carpio* | 6 | 63 ± 8.3 | 21.6 ± 0.6 | 312.2 | 0.5 | 0.2 | 6 |
| *Pseudorasbora parva* | 8 | 62.4 ± 5.7 | 21.1 ± 0.4 | 209 | 0.8 | 0.4 | 8–10 |
| *Rhodeus amarus* | 12 | 30.7 ± 2.4 | 21.1 ± 0.4 | 20 | 1.5 | 7.6 | 8–18 |

glochidia and juvenile mussels recovered from fish) in relation to the fish origin (native and non-native), region (central and peripheral) and the interaction of these two factors. We then used Fisher's exact test (with adjusted *P*-values by Bonferroni correction) to compare the proportion of suitable hosts between native and non-native fish species in both regions and overall. Only the species with recorded transformation rates of over 3% of the initially attached glochidia were considered to be suitable hosts because of the general theoretical assumption that species with extremely low transformation rates are unlikely to serve as viable hosts in nature.

## RESULTS

*Anodonta anatina* glochidia successfully developed on 16 of 17 native fish species tested (94%). In contrast, only 2 of 10

of non-native species were considered to be suitable hosts. Similar trends were recorded in both the central and peripheral regions. The transformation rates of the initially attached glochidia ranged between 0% (several non-native fish species) and 73.4% (Rudd – *Scardinius erythrophthalmus*; L., 1758) (Fig. 1). The average transformation rate (±SD) was 33.6 ± 20.3% and 6.0 ± 15.4% for native and non-native species tested, respectively (overall mean transformation rate 23.4 ± 22.9%). Fully developed juveniles were recovered from the monitoring tanks between 6 and 24 days after the infestation. The sum of the mean daily temperatures during juvenile development varied with fish species and ranged from 157 ± 24 to 358 ± 5 degree days (mean ± SD). After the end of the experiments, no glochidia were found to be attached to the experimental fishes. Neither the glochidia transformation rate ($r_s = 0.004$, $S = 3262$, $P = 0.98$) nor the
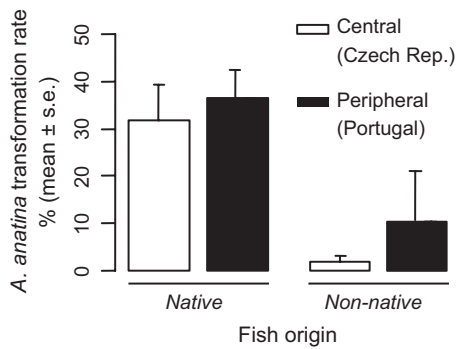
Figure 1 Transformation rate of *Anodonta anatina* glochidia on native versus non-native fish species in both the central (Czech Republic) and peripheral (Portugal) regions.

number of initially attached glochidia ($r_s = 0.13$, $S = 2848$, $P = 0.52$) was significantly related to the mean temperature during the experiment. Detailed results of the host compatibility tests are shown in Table 1. Fisher's exact test confirmed a significantly higher proportion of suitable hosts among the native fish species in both regions and overall ($P < 0.05$, $P < 0.05$, $P < 0.001$, respectively). The generalized linear model showed that transformation rate of glochidia was significantly higher for native fish species ($F_{1,24} = 13.6$, $P < 0.01$). The region (central and peripheral) did not significantly improve the model ($P > 0.05$).

Regarding the high influx of non-native fish species (generally poor hosts) and the extirpations of native fish species (generally useful hosts) in both regions during the last 160 years (Fig. 2a,b), the estimated proportion of suitable host species has steeply declined (Fig. 2c). While *A. anatina* may be expected to have been compatible with 94% (95% confidence interval: 83–100%) of fish fauna before the arrival of non-native species, we predicted that only 67% (95% confidence interval, 53–83%) of fish species are immunologically suitable hosts nowadays.

## DISCUSSION

We found significant differences in the ability of *A. anatina* glochidia to parasitize native and non-native fish species. As a result, the increasing presence of non-native species within fish communities across Europe likely decreases the availability of the mussel hosts. Hence, using the example of a native freshwater mussel, we showed that biotic homogenization of host communities may negatively interact with general life history traits such as host specificity of local affiliate species.

### Host specificity of *A. anatina*

Our results indicate a strong co-evolutionary signal of host compatibility between the studied mussel and fish species. Similar to previous studies (Weber, 2005), we corroborated the ability of *A. anatina* to parasitize almost every native fish species that is exposed to its infective glochidia. This result
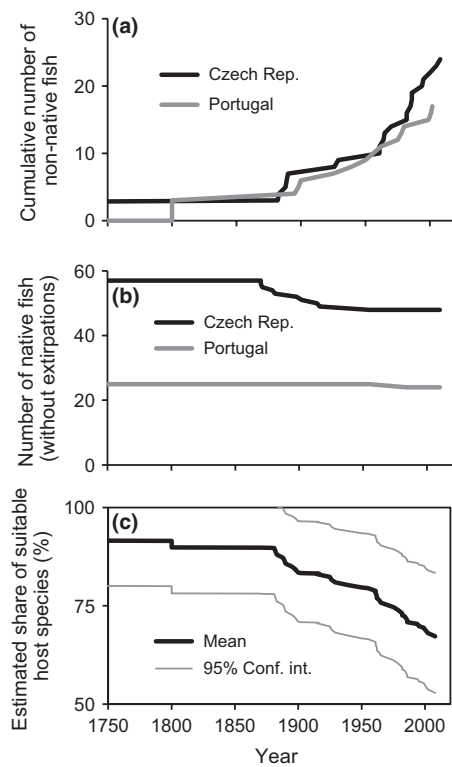


Figure 2 Fish introductions (a) and extirpations (b) in Czech and Portuguese inland free water bodies during the last 160 years (Almaça, 1995; Almaça & Elvira, 2000; Lusk *et al.*, 2004; Ribeiro *et al.*, 2009; Musil *et al.*, 2010), and the estimated proportion of suitable hosts in both regions (c).

corresponds with low host specificity in most Anodontinae species, where many *Anodonta* species are able to use a large number of fish species and even amphibians (Bauer, 2001b).

In contrast, the developmental success of *A. anatina* on non-native fish species from distant geographical regions was significantly less than the development success on native fish species. Although there are no clear rules governing host compatibility between host and affiliate species from distinct regions (Poulin, 2007), the absence of common evolutionary history prevents formation of specific defence (host) or utilization (affiliate species) mechanisms. Instead, only non-specific or adopted mechanisms with unclear results for the novel host–affiliate relationship can be used. As a result, the context of evolutionary history of both partners (including a possible competition among parasites) in their native regions is likely important (Hoberg & Brooks, 2008). Invasive species native to areas with a high diversity and abundance of parasites may allocate more resources to defence systems (Schmid-Hempel & Ebert, 2003) and therefore be more resistant to novel parasites in their invaded ranges. For example, East Asian fish and their macroparasites seem to be successful world-wide invaders partially because of their high investment in immune and attack systems (Taraschewski, 2006).

Another important issue determining the co-evolutionary dynamics of host–affiliate relationship between particular

taxonomical groups are the costs and benefits to both partners (Sheldon & Verhulst, 1996). When the costs to hosts are low compared to the benefits to affiliates, the selection power for host defence may be relatively weak (Sadd & Schmid-Hempel, 2009). This is probably also the case of *A. anatina* and the other species of unionid bivalves, which cause only weak nutritional and fitness costs to their hosts (Fisher & Dimock, 2002; Crane *et al.*, 2011); nevertheless, these species cannot complete its development without hosts. As a result, selection for defence against parasitizing glochidia may by weak, leading to a rapid adaptation of mussels to their local fish species. On the other hand, some groups of affiliates can effectively infect novel host species from distinct areas, and host specificity can break down when new hosts and parasites are brought together (Poulin & Keeney, 2008). Indeed, parasites transferred as biological control agents against a particular host species (in part because of their high host specificity) can infect non-target hosts in recipient areas (Poulin, 1992). In the case of unionid bivalves, there are also species that use a wide range of hosts including non-native species from distant regions (Trdan & Hoeh, 1982; Watters & O'Dee, 1998), which may even facilitate the spreading of these bivalves world-wide (Douda *et al.*, 2012b).

Genetic differentiation of species throughout a region is other potentially important factor that influences the host relationships of natural populations (Serb & Barnhart, 2008; Geist & Kuehn, 2008; Zanatta & Wilson, 2011) and might cause differences in host compatibilities in distant areas. Regarding the fact of high genetic differentiation of particular unionid taxa across Europe (Nagel & Badino, 2001), there may exists locally specific adaptations to different host species. Specifically, there are indications of high endemism of unionids within the Iberian Peninsula (Reis & Araujo, 2009). Hence, despite the fact that we have genetically proven identification of *A. anatina* in both studied areas (PCR/RFLP following Gerke & Tiedemann, 2001 – M. Lima and E. Froufe, unpublished data), host compatibility may differ in both areas. Any extrapolation regarding the classification of host and non-host species for *A. anatina* across Europe must be done with cautions. Nevertheless, in terms of our general conclusions, it is important to emphasize that the finding of significant differences in the ability of *A. anatina* glochidia to parasitize the native and non-native fish species were highly congruent in both the central and peripheral parts of the mussel's distribution range regardless of the differences in local species pools.

Another important aspect of host specificity evaluation is the fact that laboratory experiments provide only a partial view of natural world, which may differ in infection rates, spatial position of glochidia attachment, environmental conditions, and there are also possible effects caused by the use of artificially reared fish. Hence, despite the well-developed protocols for experimental evaluation of host compatibility of unionid bivalves, further data are needed to clarify the role of native and non-native host species in natural conditions. Most importantly, while the laboratory data typically evaluates only the immunological compatibility (e.g. transformation success of glochidia), the probability of encountering the host (spatial and temporal compatibility) is also critical in natural conditions. Regarding the interpretation of our results, not all fish species identified as suitable hosts in our experiments are likely to be really exploited as hosts in natural habitats (c.f. Araujo *et al.*, 2005). Nevertheless, the observed striking pattern of differences in immunological compatibility between the native and non-native species may strongly delimits available host pool in novel communities.

## The effects of biotic homogenization

Host specificity is a critical factor related to the probability of extinction of affiliate species (Poulin & Keeney, 2008). The higher degree of host specialization may provide numerous advantages through enabling specific adaptations that are beneficial to species performance (e.g. Vaughn, 2012). Nevertheless, predictions based on host extinction probabilities generally demonstrate that host generalists are more resistant to host community degradation, while specialists vitally depend on their exclusive hosts (Koh *et al.*, 2004; Taylor & Moir, 2009). Because the mode of glochidium infection is passive (glochidium has no capacity to actively choose the host on which they settle), we can expect density-dependent effects of host resource availability (Strayer, 2008). Indeed, there are numerous examples of unionid bivalve declines following the impairment of its hosts. For example, the reproduction of *Fusconaia ebena* (Lea 1831) is reported to be unsuccessful after its host disappeared from the upper Mississippi River (Kelner & Sietman, 2000). Also, the probability of local extirpations of Central European *Unio crassus* (Philipsson 1788) was higher at sites with a decreased relative abundance of its suitable hosts (Douda *et al.*, 2012a). It is evident that not only the presence or absence of suitable hosts but also their abundance may influence an affiliate's decline or extirpation (Haag & Warren, 1998; McNichols *et al.*, 2010). In this view, balance between the rates of metamorphosis and chances of encountering a host must be considered because the community structure and density may change in invaded sites. Nevertheless, the overall absolute fish biomass will probably remain similar (in a long-term perspective) because of the carrying capacity of habitat.

Historically, the ability of *A. anatina* to adapt to local fish faunas was likely fast enough to allow it to use the majority of available fish species from its natural range. However, recent changes in host community composition are probably too fast to allow for necessary mussel adaptations, and/or the incoming fish species may be generally less suitable hosts, as discussed above. It suggests that the 'winners' and 'losers' of biotic homogenization are not similar in ability to serve as a host for compensation to occur. Consequently, host generalism maintained within historical host communities may not be realized within new communities, and species may become partial host specialists. In fact, we suppose that the absolute

broadness of host specificity (restricted infectivity) remains unchanged within the evolutionary short time of recent biotic homogenization. But the locally co-evolved partnerships cannot be fully capitalized when the available pool of host is abruptly changing. Our estimations of host availability for *A. anatina*, based on the recorded host qualities of native versus non-native fishes, indicate a considerable decrease in the mean relative proportion of suitable host species between historical (94%) and present (67%) fish fauna. This situation could be even worse for other unionoid species that are much more specific in host compatibility (e.g. *Margaritifera margaritifera* only uses *Salmo trutta* and *Salmo salar* as a host; Bauer, 2001b). In our study system, biotic homogenization should be mainly viewed as a gradual process of species introductions (i.e. dilution of suitable hosts' species pool). Both studied regions have been subjected to numerous introductions of non-native species (Almaça, 1995; Almaça & Elvira, 2000; Lusk *et al.*, 2004; Clavero & Garcia-Berthou, 2006; Ribeiro *et al.*, 2009; Musil *et al.*, 2010), and some of these reach extremely high relative abundances. This may be particularly the case for *Pseudorasbora parva* and *Carassius auratus* in the Czech Republic (Halačka *et al.*, 2003; Gozlan *et al.*, 2010) and *Cyprinus carpio*, *Gobio lozanoi*, *Lepomis gibbosus* and *Micropterus salmoides* in Portugal (Sousa *et al.*, 2008; Comesaña & Ayres, 2009). As a result, these species occupy important niches formerly used by native functional hosts (whose abundances are mostly declining) and may be maladaptively used by native affiliate species. The inadequate adaptation of glochidia to exploit non-native species can directly decreases the ability of mussels to produce viable offspring in invaded sites because glochidia production is limited by the trade-off principles of mussel's resource allocation policy (Bauer, 1994, 1998). Obviously, fish density data would be needed to fully quantify the degree of possible limitation by host availability despite our presence–absence data well illustrate the progress of fish invasions throughout Europe. Regarding the fact that several invasive species reach high relative abundances in both studied regions, we suppose that our conclusions based on presence data are even rather conservative in terms of the estimated proportion of native and non-native fish species. Host dilution process may be stronger or weaker according to particular site-specific status of the fish community, although in general view, local situations are likely to correspond with the calculations constructed for regional species pool.

Another important issue is the possible within-species differences in host quality. Available literature on the subject (e.g. Rogers *et al.*, 2001; Taeubert *et al.*, 2010) indicates that the compatibilities of particular host fish strains may differ within one species. This fact might further enhance the negative impacts of biotic homogenization on host resources, not detectable at species level. In this view, our conclusions based on the species-level evaluation are, again, rather conservative estimates of the real host resource losses (or dilution). In this way, even more thorough insights at population level would be needed to adopt appropriate conservation or management actions at particular sites.

The fact that mixing regional biotas may lead to the excessive loss of host species even for host generalists may have broad consequences for their population dynamics. Similar to other taxonomic groups, unionid host generalists typically produce lower numbers of offspring and lack active host attraction strategies (Bauer, 1994; Barnhart *et al.*, 2008). Therefore, their ability to use almost every encountered fish individual as a host might be necessary to maintain their reproductive efficiency. Unfortunately, this need may not be met after biotic homogenization, which may subsequently cause even more serious threat to affiliate species than previously thought.

The conservation and management strategies for narrowly specialized unionid bivalves largely acknowledge the critical role of host–affiliate relationships for species survival (e.g. Geist, 2010; Schwalb *et al.*, 2011; Douda *et al.*, 2012a). On the other hand, species considered as host generalists are often supposed to be safe from host limitation despite their observed population declines. Our results indicate that an improved understanding of host–relationships and changes in the available host species pool may be essential for the selection of appropriate conservation and management measures, regardless the degree of host specificity. The employment of other methods (genetics, behavioural ecology) is also needed to fully incorporate these aspects into the current concepts of freshwater biodiversity conservation (Geist, 2011).

In conclusion, our study demonstrates that there are substantial spatial and temporal limitations for the applicability of host specificity appraisals. Affiliate species rated as broad host generalists in relation to a native species pool may in fact became host specialists during the process of human-induced biotic homogenization. Conservation strategies of endangered affiliate species need to incorporate the biogeographical context of host–affiliate relationships and particularly the consequences of biotic homogenization.

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## BIOSKETCH

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