

## RESEARCH ARTICLE

# Invasive Chinese pond mussel *Sinanodonta woodiana* threatens native mussel reproduction by inducing cross-resistance of host fish

Seth W. Donrovich<sup>1</sup> | Karel Douda<sup>1</sup> | Věra Plechingerová<sup>1</sup> | Kateřina Rylková<sup>1</sup> | Pavel Horký<sup>1</sup> | Ondřej Slavík<sup>1</sup> | Huan-Zhang Liu<sup>2</sup> | Martin Reichard<sup>3</sup> | Manuel Lopes-Lima<sup>4,5</sup> | Ronaldo Sousa<sup>6</sup>

<sup>1</sup>Department of Zoology and Fisheries, Czech University of Life Sciences Prague, Prague, Czech Republic

<sup>2</sup>The Key Lab of Aquatic Biodiversity and Conservation, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China

<sup>3</sup>Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic

<sup>4</sup>CIBIO/InBIO - Research Center in Biodiversity and Genetic Resources, University of Porto, Vairão, Portugal

<sup>5</sup>CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

<sup>6</sup>CBMA - Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Portugal

## Correspondence

Karel Douda, Czech University of Life Sciences Prague, Department of Zoology and Fisheries, Kamýcká 129, Prague, CZ 165 00, Czech Republic.

Email: k.douda@gmail.com

## Funding information

Czech Science Foundation, Grant/Award Number: 13-05872S

## Abstract

1. The effects of invasive alien species (IAS) on host–affiliate relationships are often subtle and remain unnoticed or insufficiently quantified. The global decline of freshwater unionid mussel species has been attributed to many causes, but little is known about the interactions of IAS, with their complex life cycle, which includes an obligatory parasitic stage (the glochidium) that develops on fishes.
2. The capacity of a European freshwater mussel, *Anodonta anatina*, to develop on its widespread fish host, *Squalius cephalus* was tested experimentally, after previous infestations by the IAS, *Sinanodonta (Anodonta) woodiana*. The initial attachment of glochidia, the length of the parasitic period, and the metamorphosis success rate of *A. anatina* glochidia were compared among treatments of different priming infestation intensities.
3. The metamorphosis success rate of the native *A. anatina* glochidia was strongly reduced (Wilcoxon Signed-Rank Test,  $P < 0.001$ ) and declined by 42.1 and 45.4% on fish hosts that were previously exposed to *S. woodiana* by single and multiple priming infestations, respectively, in comparison with the control group. Such cross-resistance is expected to decrease significantly the quality of the host resources available to native mussels.
4. This study provides the first evidence of the host-mediated adverse impact of invasive *S. woodiana* on native mussel species. These results also highlight the importance of potential competition for hosts between threatened groups of affiliate species and their invasive counterparts, which should be reflected in conservation strategies.

## KEYWORDS

adaptive immunity, *Anodonta anatina*, competition, freshwater, glochidia, host–parasite relationships, invasive alien species

## 1 | INTRODUCTION

The abundance and diversity of freshwater mussels are severely decreasing worldwide, with nearly half of the species currently threatened. This situation may have profound consequences at several ecological levels, from individuals to ecosystems (Bogan, 1993; Burlakova et al., 2011; Lopes-Lima et al., 2014; Walker, Jones, & Klunzinger, 2014). For example, the decline of freshwater mussels

may impair several ecosystem functions because these species have high filtration rates, providing an important link between the water column and the benthic zone, and also playing an important role in nutrient cycling, substrate stability, bioturbation, and controlling the levels of suspended solids (Bauer & Wachtler, 2001; Bogan, 1993; Lopes-Lima et al., 2017; Vaughn & Hakenkamp, 2001; Williams, Warren, Cummings, Harris, & Neves, 1993). In addition, freshwater mussels are usually considered suitable bioindicators of

overall ecosystem health and are also thought to be keystone species, as they increase species richness and overall biodiversity when present in an ecosystem (Aldridge, Fayle, & Jackson, 2007; Spooner et al., 2013).

Although the reasons for freshwater mussel decline are numerous and idiosyncratic, one of the causes of most concern is related to the introduction (deliberate or accidental) of invasive alien species (IAS). The number of freshwater IAS has been increasing rapidly in both tropical and temperate latitudes and rigorous studies of the impacts of IAS are often lacking (Tricarico, Junqueira, & Dudgeon, 2016). It is supposed that introductions of other freshwater bivalves are particularly problematic for native freshwater mussels (Baker & Levinton, 2003; Ricciardi, Neves, & Rasmussen, 1998; Sousa, Novais, Costa, & Strayer, 2014; Sousa, Pilotto, & Aldridge, 2011). The introduction of bivalve species can lead to competition with native species for space, nutrients, food, and possibly also for host fish, the latter of which are necessary for completion of the freshwater mussel life cycle (Arey, 1932; Barnhart, Haag, & Roston, 2008; Kat, 1984; Novais, Dias, & Sousa, 2016; Sousa et al., 2014).

Unionid freshwater mussels produce parasitic larvae (glochidia) that must attach to fish to complete their development into juveniles (Dillon, 2000). To form a successful host fish and freshwater mussel relationship, three conditions are required: initial contact between glochidia and the host fish, physiological suitability of the host for attachment and glochidia development, and resistance of the glochidia to the host's immune responses (Neves, Weaver, & Zale, 1985). In each reproductive year, many glochidia are released, so the first factor is dependent on the distinct infestation strategies of the mussels (Barnhart et al., 2008), ecosystem conditions, fish microhabitat preferences, fish behaviour and fish abundance. If the glochidia fail to attach to a host fish, they will eventually sink to the substrate, where opportunities for attachment are highly unlikely and the glochidia perish (Jansen, Bauer, & Zahner-Meike, 2001). Unsuccessful infestations can take place either when the host fish contains insufficient chemical or nutritional requirements for metamorphosis to occur or as a result of direct immune system rejection by the host fish (Neves et al., 1985). In these cases, glochidia may fail to become encysted or are sloughed off before transformation (Jansen et al., 2001). Alternatively, failure to metamorphose may result from the glochidia being abnormally encysted before detachment (Arey, 1932; Rogers-Lowery, Dimock, & Kuhn, 2007).

The fish immune system includes innate and adaptive components (Lieschke & Trede, 2009). Each of these acts as a means of protection against parasites, including glochidia. Innate immunity involves the general defence mechanisms that are continuously present in the fish as a response to foreign substances. Adaptive immunity arises as a response to a specific antigen, where the strength of the response is greatly amplified by previous contact with that same antigen. Several studies have found that previously infested host fish contain large amounts of specific antibodies produced in response to glochidial infestations (Dodd, Barnhart, Rogers-Lowery, Fobian, & Dimock, 2006; O'Connell & Neves, 1999; Rogers-Lowery et al., 2007). Because adaptive immunity can inhibit or prevent secondary infestations in host fish, it has been

suggested that particular mussel species can compete for host resources by inducing cross-resistance of host fish (Dodd, Barnhart, Rogers-Lowery, Fobian, & Dimock, 2005; Strayer, 2008). Competition for host fish is most likely a common and natural mechanism in freshwater mussel assemblages (Strayer, 2008). However, it could have detrimental consequences for native mussel species when induced by IAS.

There is only limited knowledge on the specific impacts that invasive mussels have on native mussels with regard to competition for host fish. In this context, one of the most potentially hazardous species is *Sinanodonta* (*Anodonta*) *woodiana* (Lea 1834), commonly known as the Chinese pond mussel. *Sinanodonta woodiana* is native to two river basins in China, the Yangtze and Amur rivers, where it was primarily located before its expansion to different parts of the world, including Southeast Asia, Europe, North America, and the Caribbean (Kraszewski, 2007; Watters, 1997; Zieritz et al., 2016). This species is spreading quickly throughout European waters and has reached high densities in many rivers and standing water bodies, such as the Cris/Koros River basin in Romania and Hungary (Sarkany-Kiss, 1997; Sarkany-Kiss, Sirbu, & Hulea, 2000), Lake Balaton in Hungary (Benkó-Kiss, Ferincz, Kováts, & Paulovits, 2013), the Danube and Tisza rivers in Hungary (Bódis, Tóth, & Sousa, 2014, 2016), lowland rivers in Serbia (Paunovic, Csányi, Simic, Stojanovic, & Cakic, 2006) and the Czech Republic (Douda, Vrtílek, Slavík, & Reichard, 2012), channels with soft substrate in Italy (Cappelletti, Cianfanelli, Beltrami, & Ciutti, 2009), inter-basin waterways in the Iberian peninsula (Pou-Rovira et al., 2009), and warmer water bodies in Poland (Kraszewski & Zdanowski, 2007). Reproduction in *S. woodiana* occurs in autumn and the glochidia are released in summer in European waters (Douda et al., 2012; Sarkany-Kiss et al., 2000) to begin parasitic life on a host for a period that varies according to environmental conditions, mainly water temperature (Afanasjev, Zdanowski, & Kraszewski, 2001). *Sinanodonta woodiana* produce glochidia two or three times per year (Sarkany-Kiss et al., 2000) in high numbers (Wachtler, Dreher-Mansur, & Richter, 2001) and successfully parasitize a wide spectrum of European native fish species (Douda et al., 2012).

The aim of this study was to quantify the capability of a native European fish to host native mussel glochidia following previous infestation by the invasive *S. woodiana*. The null hypothesis tested was that a priming infestation by an invasive unionid mussel has no detectable effect on the subsequent development of native mussel glochidia. *Squalius cephalus* (Linnaeus 1758), European chub, was chosen as the host species because it has the widest distributional overlap with *S. woodiana* and *Anodonta anatina* (Linnaeus 1785), and high local abundance in most fish assemblages in Central Europe (Douda et al., 2012; Kottelat & Freyhof, 2007; Lopes-Lima et al., 2017). *Squalius cephalus* were artificially infested (primed) with glochidia of *S. woodiana* at varying intensities. The host fish were then re-infested under laboratory conditions with glochidia of the native duck mussel, *A. anatina* to determine the success rate of juvenile mussel metamorphosis. Developmental success of glochidia was compared among treatment groups, and the potential conservation implications for native freshwater mussels are discussed.

## 2 | METHODS

### 2.1 | Study species

Parasitic larvae of the invasive *S. woodiana* were obtained from an established population in its non-native Central European range. Gravid females were collected in the Kyjovka River (Czech Republic, 48°46'42.07"N, 17°0'57.97"E) and were transferred to stock tanks (six outdoor aerated fiberglass pools with a total water volume of 5000 L) at the Czech University of Life Sciences Prague on 24 July 2015. Gravid *A. anatina* specimens were collected on 6 November 2015 from a slow-moving section of the Sázava River (Czech Republic, 49°51'23.59"N, 14°42'0.01"E). The collection was completed using the same procedure and equipment as for the collection of *S. woodiana*.

Glochidia were obtained from the parent mussels with ripened larvae by flushing the marsupia with water using a syringe immediately before each experimental infestation. The viability of glochidia was verified by quantifying the closing response to sodium chloride in subsamples (ASTM E2455-06, 2013). The glochidia from between four and six different gravid females with a glochidia viability exceeding 90% were pooled and used for inoculation of all fish to standardize the potential for infection of all study host individuals. The actual concentration of the inoculation bath was assessed by counting the number of viable glochidia in 10 × 10 mL water samples that were removed by a syringe from the baths over the course of inoculation.

The test host fish species was *S. cephalus*. This host species has a wide geographical distribution, large ecological tolerance and high local population densities (Musil, Horký, Slavík, Zbořil, & Horká, 2012), ensuring the potential for natural incidence of the initial contact between glochidia of both study mussel species and host individuals (Blažek & Gelnar, 2006). On the other hand, metamorphosis success rate of the native *A. anatina* glochidia is relatively lower on *Squalius cephalus* compared with some other potential host species (Douda, 2015; Douda et al., 2014). This host species was selected because it is a known host of both *S. woodiana* and *A. anatina* and is widely present, and because the species probably represents both a stable and important host resource for *A. anatina* in central Europe. The fish used in the experiment were hatchery-reared juveniles obtained from a local fish supplier (Vodňany, Czech Republic) and had no previous contact with glochidia. Fish individuals of a similar size (1+ year old; mean standard length 128 mm, range 101–144 mm; mean mass 27.5 g, range 13.4–41.6 g) were maintained in an aquarium recirculation system composed of four connected holding tanks (320 L each) with a 10% exchange of fresh water every day. Each tank had an aeration tube, and the water was purified using biological filters (Fluval FX6, Hagen) and a UV sterilizer (UV07, Resun).

All fish were acclimated for at least 1 month in the laboratory holding system (dechlorinated tap water, 12-h light cycle). Temperature in the system was recorded with a HOBO data logger (Onset, USA) at 15 min intervals and was  $22.9 \pm 1.4^\circ\text{C}$  (mean  $\pm$  SD) over the course of the experiment. Fish were fed daily *ad libitum* during the acclimation period and the experiment, using commercial fish pellets for cyprinids (Biomar Group, Denmark). The fish were tagged 10 days before the start of the experiment, after first anaesthetizing

them with 2-phenoxyethanol (0.2 mL L<sup>-1</sup>; Merck KGaA, Germany). Passive integrated transponders (PITs; Trovan ID100, 0.1 g in air, 12 × 2.1 mm; EID Aalten B.V., Aalten, the Netherlands) were then inserted into the dorsal musculature using a syringe.

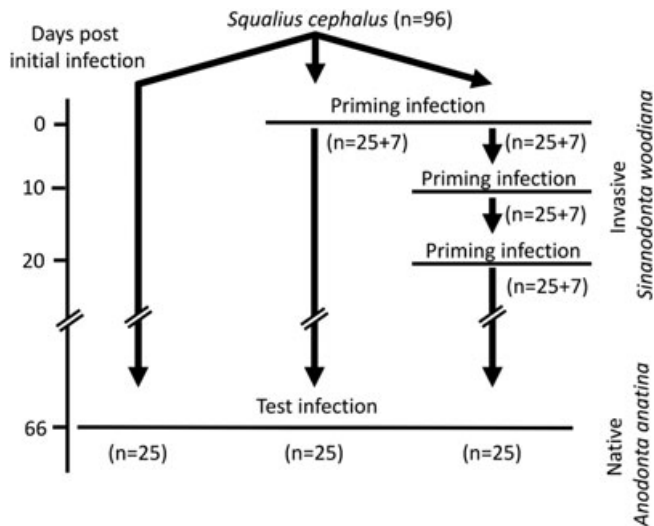
### 2.2 | Laboratory infestations

Three different infestation treatments (reported hereafter as 'naïve', 'single priming' and 'multiple priming') were used to compare the effects of previous contact of fish with glochidia. The multiple priming treatment was designed to reach the maximum levels of infestation reported in literature (tens of glochidia per gram of fish body weight) and repeated contact with glochidia throughout the season to mimic natural conditions. The single priming treatment received an identical glochidia load during the first infestation event, but the fish were not infested in subsequent inoculations. The naïve group obtained no glochidia, but the fish were handled in the same way as the infested fish during the experiment.

Immediately before the start of the first infestation, fish were randomly divided into the three treatment groups (60–75 individuals in each) and their PIT code numbers were recorded. The fish were infested in an aerated common bath suspension of dechlorinated tap water (0.5 L per fish) and live glochidia. The water was stirred manually before and during the infestation to maintain a homogeneous suspension of glochidia in the water. This process was sustained continuously for 15 min to provide sufficient time for the glochidia to attach to host fish. After the designated infestation time, the fish were transferred to another bath of dechlorinated tap water for 30 min to rinse off glochidia that had not attached.

Fish from all treatment groups were subsequently pooled, returned to the initial holding system, and kept under the above-described conditions between infestation events, which were performed using the same methods. Fish were classified by their PIT codes and separated into their respective infestation treatment groups immediately before each of the subsequent infestations. In total, fish from the multiple priming treatment group were infested three times in 10-day intervals after the first infestation, with the last infestation taking place 46 days before the experimental infestation by native *A. anatina* (see details in Figure 1). Mean ( $\pm$  SE) glochidia bath densities used for the infestations were  $5540 \pm 746$ ,  $7200 \pm 1592$  and  $7700 \pm 2113$  glochidia per litre in the first, second and third consecutive priming infestations, respectively.

Forty-six days after the last priming infestation (after all *S. woodiana* glochidia detached and before the *A. anatina* infestation), the three distinct groups of *S. cephalus* were identified by their tag numbers, and 25 individuals were randomly selected from each group to be infested by glochidia ( $7433 \pm 1614$  glochidia per litre) from *A. anatina* (a single experimental infestation). All fish were infested in a common bath suspension to ensure constant conditions during inoculation. The infestation procedure followed the same protocol used for *S. woodiana*. The length and weight of the experimental fish were recorded at the end of the experiment, and the relative body weight (the condition factor, K) was calculated using the equation:  $K = 100 \times \text{somatic weight (g)} / (\text{standard length [cm]})^3$  to express the general condition of the fish individuals (Ricker, 1975).



**FIGURE 1** The timing of priming (*S. woodiana*) and experimental (*A. anatina*) infestations. Numbers of host fish individuals used are stated in parentheses

### 2.3 | Monitoring of glochidia development on experimental fish

After the experimental glochidial infestation, the *S. cephalus* individuals were transferred into 18-L tanks. Each of the 75 tanks contained one fish from an unknown group, chosen at random. The tanks were filled with dechlorinated tap water and maintained at ambient laboratory temperature  $19.3 \pm 1.4^\circ\text{C}$  (mean  $\pm$  SD). The monitoring process took place daily for 22 days after the infestation. During the experiment, it was determined that all the fish were in adequate health, and there were no mortalities for the duration of the infestation and monitoring processes. The bottom of each aquarium was covered with a net (mesh size 3 mm) to prevent juvenile predation by host fish, and the water was partially exchanged (approximately 80% of the total water volume) and examined for the presence of glochidia and juvenile mussels daily by siphoning the tank. Glochidia and juvenile mussels were collected from siphoned water using filters (mesh size  $139 \mu\text{m}$ ) and were identified under a stereomicroscope at 10–40 $\times$  magnification and were counted. Glochidia were recorded as living juveniles if foot activity or valve movement was observed. These methods enabled an estimate of both the absolute number of glochidia attached to the fish during the course of the experiment and the metamorphosis success rate of the attached glochidia (Dodd et al., 2005). The same procedure was used to monitor glochidial success during the priming infestations with *S. woodiana*, using seven extra fish individuals from each of the single priming and multiple priming treatments to verify the effectiveness of the priming infections. Monitoring of these control fish indicated that priming infestations were successful, and *S. woodiana* juveniles were recovered from all priming infestations performed. The total number of *S. woodiana* glochidia attached to the experimental fish during priming infections was  $30.5 \pm 11.6$  and  $101.0 \pm 39.4$  glochidia per gram of fish body weight in the single priming and multiple priming treatments, respectively. The metamorphic success of *S. woodiana* was relatively low (cf. Douda et al.,

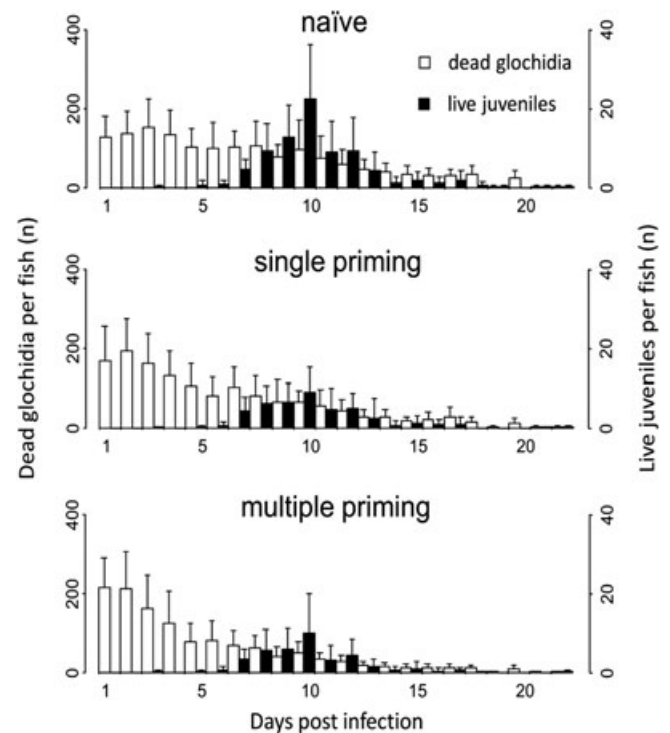
2012) during priming infections as  $4.6 \pm 2.3$  ( $15.3 \pm 0.5\%$ ) and  $10.5 \pm 3.7$  ( $10.6 \pm 1.8\%$ ) juveniles per gram of fish body weight were recovered in the single priming and multiple priming treatments, respectively.

### 2.4 | Statistical analysis

Because the data on glochidial parasitization did not meet the criteria for parametric statistics (visual inspection, Shapiro–Wilk test), and in consideration of the sample size tested ( $n = 75$ ), non-parametric tests were used throughout the study. The groups were analysed for potential differences using the non-parametric Kruskal–Wallis test. When the results of the Kruskal–Wallis test were significant, pairwise comparisons were conducted according to the two-tailed unpaired Wilcoxon–Mann–Whitney test and were adjusted for multiple comparisons using the Bonferroni correction. All statistical analyses were performed in R version 3.0.3 (R Development Core Team, 2014).

## 3 | RESULTS

Clear differences were found in hosting capabilities between naïve and primed host fish by invasive *S. woodiana* (Figure 2). The total number of initially attached glochidia per gram was slightly lower in primed host fish (Kruskal–Wallis test,  $n = 75$ ,  $H = 7.9$ ,  $df = 2$ ,  $P < 0.05$ ), and the post hoc test showed that the difference arose from the contrast between naïve and multiple priming treatments (Wilcoxon signed-rank test,



**FIGURE 2** Developmental dynamics of freshwater mussel *A. anatina* glochidia on naïve (a), ‘single priming’ (b), and ‘multiple priming’ (c) *S. cephalus* with glochidia of *S. woodiana*. Bars represent mean ( $\pm$ SD) glochidia or juveniles detached from fish in the respective day after infestation

$P < 0.05$ ). The number of successfully metamorphosed juveniles per gram of fish body weight was significantly lower for both the single and multiple priming treatment than for the naïve host treatment (Wilcoxon signed-rank test,  $P < 0.001$ ). Consequently, the metamorphosis success rate of *A. anatina* glochidia was strongly reduced in both treatments with primed fish (Wilcoxon signed-rank test,  $P < 0.001$ ). Glochidia on naïve host fish had nearly twice the metamorphosis success ( $5.1 \pm 2.0\%$ ) in developing into juvenile mussels compared with glochidia infesting hosts from single (metamorphosis success  $2.9 \pm 1.4\%$ ) and multiple (metamorphosis success  $2.8 \pm 1.7\%$ ) priming treatments (mean  $\pm$  SD, Figure 3). Hence, mean glochidial metamorphosis success was reduced in comparison with naïve host fish by 42.1 and 45.4% for groups previously infested with *S. woodiana* once and three times, respectively. Total numbers (mean  $\pm$  SD) of live *A. anatina* juveniles recovered per individual fish decreased from  $80 \pm 35$  individuals on naïve hosts to  $42 \pm 22$  (single priming) and  $36 \pm 26$  (multiple priming) individuals (Figure 3).

The length of the parasitic period of successfully metamorphosed juveniles did not differ among treatments (Kruskal–Wallis test,  $n = 75$ ,  $H = 2.1$ ,  $df = 2$ ,  $P > 0.05$ ). Nevertheless, because of increased detachment rates of unsuccessful glochidia from host fish during the initial days after infestation (Figure 2), the parasitic period (mean  $\pm$  SD) of

dead glochidia was significantly reduced from  $7.34 \pm 1.26$  days in naïve to  $6.15 \pm 1.36$  and  $5.20 \pm 0.89$  days in single and multiple priming treatments, respectively (Kruskal–Wallis test,  $n = 75$ ,  $H = 28.9$ ,  $df = 2$ ,  $P < 0.001$ ) (Figure 3).

The comparison of fish somatic parameters measured at the end of the experiment revealed no statistically significant differences in fish body weight or condition factor among treatments (Kruskal–Wallis test, all  $P > 0.05$ ). The mean  $\pm$  SD condition factor was  $0.78 \pm 0.05$  in primed fish and  $0.79 \pm 0.05$  in naïve fish.

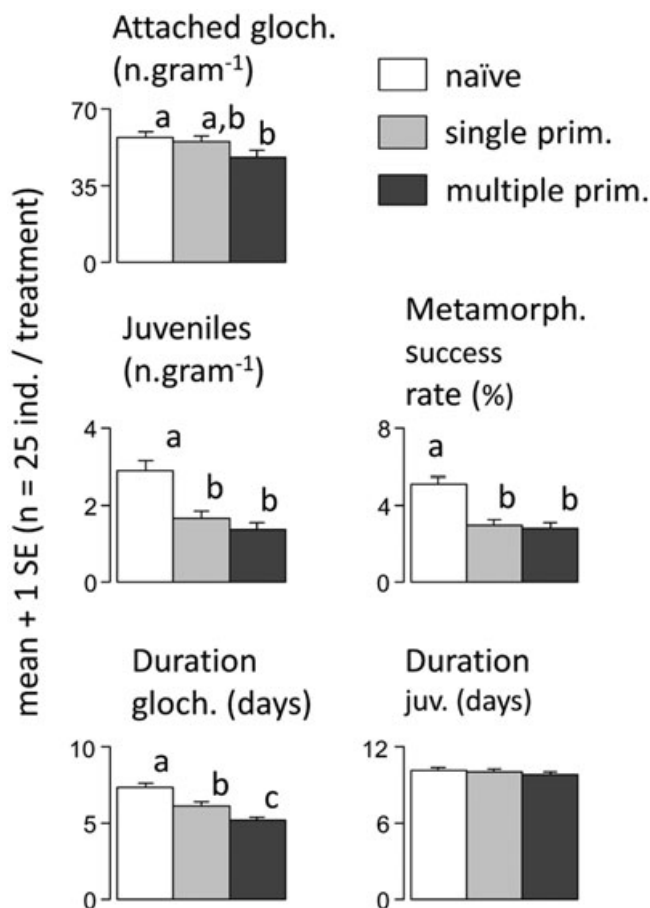
## 4 | DISCUSSION

This study documents for the first time a non-specific resistance of host fish to glochidia, induced by the invasive unionid *S. woodiana*. The transformation success rate of *A. anatina* was significantly reduced after priming by the invasive *S. woodiana* compared with naïve hosts. Such cross-resistance can be expected to decrease the quality of host resources available to native mussels in natural habitats.

### 4.1 | Adaptive immunity

Based on the results of previous studies on native unionids, the observed cross-resistance can be attributed to the mechanism of acquired immunity (Rogers & Dimock, 2003). Several studies have found that previously infested host fish can develop specific antibodies against native glochidia (Dodd et al., 2006). The response time of antibody formation for resistance varies among species of fish (Rogers-Lowery et al., 2007). Furthermore, the production of antibodies increases both with maturity and rising temperatures (O'Connell & Neves, 1999). Acquired immunity in fish against a specific parasite can persist for months or years, or in some cases perhaps even permanently (Dodd et al., 2006). At the intra-specific level, the previous attachment of glochidia of the broken-rays mussel *Lampsilis reeveiana* (Lea 1852), on large-mouth bass *Micropterus salmoides* (Lacepede 1802) decreased metamorphosis success with each successive infestation from 67.9% to 38.1% to 28.0% over three infestations (Dodd et al., 2006). These findings can be extended to the inter-specific level for species from different areas of origin, as the metamorphosis success rate of *A. anatina* significantly declined after just one priming infestation with the glochidia of the invasive *S. woodiana*.

Nevertheless, it should be mentioned that adaptive immunity is not the only possible explanation for the observed decline in host quality after the priming infestation with glochidia. Previous studies have shown that glochidia of freshwater mussels can have various effects on fish health status and behaviour (Crane, Fritts, Mathis, Lisek, & Barnhart, 2011; Horký, Douda, Maciak, Závorka, & Slavík, 2014; Taeubert & Geist, 2013; Thomas, Taylor, & Garcia de Leaniz, 2013) that could possibly influence glochidial success during the next infestation. These effects were also corroborated with *S. woodiana* in a study that found a significant effect of their glochidia on host condition factor, plasma ion concentration and enzyme activities during the parasitization period (Douda et al., 2017). However, the experimental infestation reported here was performed after a relatively long period



**FIGURE 3** Differences in the total number of glochidia attached, number of developed juveniles, metamorphosis success rate and duration of parasitism (dead glochidia, live juveniles) between treatment groups. Different letters (a, b, c) indicate significant differences between groups (Wilcoxon–Mann–Whitney test,  $P < 0.05$ )

after the priming infestation (46 days), and no negative effects of the priming infestations on fish condition were detected.

## 4.2 | Potential impacts of cross-resistance on native mussel species

The experimental results reported here suggest that the presence of *S. woodiana* can have adverse effects on reproduction and the overall status of freshwater mussels in European waters. This IAS lives in various aquatic habitats, such as rivers, lakes, streams, ponds, and reservoirs. It is most commonly observed in lowland freshwater environments, such as ponds, oxbow lakes, canals located on floodplains, and rivers that are slowly to moderately moving (0.05 to 0.3 m s<sup>-1</sup>), on muddy sediments where other native unionids are commonly present (Beran, 2008; Kraszewski & Zdanowski, 2007; Sarkany-Kiss et al., 2000). This species has been observed living in sympatry with native mussel species, such as the painter's mussel *Unio pictorum* (Linnaeus 1758), the thick-shelled river mussel *Unio crassus* (Philipsson, 1788), the swollen river mussel *Unio tumidus* (Philipsson, 1788), the depressed river mussel *Pseudanodonta complanata* (Rossmässler, 1835), *A. anatina*, and other mollusc species in the silt-clay substrate of lowland areas of the Danube River (Bódis et al., 2011, 2016; Paunovic et al., 2006). The habitat preferences of *S. woodiana* are similar to those of several native freshwater mussels, which can further increase the risk of host competition with native unionid species. The shells of *S. woodiana* are also comparable in size (typically larger) with those of native species (Afanasjev et al., 2001; Hliwa et al., 2015; Kraszewski, 2007) and they are tolerant of a wide range of environmental conditions (Corsi et al., 2007; Douda et al., 2012), including polluted and low oxygen ecosystems (Sarkany-Kiss et al., 2000). They are host-generalists (Douda et al., 2012), which can give this species a competitive advantage over other mussels. More importantly, the brooding season and the release of glochidia from *S. woodiana* occurs in advance of several native mussel species, with *S. woodiana* releasing glochidia in the summer, in multiple broods per year (Afanasjev et al., 2001; Douda et al., 2012; Sarkany-Kiss et al., 2000). Native *Anodonta* species release glochidia mainly at the end of winter or early spring (from long-term brooding) (Hinzmann et al., 2013). Thus, glochidia from *S. woodiana* are able to infest potential juvenile hosts before other anodontine glochidia.

No data are available on the population dynamics of native mussel species after the introduction of the invasive *S. woodiana*. Nevertheless, adverse effects are likely, and *S. woodiana* has already been identified as the major cause of decline of native *A. anatina* in Lake Balaton in Central Hungary, where its relative abundance has already dropped from 17.8 to 8.6% (Benkó-Kiss et al., 2013). In Serbia, *S. woodiana* has already been shown to outnumber native freshwater mussels by a ratio of 2:1 (Paunovic et al., 2006). In Italy, it has completely replaced some native mussel species (especially *A. anatina*) in ecosystems throughout the country (Cappelletti et al., 2009), and the same situation has been observed recently in southern France (Vincent Prié, personal communication). It is important to note that in addition to the mechanism described in this study (host competition), there are also other hypotheses for the observed reductions in native mussel species after the arrival of *S. woodiana*. For example, the species may compete for space

and food resources, change some abiotic features in the sediment, or spread diseases and parasites (Sousa et al., 2014); however, all these mechanisms remain untested.

Further studies are needed to monitor population trends of native bivalves at sites invaded by *S. woodiana*, including the study of additional combinations of fish and mussel species. In particular, the host species with higher metamorphosis success rate of *A. anatina* glochidia should be tested for cross-resistance effects because it is still possible that other hosts are not equally affected by a prior infection by *S. woodiana*. In terms of the assessment of the potential consequences of cross-resistance, more data are needed regarding the strength of the effect, in addition to the duration of the acquired immunity induced by *S. woodiana* under natural conditions. There are currently no comprehensive data on *S. woodiana* glochidia abundance on comparably-sized fish. Recently, few parasitizing *S. woodiana* glochidia per gram of fish body weight have been observed in the Kyjovka River (Czech Republic) and Bao'an Lake (Hubei province, China) (K. Douda, personal observation). Fukuhara (1985) found that the mean number of glochidia per host fish (*Rhinogobius brunneus*, mean body length < 30 mm, weight not provided) exceeded 20 during the main reproduction season and peaked at 52.2 glochidia of *S. woodiana* per fish in a pond in Osaka Prefecture (Japan). In light of this information, the numbers of glochidia parasitizing fish during experimental infections in this study can be supposed as environmentally relevant. Nevertheless, the relationship between the intensity of infestation and the strength of cross-resistance effects should be studied in detail in future. Similarly, the results demonstrate that the number of successful juveniles was reduced, but the quality of juveniles recovered from primed host fish was not studied (viability and energy reserves of juvenile mussels can vary among different host fish) (Douda, 2015).

## 4.3 | Conservation implications of cross-resistance

The adverse impact of *S. woodiana* priming infestations on the development of native species' glochidia reported in this study is the first evidence of a host-mediated negative impact of this IAS on native mussel species. Despite intense concern regarding the potential adverse impacts of *S. woodiana* on biodiversity and ecosystem functioning (Lopes-Lima et al., 2017; Sousa et al., 2014; Watters, 1997), in addition to observational data suggesting that a decline in the relative density of native mussels occurs following *S. woodiana* invasion, negative effects were only speculative and were not supported by quantitative data. Consequently, documenting this clear impact on the developmental success of the glochidia of a native species provides a solid basis for conservation and natural resource management decisions, and can also serve as a strong argument for the active control of *S. woodiana* invasions.

Until now, *A. anatina* has received little attention from a conservation point of view owing to its wide distribution and high abundance. However, there is a recent pattern of decline in this species associated with unspecified human influences and the spread of *S. woodiana* (Lopes-Lima, 2014). This, combined with the data provided in the present study, advocates for the strict surveillance of *A. anatina* populations and a reassessment of its conservation status in consideration of the evidence regarding this new threat.

Because host availability and compatibility is a critical factor in the management of populations of endangered unionids (Douda et al., 2014; Haag & Stoeckel, 2015; Schwab, Cottenie, Poos, & Ackerman, 2011), the comprehensive understanding of fish immunology is important for conservation efforts. Information regarding the immunological capabilities of host fish could benefit endangered or threatened unionid species (O'Connell & Neves, 1999). Specifically, full awareness of the duration, frequency, and mechanisms of adaptive immunity could aid in the propagation of endangered freshwater mussel species worldwide (Dodd et al., 2006). Understanding these timeframes can lead to efficient infestations of host fish in breeding programmes and thereby contribute to artificial reproduction. More importantly, understanding the mechanisms influencing host compatibility (including the adverse effects of IAS) is essential for ensuring adequate host resources for endangered mussel species in natural habitats.

The results of this study can contribute to research efforts on the potential effects of an IAS on native mussel species. These findings can help identify the role of fish immunology on secondary glochidial infestations and determine the propensity of native fish species to host multiple mussel species in succession. Multiple glochidial releases are common for *S. woodiana* under natural conditions, and the results indicate that the likelihood of native juvenile freshwater mussels developing in ecosystems containing this invasive species can be significantly reduced. These results can potentially serve to accelerate conservation efforts for native freshwater mussels in Europe and to help protect them against the impacts of IAS such as *S. woodiana*.

## ACKNOWLEDGEMENTS

All experimental procedures were approved by the Expert Committee for Animal Conservation (research protocol no. MSMT-31220/2014-6). The research was funded by the Czech Science Foundation (13-05872S).

## REFERENCES

- Afanasjev, S. A., Zdanowski, B., & Kraszewski, A. (2001). Growth and population structure of the mussel *Anodonta woodiana* (Lea, 1834) (Bivalvia, Unionidae) in the heated Konin lakes system. *Archives of Polish Fisheries*, 9, 123–131.
- Aldridge, D. C., Fayle, T. M., & Jackson, N. (2007). Freshwater mussel abundance predicts biodiversity in UK lowland rivers. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 17, 554–564.
- Arey, L. B. (1932). The formation and structure of the glochidial cyst. *Biological Bulletin*, 62, 212–221.
- ASTM E2455-06. (2013). *Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels*. West Conshohocken, PA, USA: ASTM International.
- Baker, S. M., & Levinton, J. S. (2003). Selective feeding by three native North American freshwater mussels implies food competition with zebra mussels. *Hydrobiologia*, 505, 97–105.
- Barnhart, M. C., Haag, W. R., & Roston, W. N. (2008). Adaptations to host infection and larval parasitism in Unionida. *Journal of the North American Benthological Society*, 27, 370–394.
- Bauer, G., & Wachtler, K. (2001). Environmental relationships of naiads: Threats, impact on the ecosystem, indicator function. In G. Bauer, & K. Wachtler (Eds.), *Ecology and evolution of the freshwater mussels Unionida*. (pp. 311–316). Heidelberg, Germany: Springer.
- Benkő-Kiss, Á., Ferincz, Á., Kováts, N., & Paulovits, G. (2013). Spread and distribution pattern of *Sinanodonta woodiana* in Lake Balaton. *Knowledge and Management of Aquatic Ecosystems*, 408, 09.
- Beran, L. (2008). Expansion of *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) in the Czech Republic. *Aquatic Invasions*, 3, 91–94.
- Blažek, R., & Gelnar, M. (2006). Temporal and spatial distribution of glochidial larval stages of European unionid mussels (Mollusca: Unionidae) on host fishes. *Folia Parasitologica*, 53, 98–106.
- Bódis, E., Nosek, J., Oertel, N., Tóth, B., Hornung, E., & Sousa, R. (2011). Spatial distribution of bivalves in relation to environmental conditions (middle Danube catchment, Hungary). *Community Ecology*, 12, 210–219.
- Bódis, E., Tóth, B., & Sousa, R. (2014). Massive mortality of invasive bivalves as a potential resource subsidy for the adjacent terrestrial food web. *Hydrobiologia*, 735, 253–262.
- Bódis, E., Tóth, B., & Sousa, R. (2016). Freshwater mollusc assemblages and habitat associations in the Danube River drainage, Hungary. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 332, 319–332.
- Bogan, A. E. (1993). Fresh-water bivalve extinctions (Mollusca, Unionida) – a search for causes. *American Zoologist*, 33, 599–609.
- Burlakova, L. E., Karatayev, A. Y., Karatayev, V., May, M. E., Bennett, D. L., & Cook, M. J. (2011). Biogeography and conservation of freshwater mussels (Bivalvia: Unionidae) in Texas: Patterns of diversity and threats. *Diversity and Distributions*, 17, 393–407.
- Cappelletti, C., Cianfanelli, S., Beltrami, M. E., & Ciutti, F. (2009). *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae): A new non-indigenous species in Lake Garda (Italy). *Aquatic Invasions*, 4, 685–688.
- Corsi, I., Pastore, A. M., Lodde, A., Palmerini, E., Castagnolo, L., & Focardi, S. (2007). Potential role of cholinesterases in the invasive capacity of the freshwater bivalve, *Anodonta woodiana* (Bivalvia: Unionacea): A comparative study with the indigenous species of the genus, *Anodonta* sp. *Comparative Biochemistry and Physiology, Part C*, 145, 413–419.
- Crane, A. L., Fritts, A. K., Mathis, A., Lisek, J. C., & Barnhart, M. C. (2011). Do gill parasites influence the foraging and antipredator behaviour of rainbow darters, *Etheostoma caeruleum*? *Animal Behaviour*, 82, 817–823.
- Dillon, R. T. (2000). *The ecology of freshwater molluscs*. Port Chester, NY, USA: Cambridge University Press.
- Dodd, B. J., Barnhart, M. C., Rogers-Lowery, C. L., Fobian, T. B., & Dimock, R. V. (2005). Cross-resistance of largemouth bass to glochidia of unionid mussels. *Journal of Parasitology*, 91, 1064–1072.
- Dodd, B. J., Barnhart, M. C., Rogers-Lowery, C. L., Fobian, T. B., & Dimock, R. V. (2006). Persistence of host response against glochidia larvae in *Micropterus salmoides*. *Fish and Shellfish Immunology*, 21, 473–484.
- Douda, K. (2015). Host-dependent vitality of juvenile freshwater mussels: Implications for breeding programs and host evaluation. *Aquaculture*, 445, 5–10.
- Douda, K., Sell, J., Kubíková-Peláková, L., Horký, P., Kaczmarczyk, A., & Mioduchowska, M. (2014). Host compatibility as a critical factor in management unit recognition: Population-level differences in mussel-fish relationships. *Journal of Applied Ecology*, 51, 1085–1095.
- Douda, K., Velišek, J., Kolářová, J., Rylková, K., Slavík, O., Horký, P., & Langrová, I. (2017). Direct impact of invasive bivalve (*Sinanodonta woodiana*) parasitism on freshwater fish physiology: Evidence and implications. *Biological Invasions*, 19, 989–999.
- Douda, K., Vrtílek, M., Slavík, O., & Reichard, M. (2012). The role of host specificity in explaining the invasion success of the freshwater mussel *Anodonta woodiana* in Europe. *Biological Invasions*, 14, 127–137.
- Fukuhara, S. (1985). Glochidium parasitic period, host-fish and parasitic site of *Anodonta woodiana* in small pond. *Venus*, 45, 43–52.

- Haag, W. R., & Stoeckel, J. A. (2015). The role of host abundance in regulating populations of freshwater mussels with parasitic larvae. *Oecologia*, *178*, 1159–1168.
- Hinzmann, M., Lopes-Lima, M., Teixeira, A., Varandas, S., Sousa, R., Lopes, A., ... Machado, J. (2013). Reproductive cycle and strategy of *Anodonta anatina* (L., 1758): Notes on hermaphroditism. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *319*, 378–390.
- Hliwa, P., Zdanowski, B., Dietrich, G. J., Andronowska, A., Krol, J., & Andrzej, C. (2015). Temporal changes in gametogenesis of the invasive Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) from the Konin Lakes system (Central Poland). *Folia Biologica*, *63*, 63–67.
- Horký, P., Douda, K., Maciak, M., Závorka, L., & Slavík, O. (2014). Parasite-induced alterations of host behaviour in a riverine fish: The effects of glochidia on host dispersal. *Freshwater Biology*, *59*, 1452–1461.
- Jansen, W., Bauer, G., & Zahner-Meike, E. (2001). Glochidial mortality in freshwater mussels. In G. Bauer, & K. Wachtler (Eds.), *Ecology and evolution of the freshwater mussels Unionoida*. (pp. 185–212). Berlin/Heidelberg: Springer-Verlag.
- Kat, P. W. (1984). Parasitism and the Unionacea (Bivalvia). *Biological Reviews of the Cambridge Philosophical Society*, *59*, 189–207.
- Kottelat, M., & Freyhof, J. (2007). *Handbook of European freshwater fishes*. Cornol, Switzerland: Publications Kottelat.
- Kraszewski, A. (2007). The continuing expansion of *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) in Poland and Europe. *Folia Malacologica*, *15*, 65–69.
- Kraszewski, A., & Zdanowski, B. (2007). *Sinanodonta woodiana* (Lea, 1834) (Mollusca) – a new mussel species in Poland: Occurrence and habitat preferences in a heated lake system. *Polish Journal of Ecology*, *55*, 337–356.
- Lieschke, G. J., & Trede, N. S. (2009). Fish immunology. *Current Biology*, *19*, 678–682.
- Lopes-Lima, M. (2014). *Anodonta anatina*. The IUCN Red List of Threatened Species 2014: e.T155667A21400363.
- Lopes-Lima, M., Sousa, R., Geist, J., Aldridge, D. C., Araujo, R., Bergengren, J., ... Van Damme, D. (2017). Conservation status of freshwater mussels in Europe: State of the art and future challenges. *Biological Reviews*, *92*, 572–607.
- Lopes-Lima, M., Teixeira, A., Froufe, E., Lopes, A., Varandas, S., & Sousa, R. (2014). Biology and conservation of freshwater bivalves: Past, present and future perspectives. *Hydrobiologia*, *735*, 1–13.
- Musil, J., Horký, P., Slavík, O., Zbořil, A., & Horká, P. (2012). The response of the young of the year fish to river obstacles: Functional and numerical linkages between dams, weirs, fish habitat guilds and biotic integrity across large spatial scale. *Ecological Indicators*, *23*, 634–640.
- Neves, R. J., Weaver, L. R., & Zale, A. V. (1985). An evaluation of host fish suitability for glochidia of *Villosa vanuxemi* and *V. nebulosa* (Pelecypoda: Unionidae). *American Midland Naturalist*, *113*, 13–19.
- Novais, A., Dias, E., & Sousa, R. (2016). Inter- and intraspecific variation of carbon and nitrogen stable isotopes ratios in freshwater bivalves. *Hydrobiologia*, *765*, 149–158.
- O'Connell, M. T., & Neves, R. J. (1999). Evidence of immunological responses by a host fish (*Ambloplites rupestris*) and two non-host fishes (*Cyprinus carpio* and *Carassius auratus*) to glochidia of a freshwater mussel (*Villosa iris*). *Journal of Freshwater Ecology*, *14*, 71–78.
- Paunovic, M., Csányi, B., Simic, V., Stojanovic, B., & Cacic, P. (2006). Distribution of *Anodonta* (*Sinanodonta*) *woodiana* (Lea, 1834) in inland waters of Serbia. *Aquatic Invasions*, *1*, 154–160.
- Pou-Rovira, Q., Araujo, R., Boix, D., Clavero, M., Feo, C., Ordeix, M., & Zamora, L. (2009). Presence of the alien Chinese pond mussel *Anodonta woodiana* (Lea, 1834) (Bivalvia, Unionidae) in the Iberian Peninsula. *Graellsia*, *65*, 67–70.
- R Development Core Team. (2014). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Ricciardi, A., Neves, R. J., & Rasmussen, J. B. (1998). Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion. *Journal of Animal Ecology*, *67*, 613–619.
- Ricker, W. E. (1975). Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada*, *191*, 1–382.
- Rogers, C. L., & Dimock, R. V. (2003). Acquired resistance of bluegill sunfish *Lepomis macrochirus* to glochidia larvae of the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) after multiple infections. *Journal of Parasitology*, *89*, 51–56.
- Rogers-Lowery, C. L., Dimock, R. V., & Kuhn, R. E. (2007). Antibody response of bluegill sunfish during development of acquired resistance against the larvae of the freshwater mussel *Utterbackia imbecillis*. *Developmental and Comparative Immunology*, *31*, 143–155.
- Sarkany-Kiss, A. (1997). The present-day situation of the Unionidae (Mollusca: Bivalvia) in the Transylvanian tributaries of the Tisa River (Romania). *Travaux du Museum National d'Histoire Naturelle 'Grigore Antipa'*, *37*, 213–224.
- Sarkany-Kiss, A., Sirbu, I., & Hulea, O. (2000). Expansion of the adventive species *Anodonta woodiana* (Lea, 1834) (Mollusca, Bivalvia, Unionoidea) in central and eastern Europe. *Acta Oecologica*, *7*, 49–57.
- Schwalb, A. N., Cottenie, K., Poos, M. S., & Ackerman, J. D. (2011). Dispersal limitation of unionid mussels and implications for their conservation. *Freshwater Biology*, *56*, 1509–1518.
- Sousa, R., Novais, A., Costa, R., & Strayer, D. L. (2014). Invasive bivalves in fresh waters: Impacts from individuals to ecosystems and possible control strategies. *Hydrobiologia*, *735*, 233–251.
- Sousa, R., Pilotto, F., & Aldridge, D. C. (2011). Fouling of European freshwater bivalves (Unionidae) by the invasive zebra mussel (*Dreissena polymorpha*). *Freshwater Biology*, *56*, 867–876.
- Spooner, D. E., Frost, P. C., Hillebrand, H., Arts, M. T., Puckrin, O., & Xenopoulos, M. A. (2013). Nutrient loading associated with agriculture land use dampens the importance of consumer-mediated niche construction. *Ecology Letters*, *16*, 1115–1125.
- Strayer, D. L. (2008). *Freshwater mussel ecology: A multifactor approach to distribution and abundance*. Berkeley, CA, USA: University of California Press.
- Taeubert, J. E., & Geist, J. (2013). Critical swimming speed of brown trout (*Salmo trutta*) infested with freshwater pearl mussel (*Margaritifera margaritifera*) glochidia and implications for artificial breeding of an endangered mussel species. *Parasitology Research*, *112*, 1607–1613.
- Thomas, G. R., Taylor, J., & Garcia de Leaniz, C. (2013). Does the parasitic freshwater pearl mussel *M. margaritifera* harm its host? *Hydrobiologia*, *735*, 191–201.
- Tricarico, E., Junqueira, A. O., & Dudgeon, D. (2016). Alien species in aquatic environments: A selective comparison of coastal and inland waters in tropical and temperate latitudes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *26*, 872–891.
- Vaughn, C. C., & Hakenkamp, C. C. (2001). The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology*, *46*, 1431–1446.
- Wachtler, K., Dreher-Mansur, M. C., & Richter, T. (2001). Larval types and early postlarval biology in naiads (Unionoida). In G. Bauer, & K. Wachtler (Eds.), *Ecology and evolution of the freshwater mussels Unionoida*. (pp. 93–126). Berlin/Heidelberg: Springer-Verlag.
- Walker, K. F., Jones, H., & Klunzinger, M. W. (2014). Bivalves in a bottleneck: Taxonomy, phylogeography and conservation of freshwater mussels (Bivalvia: Unionoida) in Australasia. *Hydrobiologia*, *735*, 61–79.
- Watters, G. T. (1997). A synthesis and review of the expanding range of the Asian freshwater mussel *Anodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae). *Veliger*, *40*, 152–156.



- Williams, J. D., Warren, M. L., Cummings, K. S., Harris, J. L., & Neves, R. J. (1993). Conservation status of freshwater mussels of the United States and Canada. *Fisheries*, 18, 6–22.
- Zieritz, A., Lopes-Lima, M., Bogan, A., Sousa, R., Walton, S., Rahim, K., ... McGowan, S. (2016). Factors driving changes in freshwater mussel (Bivalvia, Unionida) diversity and distribution in Peninsular Malaysia. *Science of the Total Environment*, 571, 1069–1078.

**How to cite this article:** Donrovich SW, Douda K, Plechingerová V, et al. Invasive Chinese pond mussel *Sinanodonta woodiana* threatens native mussel reproduction by inducing cross-resistance of host fish. *Aquatic Conserv: Mar Freshw Ecosyst*. 2017;00:1–9. <https://doi.org/10.1002/aqc.2759>