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The role of adaptive resistance in a widespread freshwater mussel species

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Abstract

1. Organism features, such as size, weight, sex and age, among others, can influence the success of parasitism. For species that depend on a host to complete their life cycle, such as freshwater mussels (Bivalvia, Unionida), understanding how these traits influence parasitism can help guide management actions aimed at improving their reproductive success, ultimately benefitting their conservation.
2. In this study, the physiological compatibility between the duck mussel *Anodonta anatina* and its host fish was investigated in a simultaneous infestation experiment on four (three native and one non-native) fish species using sympatric and allopatric host strains. The success of glochidia metamorphosis in juvenile mussels was evaluated as a function of host species, strain and fish length.
3. Successful metamorphosis was achieved in almost all fish species tested and differences were detected between allopatric and sympatric strains, with higher values found in allopatric hosts. Allopatric strains of primary hosts produce more juveniles than marginal hosts and non-native species. In addition, larger fish have lower rates of metamorphosis.
4. The results confirm recent findings showing high variation in the metamorphosis success of *A. anatina* glochidia among different native and non-native hosts, both in the natural environment and in artificial laboratory infestations. Allopatric hosts were more suitable for encystment, demonstrating potential adaptive immunity, not yet observed in *A. anatina*. Nevertheless, *A. anatina* may temporarily exploit the naivety of smaller fish regardless of strain.
5. Overall, the results suggest that mussel propagation, reproduction and reintroduction efforts should consider the mechanisms that influence adaptive immunity in fish, including differences in compatibility within and between populations of sympatric and allopatric host strains. This situation should be taken into account, as successful metamorphosis is an important factor in the selection of hosts for juvenile development, especially in efforts to propagate endangered mussel species.

KEYWORDS
encystment, freshwater bivalves, glochidia, host relationships, immunity, Unionida

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1 | INTRODUCTION

Many freshwater mussel species (Bivalvia, Unionida) are currently threatened, and records of declining diversity and abundance have increased in recent years (Lopes-Lima et al., 2017; Lopes-Lima, Reis, et al., 2023; Nakamura et al., 2023; Ollard & Aldridge, 2023). This scenario is worrying, as the loss of mussel species can affect the ecological roles mediated by these organisms in freshwater ecosystems. For example, mussels influence benthic communities by modifying nutrient dynamics (Allen et al., 2012; Atkinson & Vaughn, 2015; Spooner & Vaughn, 2006). In various freshwater ecosystems, species richness and overall biodiversity increase in the presence of mussels owing to their ecosystem engineering activities (e.g. bioturbation, shells as substrate and refuge; Ilarri et al., 2015; Ilarri et al., 2018; Simeone et al., 2018; Spooner et al., 2012). Several research efforts have been undertaken worldwide to increase knowledge about mussels and to support their conservation. However, research is still needed to address issues ranging from macro factors, such as climate change, to individual autecological processes, such as the physiological compatibility of the host in the mussel–fish relationship (Aldridge et al., 2023; Ferreira-Rodríguez et al., 2019; Lopes-Lima et al., 2021; da Silva et al., 2022; da Silva et al., 2023).

The complex life cycle of freshwater mussels includes a parasitic stage, usually on fish (for a review see Modesto et al., 2018). Mussels produce parasitic larvae (glochidia) that attach to and feed on nutrients from the host fish (Denic et al., 2015), forming a cyst until they metamorphose into juveniles (Haag, 2012; Strayer, 2008). After being released and falling into the substrate, they begin their benthic life (Haag, 2012; Strayer, 2008). For the mussel–fish relationship to be successful and for the encystment of the glochidia to occur, there must be physiological compatibility between the host and the larvae (Donrovich et al., 2017; Neves et al., 1985; Rogers & Dimock, 2003; Taeubert & Geist, 2013). Three approaches have been used to evaluate the potential host fish for freshwater mussels. The first considers artificial infestations in the laboratory (Douda et al., 2013; Taeubert, Gum & Geist, 2012), the second quantifies infestations in natural environments (Dias et al., 2020; Lamand et al., 2016) and the third considers artificial and natural infestations simultaneously (Benaissa et al., 2019; Taeubert, Martinez, et al., 2012). On the one hand, studies have shown that host suitability assessed under standardized laboratory conditions can vary substantially from natural environments (Taeubert, Martinez, et al., 2012). On the other hand, studies of hosts in natural environments have complemented laboratory results (Dias et al., 2020; Douda et al., 2013). Studies that identify the response of the host's immune system using any of the above approaches should meet the necessary standards in experiments (Douda, 2013; Taeubert et al., 2013) and be considered as a first step in understanding the mussel–fish relationship, as host specificity is a key factor for mussel survival.

The host immune response to parasitism uses both the innate and adaptive defences of the fish (Lieschke & Trede, 2009). Innate immunity includes the general defence mechanisms naturally present in fish, such as responses to foreign elements, which do not require prior exposure of the host individual to parasite antigens (Barnhart et al., 2008; Donrovich et al., 2017). For successful encystment, glochidia must evade the host's innate immune barrier. This is achieved, first, by promoting the host's normal anti-ectoparasite encapsulation response, and second, by inhibiting the injection of fibres and digestive enzymes into the cyst. Adaptive immunity arises as a response to a specific antigen through the production of antibodies, with the intensity of the response being enhanced by previous exposure to the same antigen (Barnhart et al., 2008; Donrovich et al., 2017). Adaptive immunity can increase host resistance to glochidia after previous infestations (Donrovich et al., 2017; Rogers-Lowery et al., 2007), but it is the ability of glochidia to evade the innate immunity of a species that determines whether that species is a good host (Barnhart et al., 2008).

The duck mussel *Anodonta anatina* (Linnaeus, 1758) is a widely distributed species, occurring throughout Europe, north-west Africa and parts of Asia (Froufe et al., 2014; Gomes-dos-Santos et al., 2019; Hinzmann et al., 2013; Lopes-Lima et al., 2017). Currently listed as Least Concern on the IUCN Red List of Threatened Species in Europe, *A. anatina* populations have declined in many regions and countries in recent decades, including in Portugal (Lopes-Lima et al., 2014; Lopes-Lima et al., 2017; Lopes-Lima, Pereira & Pires, 2023; Nakamura et al., 2023; Ollard & Aldridge, 2023). As a result, the species is now considered as Endangered in Portugal (Lopes-Lima, Pereira & Pires, 2023). Recent studies have shown that *A. anatina* is a generalist species that uses a wide range of hosts (see fish host species in Douda et al., 2013; Huber & Geist, 2019) but is most successful on native fish, indicating that changes in native fish communities (e.g. substitution of native by non-native fish species) may threaten *A. anatina* (Dias et al., 2020; Douda et al., 2013; Huber & Geist, 2019; da Silva et al., 2023).

Lack of suitable hosts can limit the dispersal of freshwater mussels and may also be a critical factor in their decline (Schwalb et al., 2011; Vaughn, 2012). Although the importance of hosts for mussel conservation is increasingly recognized by the growing number of laboratory experiments on host suitability (Benaissa et al., 2019; Benaissa et al., 2022; Douda et al., 2017; Marwaha et al., 2019; Österling, 2015; Taeubert & Geist, 2013; Taeubert, Gum & Geist, 2012; Zieritz et al., 2012), few studies consider sympatric or allopatric host species in the same investigation (but see Douda et al., 2014; Schneider et al., 2017; Taeubert et al., 2010; Taskinen & Salonen, 2022). Elucidating the mechanisms that influence mussel–host compatibility and identifying factors associated with adaptive immunity may aid in the propagation of threatened or declining species (Dodd et al., 2006). The hypothesis tested in this study was that sympatric strains of host fish have greater resistance to *A. anatina* glochidia as a result of adaptive immunity. The premise is that the lower success rates of metamorphosis (i.e. higher immune responses) are associated with sympatric hosts and larger fish, as they have higher antibody loads from previous infections in natural environments.

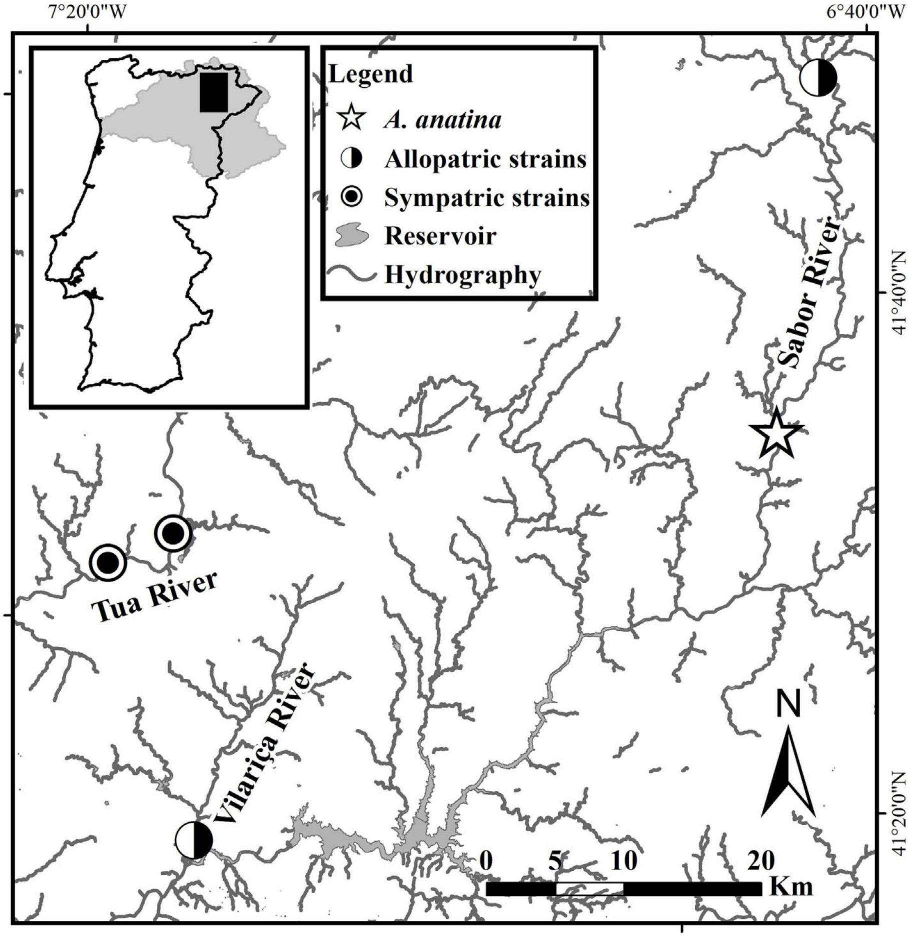


FIGURE 1 Map showing sampling locations of allopatric and sympatric strains of hosts and collection site of *Anodonta anatina*.

2 | METHODS

2.1 | Host fish collection

A. anatina hosts were captured in early January 2019, before the glochidia release period (Dias et al., 2020; Hinzmann et al., 2013), in the Douro River basin, located in north-east Portugal (Figure 1). Sympatric host strains were captured at two sites in the Tua River (7°10′6.34″W; 41°24′36.75″, 7°13′7.29″W; 41°22′54.07″) coinciding with the presence of duck mussels (Sousa et al., 2012) (Figure 1). Allopatric host strains were captured at two sites in the Vilariça (7°5′43.17″W; 41°13′1.44″) and Sabor (6°41′58.77″W; 41°47′46.23″) rivers, where duck mussels were absent, to avoid the host's adaptive immunity to *A. anatina* (Figure 1). Fish were captured with an electrofishing device (Hans Grassl ELTII, using direct current and 300–660 V). Electrofishing does not represent a risk to glochidial attachment or the success of metamorphosis into juveniles (Holliman et al., 2007). Four fish species were used in the *A. anatina* glochidia infestation experiments, including primary and marginal hosts (da Silva et al., 2022). Three species of European origin and endemic to Iberia were tested: the Iberian barbel *Luciobarbus bocagei* (Steindachner, 1864) (marginal host), the Calandino *Squalius alburnoides* (Steindachner, 1866) (primary host), the northern Iberian chub *Squalius carolitertii* (Doadrio, 1988) (primary host) and a non-native species, the bleak *Alburnus alburnus* (Linnaeus, 1758) (Table 1). The fish were transported and kept separately in the laboratory for 72 h for acclimatization, using an Aquaneering System ® with aerated water in 16 L aquaria. Hosts were examined for the presence of glochidia using subsamples of five to eight individuals, in addition to daily examination of the bottom material of the aquaria. The fish collected were similar in length (cm), which did not differ between strains (Kruskal–Wallis chi-squared = 0.002, *df* = 1, *P* = 0.956) and species (Kruskal–Wallis chi-squared = 0.170, *df* = 3, *P* = 0.982), indicating that all fish belonged to age groups 1+ and 2+ (see Supplementary material Figures S1 and S2). A total of 189 fish were used: 98 for the allopatric strain and 91 for the sympatric strain. The number of individuals per species and strain is shown in Table 1.

2.2 | Mussel collection

Adult individuals of *A. anatina* were collected on 12 January 2019 in the Sabor River, Portugal (6°40′20.642″W; 41°33′49.572″N) (Figure 1). The gravidity of the mussels was observed by gently opening the valves with crossed pliers and confirmed by the swelling of the outer demibranchs (marsupia), which indicates the presence of glochidia (Douda et al., 2013; Hinzmann et al., 2013). Five individuals of *A. anatina* were used, with a mean length of 10.04 ± 0.48 cm

TABLE 1 Results of *Anodonta anatina* host compatibility test.

Fish species	Strains	N_F	N_A	N_{FA}	*L* (cm)	*J*	MSR %	*D*
Alburnus alburnus	Allopatric	22	4	4–6	8.13 ± 1.36	0.03 ± 0.10	2.48 ± 1.54	17–27
Alburnus alburnus	Sympatric	24	4	6–6	8.05 ± 1.11	0.08 ± 0.20	3.66 ± 2.44	17–27
Luciobarbus bocagei	Allopatric	31	5	6–7	9.44 ± 2.20	0.19 ± 0.47	1.2 ± 0.76	17–24
Luciobarbus bocagei	Sympatric	24	4	2–6	9.87 ± 3.88	0.87 ± 3.14	0.86 ± 1.24	17–24
Squalius alburnoides	Allopatric	24	4	6–6	7.91 ± 0.96	0.46 ± 0.88	4.93 ± 1.64	17–31
Squalius alburnoides	Sympatric	24	4	6–6	8.62 ± 0.85	0.1 ± 0.26	1.08 ± 0.77	17–24
Squalius carolitertii	Allopatric	21	4	3–6	8.02 ± 1.47	2.28 ± 3.39	6.44 ± 2.9	17–35
Squalius carolitertii	Sympatric	19	3	5–7	8.48 ± 2.86	1.85 ± 8.09	1.16 ± 0.84	14–24

Note: Number of fish (N_F), number of aquaria (N_A), minimum and maximum number of fish per aquaria (N_{FA}), mean (±SD) values of fish total length (cm) (*L*), mean (±SD) number of juveniles per fish (*J*), mean (±SD) metamorphosis success rate (MSR g^{-1}) and duration of successful parasitism (days) (*D*). Metamorphosis success indicates (%) of attached glochidia that were recovered as live juveniles.

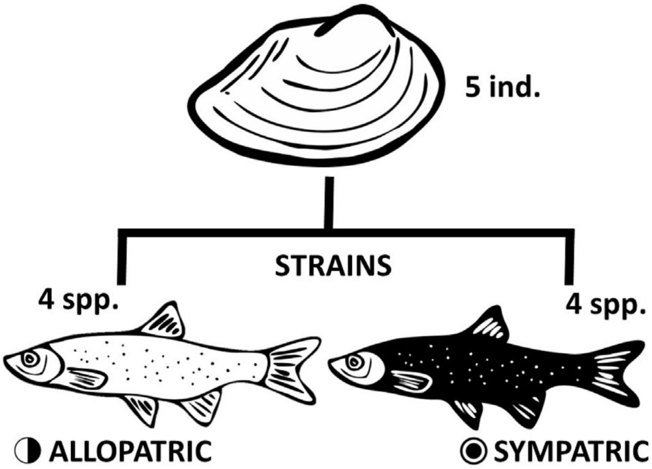


FIGURE 2 Simplified illustration of local adaptation experiment on *Anodonta anatina* fish hosts.

(mean ± SD). Mussels were transported to the laboratory and kept in 10 L aquaria with aerated river water until glochidia were released spontaneously.

2.3 | Artificial infestation

The infestation experiments were conducted from 14 January to 18 February 2019, considering guidelines proposed by Douda (2013) and Taeubert et al. (2013). The experiment was maintained with dechlorinated tap water and under identical conditions (following Taeubert et al., 2013) using an Aquaneering System ®. Glochidia subsamples were collected in aquaria containing *A. anatina* and tested for viability by adding a sodium chloride solution (Zale & Neves, 1982). Mixed glochidia from the five gravid females with viability greater than 90% were used in the experiments. The host fish were infested separately in two 40 L tanks, one containing sympatric host strains and one containing allopatric host strains (Figure 2). In each tank, the glochidia were kept in homogeneous suspension by aeration and constant agitation for 15 min (following Dodd et al., 2005). The density of glochidia in the infestation bath was 255 ± 23.1 [mean (±SD)] glochidia/120 mL in sympatric host strains and 237 ± 31.43 [mean (±SD)] glochidia/120 mL in allopatric hosts. Density was determined from six subsamples (volume of 120 mL) taken from each infestation bath. Fish were then removed from the infestation bath and transferred to an aerated glochidia-free bath, to rinse off the unattached glochidia (following Douda et al., 2012). Each species was then placed in a separate aquarium according to strain (in groups of two to seven randomly selected individuals) (Table 1), with a 3 mm net on the bottom to prevent predation on juvenile mussels. All water in the aquaria was siphoned daily through a 180 µm sieve to check for glochidia or juveniles. The retained material was evaluated and quantified under a stereoscope from the first day after infestation, ensuring knowledge of the proportion of rejected and juvenile glochidia produced by the hosts (following Douda, 2013). Live juveniles were distinguished from non-metamorphosed or dead glochidia by observing the presence of foot movements. The fish remained in the aquaria until the end of the experiment and were fed with approximately 0.200 g of commercial flake food daily. The experiment was considered complete when no glochidia or juvenile mussels were observed after a continuous period of 2 days. The weight (g) and length (cm) of each fish were measured at the end of the experiment. The mean weight and length of fish from each aquarium were used in the calculation of metamorphosis rates.

2.4 | Metamorphosis success rates

The total number of glochidia attached to the fish was used to calculate the mean metamorphosis success rate—MSR [(live juveniles produced/total number of glochidia attached to fish throughout the experiment) * 100]. The rates were normalized to the wet weight of the fish (Douda et al., 2017; Schneider et al., 2017; Taeubert & Geist, 2013), that is calculated as MSR per gram of fish weight (MSR g^{-1}).

2.5 | Data analyses

Generalized linear models (GLMs) with logit link function and quasi-binomial error structure were used to test the physiological compatibility of *A. anatina* with its hosts. The MSR g^{-1} for strains, host identity (species), mean length of the fish and the interaction between these three factors were tested. To analyse pairwise differences, that is between host species and between allopatric and sympatric strains of the same host species, multiple comparisons were performed with a Tukey post hoc test using the glht function in the multcomp R package (Hothorn et al., 2019). All analyses were performed in R (R Core Team, 2019).

3 | RESULTS

The allopatric and sympatric strains differed in the MSR g^{-1} of *A. anatina*, which was significantly higher in allopatric hosts (GLM: $F_{1,31} = 6.058$, $P = 0.019$; Table 2; Figure 3). The MSR g^{-1} (±SD) was 3.61 ± 2.69% in allopatric hosts and 1.67 ± 1.8% in sympatric hosts (global MSR g^{-1} of 2.67 ± 2.47%) (Table 1; Figure 3). The shortest and longest duration of successful parasitism was found in *S. carolitertii*, 14 days in sympatric hosts and 35 days in allopatric hosts (Table 1). The marginal host *L. bocagei* and the non-native *A. alburnus* had the same duration of parasitism for the sympatric and allopatric strains (Table 1).

The highest MSR g^{-1} (±SD) was 4.18 ± 3.52% for *S. carolitertii*, followed by *A. alburnus* with 3.07 ± 1.99% and *S. alburnoides* with 3 ± 2.37% (Table 1). *L. bocagei* had the lowest MSR g^{-1} (±SD), 1.03 ± 0.98% (Table 1). The species showed significant differences in MSR g^{-1} (GLM: $F_{3,29} = 3.755$, $P = 0.021$; Figure 4; see also Supplementary Materials Appendix Table S1). Only *L. bocagei* and *S. carolitertii* differed in MSR g^{-1} ($P = 0.015$; Figure 4). The highest MSR g^{-1} occurred in smaller fish (GLM: $F_{1,31} = 7.732$, $P = 0.009$) (Table 2; Figure 5). In the integrative models, only strains and species were significant (GLM: $F_{3,25} = 4.710$, $P = 0.009$; Table 2). *Squalius carolitertii* ($P = 0.048$) and *S. alburnoides* ($P = 0.049$) showed significant differences in the MSR g^{-1} between strains (Figure 4). The integrative models of strains and length (GLM: $F_{1,29} = 3.526$, $P = 0.070$), species and length (GLM: $F_{3,25} = 0.808$, $P = 0.501$) and strains, species and length (GLM: $F_{3,17} = 0.570$, $P = 0.642$) were not significantly different (Table 2).

4 | DISCUSSION

The results suggest that the sympatric hosts of *A. anatina* have enhanced resistance to glochidial infection and that the intensity of the immune response to glochidial attachment varies between individuals and populations. Allopatric hosts and smaller fish produce more juveniles, suggesting that *A. anatina* can temporarily exploit the naiveness of host populations and individuals with which it had no recent co-evolutionary history.

Allopatric host strains were twice as successful for *A. anatina* glochidia metamorphosis as sympatric host strains. Sympatric host strains for the Unionida have been shown to develop resistance to glochidia and reduce the number of juveniles produced after exposure to repeated infestations (Donrovich et al., 2017; Rogers & Dimock, 2003; Strayer, 2008). For example, in North America hosts that co-occur with *Lampsilis reeveiana* (Lea, 1852) and *Utterbackia imbecillis* (Say, 1829) have slower cyst formation and higher levels of antibodies against glochidia (Dodd et al., 2006; Rogers-Lowery & Dimock, 2006). Studies in Europe have also shown that brown trout *Salmo trutta* and Atlantic salmon *Salmo salar* strains that do not co-occur with *Margaritifera margaritifera* have higher rates of glochidial metamorphosis (Österling & Larsen, 2013; Taeubert et al., 2010). Acquired immunity to glochidia has not been observed in *A. anatina* because previous experiments only used allopatric hosts (Douda et al., 2013; Huber & Geist, 2019) or quantified the number of glochidia in sympatric hosts (Dias et al., 2020). Here, it is shown that allopatric strains are better at encysting *A. anatina* glochidia, possibly because of a lack of adaptive immunity, suggesting that infestations on allopatric strains may contribute to higher numbers of juveniles. The results also suggest that factors other than immune defence, such as host length and species (discussed below), may influence juvenile recruitment of *A. anatina*.

The success of glochidia metamorphosis differed between most of the hosts that were used in the experiment. Primary hosts produced three times more juveniles than marginal hosts. The results found here confirm recent studies that have shown high variation in the metamorphosis success of *A. anatina* glochidia in different hosts and its ability to parasitize a wide range of native and non-native species (Dias et al., 2020; Douda et al., 2013; Huber & Geist, 2019; Weber, 2005). In Europe, *A. anatina* has the largest glochidia compared with other European mussel species, characterized by

TABLE 2 Results of the generalized linear models of the compatibility test of *Anodonta anatina* with strains, host species and length.

Response	Effect	*df*	F	P
Metamorphosis success rate (MSR g^{-1})	Strains	1	6.058	**0.019**
	Species	3	3.755	**0.021**
	Length	1	7.732	**0.009**
	Strains × Species	3	4.710	**0.009**
	Strains × Length	1	3.526	0.070
	Species × Length	3	0.808	0.501
	Strains × Species × Length	3	0.570	0.642

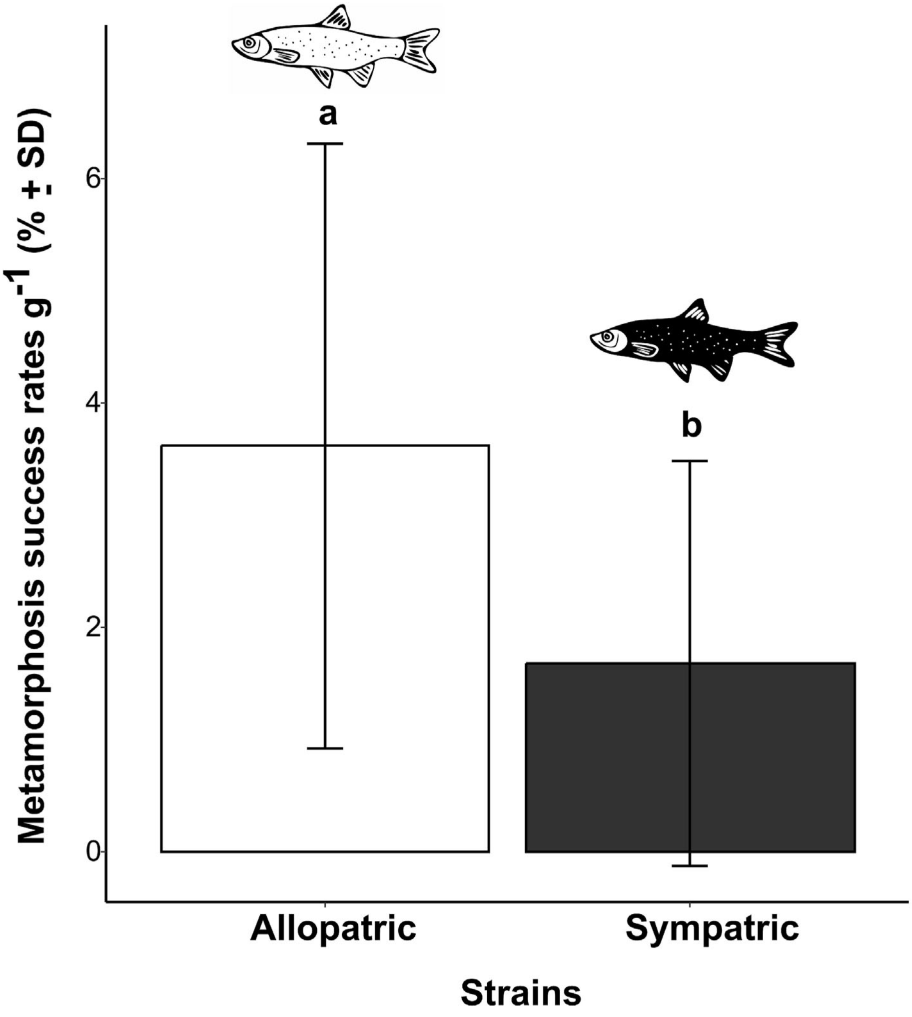


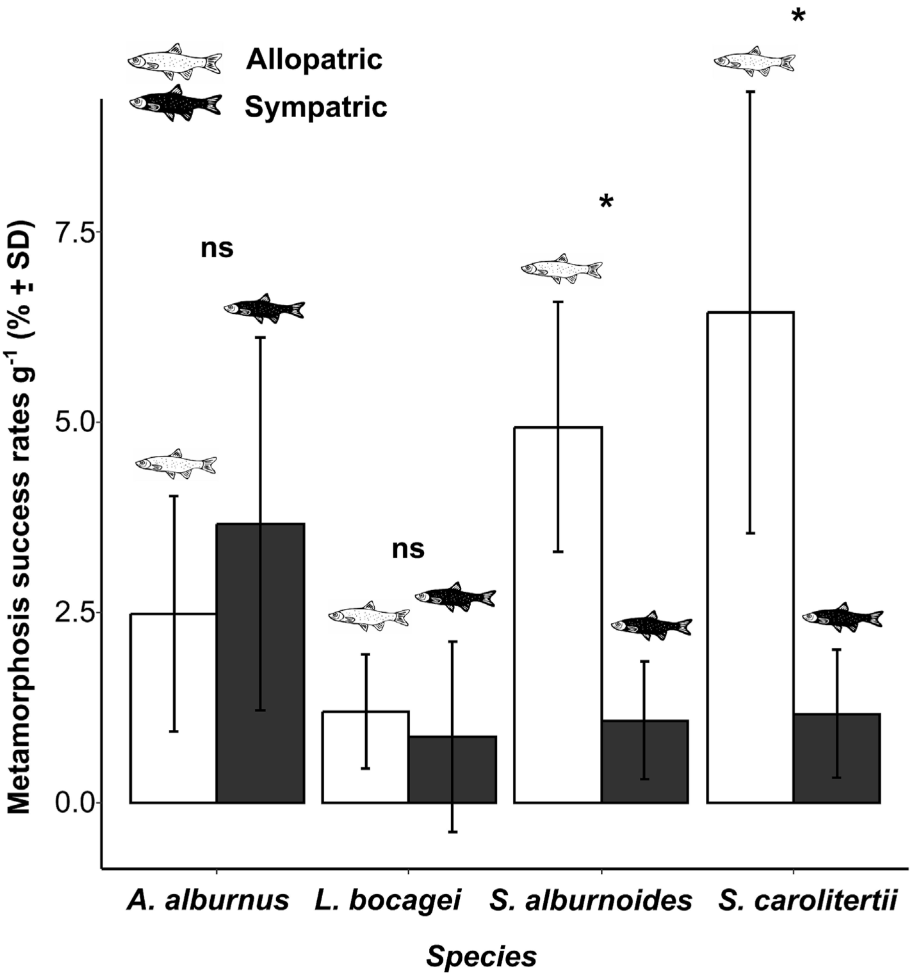
FIGURE 3 Mean (±SD) metamorphosis success rates (MSR g^{-1}) of *Anodonta anatina* in strains of allopatric and sympatric hosts tested. Boxes with different index letters (a, b) are statistically significantly different from each other (Tukey's HSD test, $P < 0.05$).

narrow hooks, typical for glochidia that attach to external tissues such as fins and lips instead of gills (Ćmiel et al., 2021; Lopes-Lima et al., 2016). Related to this feature, *A. anatina* glochidia can cause damage and reduced fitness in fish at high infestation levels, owing to their large size (Douda et al., 2013). The large size and shape of *A. anatina* glochidia may also be related to their low host specificity, as larger larvae require less time in the host and their hooks allow an easier attachment to all types of fish tissue. The laboratory results confirm previous host fish determinations made in the laboratory and in the field by Douda et al. (2013) and Dias et al. (2020), respectively. All species that had been identified previously as suitable hosts also produced juveniles in the experiment. Approaches that take into account the number of juveniles produced in laboratory experiments, as well as those obtained from naturally infested fish in a natural environment, can be a good way to identify suitable hosts.

Metamorphosis success was significantly higher in allopatric strains only for the *A. anatina* primary hosts. For example, allopatric *Squalius alburnoides* and *S. carolitertii* populations had four to five times more juveniles than those in sympatry. The results suggest that the low dependence on primary host species in sympatric strains, such as *S. alburnoides* and *S. carolitertii*, is not maintained in allopatric strains. These species have already been identified as some of the most suitable hosts for *A. anatina* in experiments with allopatric strains (Douda et al., 2013) and in sampling of sympatric strains in natural environments (Dias et al., 2020). Sympatric strains of *S. alburnoides* and *S. carolitertii* probably underwent strong selection for genotypes more able to avoid *A. anatina* parasitism at some point in mussel–fish coevolution, as only the allopatric strains of these two hosts appear to be highly susceptible to glochidia. However, differences in the compatibility of *A. anatina* populations with suitable hosts may go beyond co-occurrence and the condition factor of the fish species and may also be explained by distinct genetic strains of host populations, as already observed for other freshwater mussel species (Douda et al., 2014; Karlsson et al., 2014; but see also Geist & Kuehn, 2008). Therefore, studies on the intraspecific genetic diversity of host populations compatible with *A. anatina* are needed to understand better the strength of the adaptive immune responses demonstrated here.

The parasitic period was longer in allopatric primary hosts than in sympatric ones. The nutrient exchange that occurs between host fish and glochidia may be limited by the shorter duration of parasitism, which in turn may affect juvenile survival and performance (Dodd

FIGURE 4 Mean (±SD) metamorphosis success rates (MSR g^{-1}) of *Anodonta anatina* in species of host fish tested by strains. Significant paired comparisons are represented by asterisks and not significant by 'ns' (Tukey's HSD test, $P < 0.05$).



et al., 2005; Marwaha et al., 2017). Studies have found a shorter duration of parasitism in fish hosts with acquired immunity (Dodd et al., 2005; Rogers & Dimock, 2003) and higher survival rates in hosts with a longer parasitic phase (Marwaha et al., 2017). In addition, the most suitable fish strains have also shown the highest glochidial growth rates (Taeubert et al., 2010). For *A. anatina*, hosts with the highest metamorphosis success rates have produced juveniles with lower lipid reserves (a proxy for energy reserves and growth) (Douda, 2015), suggesting lower survival success. In addition, allopatric strains of primary hosts have demonstrated longer persistence times relative to secondary hosts (Douda et al., 2013). Owing to a lack of data, there is no clear relationship between the duration of *A. anatina* parasitism in allopatric and sympatric strains. Determining the duration of the parasitism period is essential for the effectiveness of breeding programmes, as a longer delay in the production of juveniles requires greater maintenance in the laboratory. Further research should be conducted to test whether allopatric hosts of *A. anatina* that optimize glochidia metamorphosis also maintain high growth rates and juvenile survival.

Several factors influence host antibody levels and thus the efficiency of immune responses to glochidia. Larger fish have a greater capacity to resist *A. anatina* glochidial attachment, regardless of strain or species. Larger fish may have a more developed and efficient immune system than smaller fish, producing more effective antibodies (Rogers-Lowery et al., 2007; Rogers-Lowery & Dimock, 2006). The strength of the immune defences generally increases with increasing age or can be acquired as a result of previous infections (Donrovich et al., 2017; Uribe et al., 2011). However, *M. margaritifera* fish hosts such as *S. trutta* have shown changes in response, with naive older fish being more suitable hosts (Marwaha et al., 2019). For *A. anatina*, studies have shown that host length affects metamorphosis rates in several ways. On the one hand, larger fish may host a greater number of glochidia attached to the body because of their larger attachment surface (Dias et al., 2020). On the other hand, metamorphosis success rates are known to decrease in hosts previously exposed to other mussel species, meaning that older and larger fish should have a stronger immune response to glochidial encystment (Donrovich et al., 2017). This heterogeneity in response suggests that individual host characteristics may be one of the most important factors in the reproductive success of *A. anatina*.

Metamorphosis success rates of the non-native *A. alburnus* were similar to those of the native primary hosts *S. alburnoides* and *S. carolitertii*. Interestingly, they also did not differ between sympatric and allopatric strains. Recently, Dias et al. (2020) showed that in the

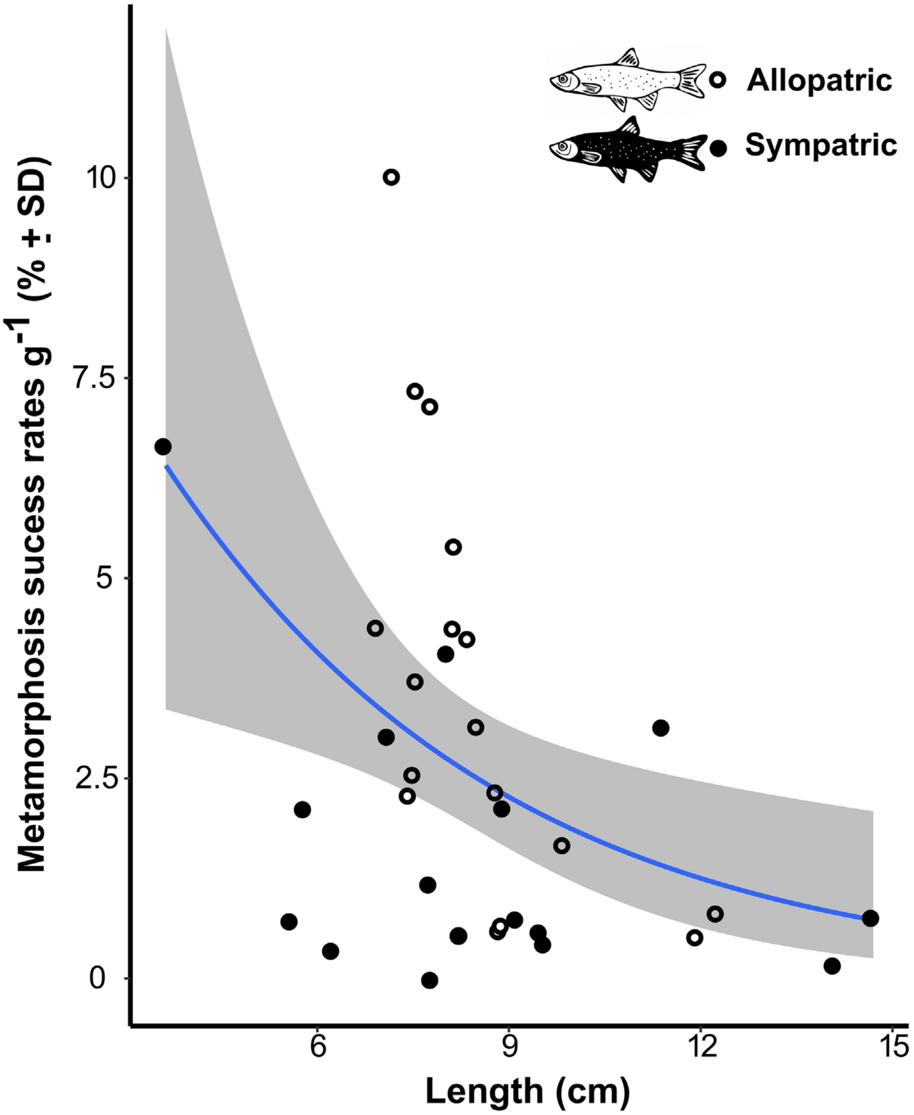


FIGURE 5 Relationship between the mean length of the fish and mean (±SD) metamorphosis success rates (MSR g^{-1}). The 95% confidence intervals are in grey.

natural environment, non-native species carry lower numbers of *A. anatina* glochidia than native species. However, in the Tua River, the number of glochidia found in *A. alburnus* was similar to that found in primary hosts (Dias et al., 2020). The metamorphosis success rates found here suggest that the *A. anatina* glochidia found in *A. alburnus* by Dias et al. (2020) had the potential to become juveniles, confirming *A. alburnus* as a suitable host for *A. anatina*. Other non-native fish species have already been identified as suitable hosts for *A. anatina* from other regions (Douda et al., 2013; Huber & Geist, 2019) but without comparing metamorphosis rates in sympatric and allopatric strains. Therefore, it was not possible to describe a general pattern for the interaction of *A. anatina* with non-native hosts. Future studies may help to understand how beneficial this relationship is for *A. anatina*, as *A. alburnus* is a successful invader throughout the Iberian Peninsula (Latorre et al., 2018). Alternatively, it may even be detrimental, becoming a sink host for glochidia in the future (Huber & Geist, 2019 for more details), owing to hybridization with other fish species (Curto et al., 2022), which may alter the immunological responses of individuals.

5 | CONCLUSION AND IMPLICATIONS FOR CONSERVATION

Data on the role of innate and adaptive immunity in mussels, quantified from concurrent experiments, are scarce. The lack of data on the basic ecology of mussels is problematic, as successful conservation efforts require knowledge of one of the key factors for their survival, including the identification of the most suitable hosts (Dias et al., 2020; Ferreira-Rodríguez et al., 2018; Lopes-Lima et al., 2021). The present study adds information on how *A. anatina* exploits fish host resources and how fish have evolved to avoid parasitism by this freshwater mussel. The information reported here may be useful in identifying the species and host populations that are most suitable for producing greater numbers of *A. anatina* juveniles in captivity. The findings suggest that allopatric primary host species and smaller individuals are best suited for *A. anatina* propagation efforts, as they have the highest metamorphosis success rates. This suggests that healthy rivers, mainly containing native fish populations with high recruitment rates, provide juvenile fish stocks that play a crucial role in the conservation of freshwater mussels. However, allopatric hosts exhibit a longer period of parasitism, which may pose a challenge to the reproduction of *A. anatina* in the laboratory, despite the increased production of juveniles. In a current scenario of mussel extinction (Lopes-Lima, Reis, et al., 2023) and homogenization of fish communities in the Iberian Peninsula (Clavero & García-Berthou, 2006; Villéger et al., 2011), the reintroduction of mussels could be one of the conservation measures needed to maintain healthy freshwater mussel populations. Furthermore, the co-evolutionary relationship between *A. anatina* mussel–fish and non-native host species such as *A. alburnus* remains unclear. The ability of *A. anatina* to adapt and make efficient use of the resources of the non-native host *A. alburnus* is not evident as the number of juveniles produced is low, which may make *A. alburnus* a potential ecological sink for glochidia in the future. However, it should be noted that there are exceptions to the generalizations made in this study, as suitable host species vary throughout the entire native range of *A. anatina*. Future experimental studies should address the possible influences of adaptive immunity to provide a full understanding of the extent of immunity demonstrated here, particularly its effects on survival, juvenile development, encystment success and metamorphosis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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