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Host fish suitability for the endangered native *Anodonta* and impacts of the invasive *Sinanodonta woodiana* on their reproductive success

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Summary

Freshwaters are very important ecosystems worldwide due to their high biodiversity and important ecosystem services they provide as well as their water resource for humans. Nevertheless, they are among the most threatened ecosystems on the planet. One of the most endangered faunal groups globally are freshwater mussels (Unionoida). Due to their important ecosystem functions, a loss of unionid mussels would have enormous consequences for freshwater ecosystems. Major threats for freshwater bivalves are habitat loss, fragmentation or degradation, climate change, overexploitation, pollution, introduction of non-native species as well as the loss of host fish species. Especially the lack of suitable host fish is a major threat since hosts are crucial for the larval development and recruitment of unionid mussels. Detailed information about host-parasite interactions between mussels and fish are missing for the most unionid species, for example also for the native European bivalves *Anodonta cygnea* (swan mussel) and *Anodonta anatina* (duck mussel), which are still in decline and population trends are decreasing. Thus, the major aim of this thesis was to analyze the host suitability of ten different native and non-native fish species for the glochidial larvae of *A. cygnea* and *A. anatina*. Furthermore, analyses about the impacts of non-native fish and mussel species on the reproductive success of both native *Anodonta* were included due to the ongoing spread of non-native organisms in freshwater ecosystems around the world.

The host-parasite interaction of the glochidial larvae of native *Anodonta* species as well as of the invasive *Sinanodonta woodiana* (Chinese pond mussel) with ten different native and non-native fish species was analyzed in three separate standardized laboratory experiments (one per mussel species). In each experiment, fish were infested with the mussels' larvae in one common infestation bath. The results revealed that both native anodontines had a different composition of host fish species and a different order of good and poor hosts. In total, *A. anatina* showed higher larval infestation rates on all tested fish species and a lower average weight-normalized glochidial loss during metamorphosis phase resulting in a higher number of successful excysted juvenile mussels (7431 individuals of *A. anatina* excysted successfully compared to 6029 juveniles of the species *A. cygnea*). Both species also differed in duration of metamorphosis and juvenile mussel excystment. Therefore, future conservation actions must be developed for both species separately, anodontines can no longer seen as one single species as it was assumed in the past.

Surprisingly, it was highlighted that one tested non-native fish species (grass carp, *Ctenopharyngodon idella*) turned out to be a very good host for native *Anodonta* larvae. In fact, it was a better host for both mussels than some of the native fish species. Thus, non-native fish are not always a threat to native mussel populations and do not forcibly have to be removed from water bodies with native mussel populations. On the contrary, the second tested non-native fish species (topmouth gudgeon, *Pseudorasbora parva*), was a very unsuitable host for both *Anodonta*. These results demonstrate that non-native fish co-occurring with mussel populations cannot generally mentioned as threat or no threat for native mussels, their impacts on the reproduction of native bivalves can differ and management efforts should consider these aspects.
The experiment with the invasive *S. woodiana* showed that the glochidial larvae of the Chinese pond mussel developed successfully on all ten tested fish species (native and non-native ones). On an average, S. *woodiana* had the highest larval attachment rate, the highest rate of juvenile mussel excystment and the shortest metamorphosis phase on the ten fish species compared to the native *Anodonta*. Thus, *S. woodiana* can clearly be seen as competitor for native mussel species regarding their host use when co-occurring in the same water bodies. The Chinese pond mussel will be a threat for the reproduction success of native freshwater mussels co-occurring in the same habitats and thus further spreading of this invasive mussel has to be prevented. People have to be informed about the invasive species and its negative impact on native bivalves to stop its dispersal due to selling *S. woodiana* in pet shops and garden centers or due to the accidental spread of fish infested with the glochidia of the Chinese pond mussel.

Successful conservation strategies for native *Anodonta* species must be developed for both *Anodonta* species and also for every population separately. Under natural conditions, fish species tested as poor hosts during laboratory experiments might be the only available hosts in a mussel habitat and therefore, fish species composition in waters with mussel populations must be considered in case of conservation measures. Thus, conservation strategies for native *Anodonta* must not only include the prevention of the spread of the invasive Chinese pond mussel, but also conservation actions for their suitable host fish species. Fisheries management must be part of mussel conservation measures in waters with mussel populations. Otherwise, the reproductive success of native mussels cannot be guaranteed in the future.

Zusammenfassung

Süßwasserökosysteme gehören durch ihre enorm hohe Biodiversität, ihre vielfältigen Ökosystemdienstleistungen und ihrer Funktion als Wasserressource zu den wichtigsten Ökosystemen des Planeten. Leider sind sie auch die gefährdetsten Ökosysteme weltweit. Eine der meist gefährdetsten Tiergruppen in Süßwasserökosystemen sind Süßwassermuscheln (Unionoida). Wegen ihren wichtigen Funktionen in Gewässern, hätte ein Aussterben dieser Tiergruppe extreme Folgen für das gesamte Gewässerökosystem. Zu den Hauptursachen des Rückgangs von Süßwassermuschelarten gehören der Verlust, die Fragmentierung oder Degradation von Habitaten, Klimawandel, Verschmutzung, die Einwanderung nicht-heimischer Arten sowie der Verlust geeigneter Wirtsfischarten. Besonders der Verlust geeigneter Wirtsfische ist ausschlaggebend für den Rückgang von Süßwassermuscheln, da die Larvenentwicklung auf geeigneten Wirtsfischen ein obligatorischer Bestandteil der Reproduktion von Unioniden ist. Dennoch fehlen detaillierte Informationen über die Wirtsfisch-Larven Interaktionen zwischen Süßwassermuscheln und Fischen für die meisten Arten von Unioniden, so zum Beispiel auch für die beiden in Europa heimischen Teichmuschelarten *Anodonta cygnea* (Schwanenmuschel, Große Teichmuschel) und *Anodonta anatina* (Entenmuschel, Gemeine Teichmuschel). Populationen beider Arten sind rückläufig und *A. cygnea* und *A. anatina* sind mittlerweile in vielen europäischen Ländern streng geschützt. Das vorrangige Ziel dieser Dissertation war die Analyse der Wirtsfisch-Eignung von zehn verschiedenen heimischen und nicht-heimischen Fischarten für die Glochidien-Larven von *A. cygnea* und *A. anatina*. Zudem wurden, wegen der steigenden Ausbreitung gebietsfremder Arten in Süßwasserökosystemen weltweit, die Einflüsse nicht-heimischer Fisch- und Muschelarten auf den Reproduktionserfolg heimischer Teichmuscheln in die Analysen integriert.

Die Wirtsfisch-Larven Interaktion zwischen Glochidien der beiden heimischen Anodonten sowie den Glochidien der invasiven Art *Sinanodonta woodiana* (Chinesische Teichmuschel) und zehn verschiedenen heimischen und eingewanderten Fischarten wurde anhand von drei getrennten Laborversuchen (ein Experiment pro Muschelart) analysiert. Alle zehn getesteten Fischarten wurden in den drei getrennt durchgeführten Experimenten in jeweils einem gemeinsamen Infektionsbad mit den Larven der jeweiligen Muschelart infiziert. Die Ergebnisse zeigten, dass die beiden heimischen Teichmuschelarten sich sowohl in ihrer Wirtsfischarten-Zusammensetzung als auch in der Reihenfolge der Eignung der Fische von guten bis schlechten Wirtsfischen unterschieden. Insgesamt zeigte die Art *A. anatina* im Gegensatz zu *A. cygnea*

höhere Infektionsraten an allen getesteten Fischen sowie einen geringeren durchschnittlichen Verlust an Larven (nach Gewichtsnormalisierung) während der Metamorphose-Phase am Fisch. Dies resultierte in einer größeren Anzahl an erfolgreich entwickelten Jungmuscheln bei *A. anatina* (7431 erfolgreich entwickelte juvenile Muscheln, im Gegensatz zu 6029 erfolgreich entwickelten Jungmuscheln bei *A. cygnea*). Des Weiteren unterschieden sich beide Arten auch in der Dauer der Metamorphose-Phase und der Zeitspanne, in der die jungen Muscheln von den Fischen abfielen. Da sich beide Arten in den genannten Punkten stark voneinander differenzierten, ist es besonders wichtig, dass Schutzmaßnahmen zukünftig für beide Anodonten getrennt entwickelt werden und Teichmuscheln nicht wie bisher als eine Art angesehen werden.

Überraschenderweise stellte sich bei den Experimenten heraus, dass eine nicht-heimische Fischart (Grasfisch, *Ctenopharyngodon idella*) sich entgegen der Annahme als guter Wirtsfisch für die Larven beider Teichmuschelarten entpuppte. *Ctenopharyngodon idella* war sogar ein besserer Wirtsfisch als einige heimische Fischarten. Daher muss die Aussage, dass eingewanderte Fischarten, aufgrund ihrer schlechten Eignung als Wirtsfische, immer eine Gefahr für heimische Muschelpopulationen darstellen, revidiert werden. Demnach müssen nicht-heimische Fische auch nicht zwangsweise aus Gewässern mit Muschelpopulationen entfernt werden. Im Gegensatz dazu war die zweite eingewanderte Fischart in den Analysen (Blaubandbärbling, *Pseudorasbora parva*) ein ungeeigneter Wirtsfisch für die beiden heimischen Teichmuschelarten. Dieses Ergebnis wiederum demonstrierte, dass nicht-heimische Fischarten, die in den gleichen Gewässern wie Muschelpopulationen vorkommen, nicht generell als gefährlich oder ungefährlich für heimische Süßwassermuscheln eingestuft werden dürfen. Ihre jeweiligen Einflüsse auf die Reproduktion heimischer Muscheln kann sich sehr stark unterscheiden. Diese Aspekte müssen in zukünftigen Managementmaßnahmen beachtet werden.

Das Experiment mit der invasiven Chinesischen Teichmuschel zeigte, dass sich die Larven von *S. woodiana* auf allen zehn getesteten Fischarten erfolgreich entwickeln konnten (sowohl auf heimischen als auch auf nicht-heimischen Fischarten). Im Durchschnitt hatte *S. woodiana* im Vergleich zu den beiden heimischen Teichmuscheln die höchsten Infektionsraten, die höchsten Raten an erfolgreich entwickelten juvenilen Muscheln sowie die kürzeste Dauer der Metamorphose-Phase an allen zehn Fischarten. Daher kann die Chinesische Teichmuschel in Bezug auf ihre Nutzung von Wirtsfischen als Konkurrenz für die heimischen Teichmuscheln gesehen werden, sobald die Art gemeinsam mit den beiden heimischen Muscheln in einem Gewässer vorkommt. *Sinanodonta woodiana* wird in Zukunft eine große Gefahr für den Reproduktionserfolg von *A. cygnea* und *A. anatina* darstellen. Demnach sollte die weitere Ausbreitung dieser Art in heimischen Gewässern vermieden werden. Es ist dabei besonders wichtig, dass die

Bevölkerung über die Chinesische Teichmuschel und ihre Gefahr für heimische Teichmuschelarten informiert wird, um vor allem die Verbreitung durch den Verkauf von *S. woodiana* im Zoohandel und in Gartencentern sowie ihre Verbreitung in Form von angehefteten Larven an Wirtsfischen zu verhindern.

Erfolgreiche Schutzmaßnahmen müssen nicht nur für beide heimischen Teichmuschelarten, sondern auch für einzelne Populationen dieser Arten getrennt entwickelt werden. Fischarten, die sich in den Laborversuchen als schlechte Wirtsfische herausstellten, könnten im Freiland die einzig verfügbaren Wirtsfischarten für die jeweilige Muschelpopulation sein. Daher muss die Fischartenzusammensetzung für jedes Muschelgewässer, für das Schutzmaßnahmen ergriffen werden, separat ermittelt werden und Beachtung finden. Schutzkonzepte müssen zudem nicht nur die Eindämmung von Populationen der Chinesischen Teichmuschel beinhalten, sondern auch Schutzmaßnahmen für geeignete Wirtsfischarten der heimischen Teichmuscheln einschließen. Das Fischereimanagement muss Teil des Muschelschutzes werden. Andernfalls kann der Reproduktionserfolg heimischer Muschelarten in Zukunft nicht gewährleistet werden.

1 Introduction

1.1 General background

Surface freshwater habitats cover only about 0.8% of the Earth's surface and contain only around 0.01% of the world's water (Dudgeon et al., 2006; Gleick, 1996). Nevertheless, freshwater ecosystems host a very high biodiversity in relation to their size, provide important ecosystem services (Dudgeon et al., 2006) and are among the most threatened ecosystems on the planet (Dudgeon et al., 2006; Lopes-Lima et al., 2018). The increase of human population and socioeconomic development exert crucial pressure on freshwater environments (Dudgeon et al., 2006; Lopes-Lima et al., 2018). Thus, the declines in biodiversity are higher in fresh waters than in terrestrial ecosystems (Sala et al., 2000). Major threats for freshwater biodiversity include habitat degradation, overexploitation, water pollution, flow modifications, climate change and the introduction of non-native species (e.g. Dudgeon et al., 2006; Geist, 2011; Lopes-Lima et al., 2017). Especially invertebrate species, like freshwater mussels, receive much less publicity than vertebrates and attract a disproportionately minor research effort (Lydeard et al., 2004), although freshwater mussels (Unionoida, Unionids) are among the most threatened faunal group globally (Haag and Williams, 2014; Ferreira-Rodríguez et al., 2019; Lopes-Lima et al., 2014; Neves et al., 1997; Nobles and Zhang, 2011; Simberloff, 2012). Around 44% of the freshwater mussel species of the Order Unionoida are classified as Near Threatened or Threatened in the 2015 IUCN Red List of Threatened Species (Lopes-Lima et al., 2017). For example, in North America only about 30 taxa have become extinct in the last 100 years and 65% of the remaining species are considered endangered, threatened, or vulnerable (Haag and Williams, 2014).

The ecologically very important group of freshwater mussels serves important ecosystem functions, e.g. biomass transfer from the water column to the benthos, reduction of water turbidity, control of the concentration and composition of suspended particles, nutrient recycling or provision of habitat for other organisms (Hinzmann et al, 2013; Lummer et al., 2016; Sousa et al., 2011, 2012; Vaughn, 2010, 2018; Vaughn and Hakenkamp, 2001). They also play an important role as bioturbators in freshwater ecosystems functioning affecting physico-chemical properties of the substrate, as well as microbial community structure (Boeker et al., 2016). Due to these useful functions, mussels are often described as ecosystem engineers (Gutiérrez et al., 2003). They also provide important services to humans, like water purification, serving as prey for

several commercial fishes or providing materials such as shells and pearls (Haag, 2012; Lopes-Lima et al., 2017). Therefore, a loss of this faunal group would have serious consequences for aquatic ecosystems.

All Unionids have a complex life cycle with an obligate parasitic phase of the larvae on a suitable host fish (e.g. Bauer, 1994; Lefevre and Curtis, 1910; Wächtler et al., 2001; Nobles and Zhang, 2011) (Fig. 1). The larvae, so called glochidia, form from fertilized eggs within the marsupia of the adult mussel (Wächtler et al., 2001). Some species are so-called short-term breeders, keeping their eggs and glochidia for only a few weeks within the marsupia, some species are long-term breeders, retaining eggs and glochidia for many months and supplying them with nutrients (Wood, 1974; Bauer, 2001b; Jansen et al., 2001). Mature glochidia are then released from the gravid female's marsupia and have to attach to a suitable host fish for their next step of development (Barnhart et al., 2008; Douda, 2015; Jansen et al., 2001; Nobles and Zhang, 2011; Taeubert et al., 2012b). One female mussel can produce thousands or millions of larvae, depending on both the size of glochidia and the size of the female mussel (Wächtler et al., 2001; Nobles and Zhang, 2011). The larvae attach to fins and gills of the fish (depending on the species and morphology of the glochidia), become encysted by host epithelial cells and develop on the host into juvenile mussels (Hochwald and Bauer, 1990; Taeubert et al., 2012b). The dispersal by host fish species is necessary for the spread and maintenance of most Unionid populations (Strayer et al., 2004). In general, juvenile fish are reported to be the most important hosts because contact with glochidia induces an immune response and results in a reduced suitability of older fish as hosts for the mussels' larvae (Bauer and Vogel, 1987; Wächtler et al., 2001). Some mussel species are host specialists, using only one or a few host fish species, others are generalists and can use a dozen hosts or more (Rashleigh and DeAngelis, 2007; Strayer et al., 2004; Taeubert and Geist, 2017).

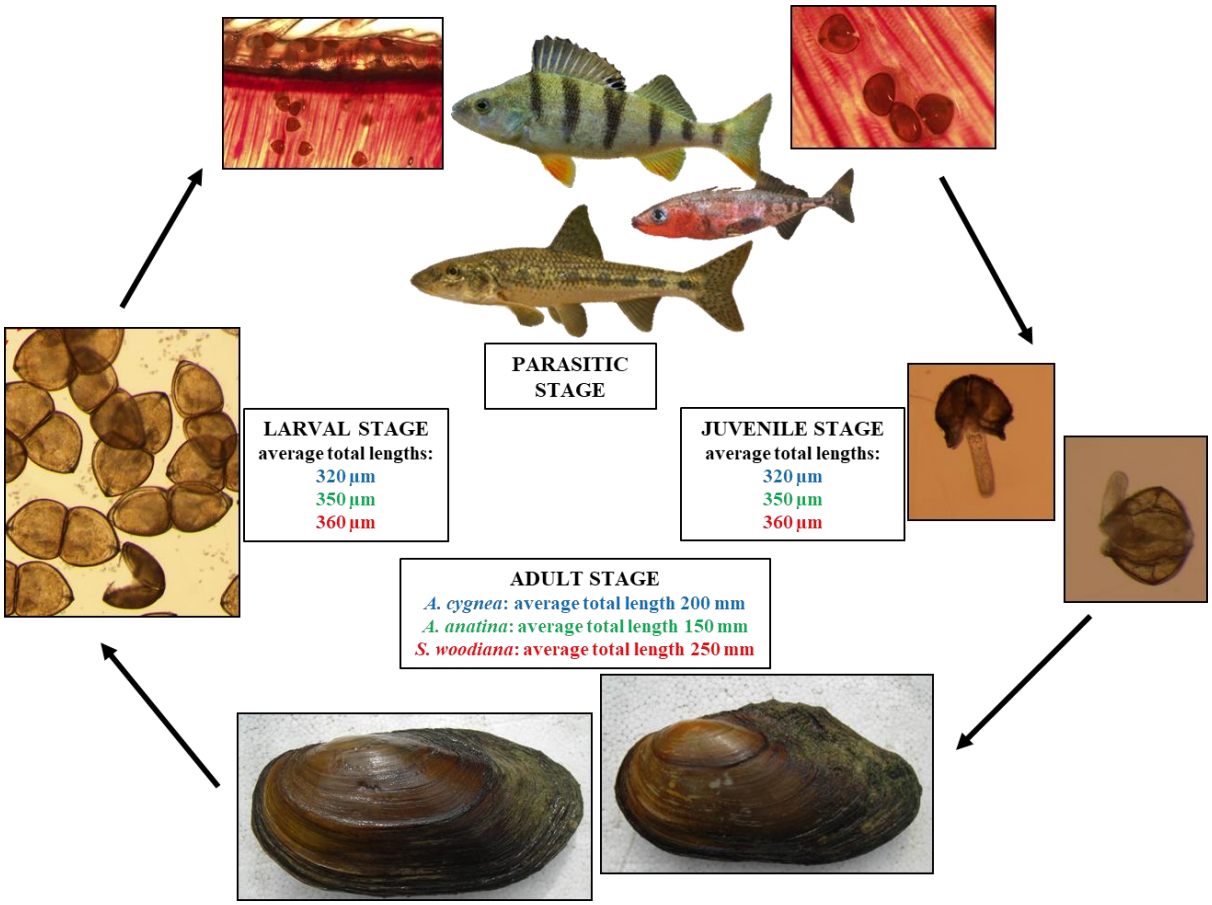

Figure 1: Life cycle of freshwater Unionids. Exemplarily shown for the native target species of this study *Anodonta cygnea* and *Anodonta anatina*. Average total lengths of adult mussels, glochidia and juvenile mussels are also shown for the invasive mussel species *Sinanodonta woodiana*.

After the metamorphosis phase on the fish, juvenile mussels excyst and live into the substratum of rivers or lakes of a waterbody for their first weeks, months or years in their life (depending on the species, Wächtler et al., 2001; Taeubert et al., 2010; Taeubert et al., 2012b).

A crucial factor in the reproduction of parasites is the rate of successful host infestation (Jansen et al., 2001). Thus, a lot of mussel communities are thought to be limited at recruitment (Young and Williams, 1984; Haag and Warren, 1998), since the loss of suitable host fish species is a major threat for freshwater mussels (Geist, 2010; Modesto et al., 2018; Stoeckl et al., 2015; Taeubert et al., 2012a). There is only limited knowledge of the host species of most bivalves worldwide (Jansen et al., 2001). The absence of this baseline data also makes the quantification of population change challenging (Aldridge, 2004). Therefore, reliable information about the ongoing decline of freshwater Unionids are hard to detect.

To date, 16 species of European Unionoida are recognized and can be separated in two families: Margaritiferidae and Unionidae (Lopes-Lima et al., 2017). In most European species very few

studies exist, either on the distribution and population structure but also on the study of their basic life cycle traits (Hinzmann et al., 2013). In general, conservation has mainly focused on the most threatened species *Margaritifera margaritifera* and *Unio crassus* and less attention has been paid to other European unionid mussel species (Lopes-Lima et al., 2017). Although, European freshwater mussels are vulnerable to all the threats mentioned before for freshwater ecosystems (Lopes-Lima et al., 2017): loss, fragmentation and degradation of habitat, overexploitation, pollution, introduction of non-native species and climate change (Dudgeon et al., 2006; Geist, 2011) as well as loss of host fish species. Thus, for the future survival of freshwater mussels, scientists, managers, politicians and the general public need to strengthen their cooperation in order to conserve all freshwater mussel species (Lopes-Lima et al., 2018). Otherwise, also the high amount of ecosystem services they provide (Vaughn, 2018) will get lost.

1.2 Native European *Anodonta* species

The European Genus *Anodonta* belongs to the Family Unionidae, including the two species *Anodonta cygnea* (swan mussel) and *Anodonta anatina* (duck mussel) (Lopes-Lima et al., 2017). Both species can be found throughout Europe until Russia, *A. anatina* also occurs in parts of Asia (Lopes-Lima, 2014a, b). There is often a misidentification of both anodontines due to their high shell plasticity and their morphological similarity (Franke, 1993; Lopes-Lima, 2014a). In the past, the proposed number of *Anodonta* species ranged from one to over 400 (Nagel and Badino, 2001). Recently, the existence of two species is widely accepted and molecular approach has confirmed this fact (Falkner et al., 2001; Källersjö et al., 2005; Nagel and Badino, 2001). This misidentification of the species over many years complicates the study of historical and actual distribution patterns extremely (Lopes-Lima, 2014a).
Anodonta cygnea prefers to inhabit standing water bodies like lakes and ponds (including artificial carp ponds) but also slow-flowing lowland rivers and oxbow lakes (Franke, 1993; Zettler et al., 2006). It can also be found in canals, drainage dykes and dam reservoirs (Killeen et al., 2004). In contrast, *A. anatina* can not only be found in standing waters, but also in flowing streams together with *U. crassus* or *M. margaritifera* (Bauer, 2001a; Franke, 1993; Zettler et al., 2006). Both *Anodonta* species often co-occur in standing waters or slow-flowing river sections (Franke, 1993; Niemeyer, 1993).

Regarding their biology and life history, the European *Anodonta* are relatively short-lived species, reaching ages of less than 30 years (Bauer, 2001b; Lopes-Lima et al., 2017; Niemeyer,

1993). They reach sexual maturity at 1-4 years (Lopes-Lima et al., 2017) and differ in their sexual strategy: *Anodonta anatina* was found to be of separate sex with very few hermaphrodites in some populations, whereas *A. cygnea* populations consist of only a few females and a large proportion of hermaphrodites (Bauer, 2001b; Franke, 1993; Teutsch, 1997). Hinzmann et al. (2013) also analyzed that *A. anatina* indicates a possible shift of its sexual strategy from essential dioecious to a more plastic sexual strategy that may be dependent on habitat characteristics. In general, both species show the same complex life cycle as described for all unionids before (Fig. 1).

Anodonta cygnea and *A. anatina* are long-term (bradytictic) breeders, carrying their glochidia for several months (over the winter) within their marsupia (Bauer, 2001b; Heard, 1998; Wächtler et al., 2001) and releasing them between late winter and spring (Graf and Cummings, 2007; Lopes-Lima et al., 2017; Niemeyer, 1993). They are able to produce around 300,000 – 400,000 glochidia per adult mussel (Claes, 1987; Niemeyer, 1993). The larvae of the *Anodonta* species are the biggest in native European species and carry strong hooks for a better attachment on the host fish, whereas *A. anatina* showing bigger glochidia than *A. cygnea* and hooks of both species also differ in their shape (Scharsack, 1994; Wächtler et al., 2001). Thus, larvae of *Anodonta* are not only able to attach to the gills of a host fish, but also to the fins and other parts of the fish's body (Bauer 2001b; Wächtler et al., 2001). *Anodonta* species are mentioned to be host fish generalists with a wide range of suitable hosts (Wächtler et al., 2001). Detailed knowledge about the host fish suitability, preference and duration of larval development on different fish species are still missing, although both species are declining and detailed knowledge about biology and life history traits would be essential for an efficient conservation (Lopes-Lima, 2014a, b; Lopes-Lima et al., 2017).

A. cygnea and *A. anatina* have been assessed as Least Concern on the IUCN Red List of Threatened Species, but local declines are still visible, global population trends are unknown and both species are protected and listed on national Red Lists in many countries (Lopes-Lima, 2014a, b). For example, the swan mussel is listed on the national Red Lists of Poland (Zając, 2005), Germany (Jungbluth and von Knorre, 2009), Ireland (Byrne et al., 2009), the Czech Republic (Farkač et al., 2005) or Norway (Byrne et al., 2009). The duck mussel is listed on the national Red List of Germany (Jungbluth and von Knorre, 2009), as well as considered Vulnerable in Austria (Reischütz and Reischütz, 2007) and Romania (Sárkány-Kiss, 2003), and Near Threatened in Spain (Verdú et al., 2006). Due to the difficulty of assessing the population structures of native anodontines (a lot of populations occur in lakes in great depths or in private fish ponds)

and the missing data about both species, further research is urgently needed for future conservation of these species (Lopes-Lima, 2014a, b).

1.3 Invasive species in freshwater ecosystems

The recent decline of freshwater mussel species is the consequence of an abundance of threats that influence aquatic ecosystems and the organisms therein. As mentioned before, one of the major threats is the introduction of non-native species (Lopes-Lima et al., 2017; Ferreira-Rodríguez et al., 2019). The introduction and spread of non-native species are a global ecological and conservation problem since invasive organisms are increasingly changing aquatic communities and ecosystems worldwide (Gurevitch and Padilla, 2004). The introduction pathways of non-native aquatic organisms are divers, these species arrive in new ecosystems for example due to shipping (dominant pathway), transport of organisms in ballast water or attached to hulls or opening of canals (serve as invasion corridors) (Keller et al., 2011).The most important pathways for non-native aquatic species to Europe (despite shipping and canals) are stocking and aquaculture or trades in ornamental (predominantly aquarium, especially important in freshwater ecosystems) (Keller et al., 2011). In general, European aquatic ecosystems contain the highest number of non-native species due to their high frequency of human access and their high connectivity to other ecosystems (Keller et al., 2011).

Regarding the kind of invading species, it has to differentiate between non-native species that arrive in new ecosystems without any negative consequences for the newly arrived aquatic ecosystems and the native organisms, and species that arrive in new ecosystems, spread widely and cause measurable environmental, economic, or human health impacts (Keller et al., 2011). The latter ones are termed invasive species (Keller et al., 2011; Kolar and Lodge, 2001). Invasive species are also described as exotic species that persist in new environments, reproduce successfully in their new habitats, and spread greatly in their distribution (Havel et al., 2015). They are often generalists that can tolerate a variety of environmental conditions and have a higher reproduction rate than native or non-invasive species (Havel et al., 2015; Keller et al., 2007, 2011). Especially freshwater ecosystems have been deeply transformed by invasive species from a wide variety of taxonomic groups (Simberloff et al., 2013; Strayer, 2010). And these invasive species can cause enormous economic impacts. For example, aquatic ecosystems of the US showed an approximate annual cost of 7.7 billion USD associated with damages caused by alien species and their control (Pimentel et al., 2005). In most cases, the ecological impacts

of invasive species are much more crucial than the economic ones. In Europe, for example, the North American signal crayfish (*Pacifastacus leniusculus*) was introduced primarily for aquaculture, has spread rapidly, and is now considered one of the major threats to the indigenous crayfish fauna (Souty-Grosset et al., 2006). The signal crayfish is host of the crayfish plague (*Aphanomyces astaci*) that cause a lethal disease to European crayfish species and threaten these native organisms enormously (Keller et al., 2011; Söderhäll and Cerenius, 1999).

The most widely introduced aquatic organisms are fish species (Leprieur et al., 2008; Modesto et al., 2018). They can cause significant changes in fish communities right up to species extinctions due to diseases or competition (Castaldelli et al., 2013; Modesto et al., 2018; Simberloff et al., 2013). The most frequent pathways for fish introductions are human activities like aquaculture, recreational and commercial fisheries, biological control, and pet and ornamental animals' industry (Gozlan, 2008; Kolar and Lodge, 2001), like for example the targeted introduction of Nile perch (*Lates niloticus*) into Lake Victoria to enhance fishery yield (Gozlan et al., 2010; Matsuishi et al., 2006; Ogutu-Ohwayo and Hecky, 1991) or the introduction of grass carp (*Ctenopharyngodon idella*) in European freshwater ecosystems for weed control (Cross, 1969). Moreover, accidental introductions of fish species in new freshwater habitats play a major role in the distribution of invasive species as seen for topmouth gudgeon (*Pseudorasbora parva*) in European freshwaters (Copp et al., 2010).

The introduction of non-native fish can have crucial negative effects on native freshwater mussels, for example shown for the introduced North American pink salmon (*Oncorhynchus gorbuscha*), which is one of the major conservation threats to Europe's largest freshwater pearl mussel population in Russia (Taeubert and Geist, 2017). The most important impacts of non-native fish on native freshwater mussel declines are the changes in fish communities leading to changes in host-parasite relationships (Modesto et al., 2018). Some studies pointed out that freshwater mussels do not use non-native fish as hosts for the development of their glochidia and that there is no successful reproduction of mussels if non-native fish replace native ones in freshwater mussel habitats (Douda et al., 2013; Modesto et al., 2018; Salonen et al., 2016; Strayer, 2008). Native freshwater mussels are not only threatened by the introduction of non-native fish, but also by the ongoing introduction of non-native freshwater mussel species. For instance, invasive mussels such as *Dreissena polymorpha*, reduce food and oxygen for native fauna (Havel et al., 2015), changed the physical, chemical and biological characteristics of water bodies (Strayer et al., 1999) or harm native mussels by attaching to their shells and hamper their filter feeding (Ferreira-Rodríguez et al., 2018, 2019), as they occur in high-volume populations in many lakes and streams throughout North America and Europe (Strayer, 1999). Due

to their simple life cycle and fast reproduction because of the high production of free-living larvae, they adapt to and spread very fast in new freshwater ecosystems (Douda et al., 2012b; Karatayev et al., 2007).

Other non-native freshwater mussels that successful invade European freshwaters can harm native mussel species not only by competing for food or space, but also by competing for suitable host fish species due to the same life cycle and reproduction strategy as native unionids. The most important representative of this category is the non-native Chinese pond mussel (*Sinanodonta woodiana*). Originally native to Asia, this species is spreading throughout Europe due to its introduction as glochidia attached to introduced Asian fish (Kondakov et al., 2018; Konečný et al., 2018). Nowadays many self-recruiting populations exist in a lot of European countries (Bahr and Wiese, 2018; Cappelletti et al., 2009; Duempelmann, 2012; von Proschwitz, 2008). *Sinanodonta woodiana* co-occurs in the same habitats as native freshwater mussels, for example as the native *A. cygnea* and *A. anatina*, with the same reproductive cycle (Soroka, 2005; Donrovich et al., 2017; Zettler and Jueg, 2006). There is still little information about the competition of native bivalves and the Chinese pond mussel for hosts and the influence of *S. woodiana* on the ongoing decline of native mussels (Sousa et al., 2014).

1.4 Objectives

Populations of native European *Anodonta* species are in decline with unknown future perspectives for both species. Their interaction with host fish species and the suitability of hosts is one major threat and is still little examined. Knowledge on the suitability of host fish populations as well as knowledge on the timing of glochidial attachment and duration of the host-dependent phase are essential for conservation management (Taeubert and Geist, 2017). Thus, the overall objective of the present thesis is to analyze the host-parasite relationship between the glochidia of the native mussel species *Anodonta cygnea* and *A. anatina* and ten different fish species. This analysis generates important information about host suitability that can be used for future conservation and management of these freshwater mussels and stop their ongoing population decline. Moreover, the impacts of non-native fish and mussel species on the reproductive success of native anodontines is evaluated.

Standardized laboratory experiments were established to study the host-parasite relationship between the glochidial larvae of *A. cygnea*, *A. anatina* and the invasive *Sinanodonta woodiana* and ten different native and non-native potential host fish species in a simultaneous infestation of the fish in one common infestation bath. An univariate statistical approach was applied with

focus on the attachment and development of the glochidia on host fish species, duration of metamorphosis and juvenile mussel excystment on different host fish, excystment of juveniles on non-native fish species and comparison between the reproduction of native and invasive mussel species.

The **first study** concentrated on the reproduction of *Anodonta cygnea* on ten different hosts and identified the suitability of native and non-native fish as hosts, characterized and compared the duration of the larval phase on different host fish species and determined the glochidial infestation at different body parts of a host. In detail, it was hypothesized that (1) glochidia of *A. cygnea* are host generalists, and suitable hosts are equally infested with similar metamorphosis success; (2) non-native fish species are unsuitable hosts for *A. cygnea*; (3) duration of larval development differs between host fish species, and (4) numbers of attached glochidia vary between different types of tissue of a single host fish.

The host-parasite interaction between ten different fish species and the larvae of the second native *Anodonta* (*A. anatina*) was the focus of the **second study** within this thesis. Thus, the major aim was to clarify the suitability of native and non-native fish for glochidial attachment and development of *A. anatina* and to compare the results of this study with the results of the first study with the larvae of *A. cygnea*. The following hypotheses were tested: (1) Suitable host fish species are not equally infested, they differ in duration and success of metamorphosis and juvenile mussel excystment; (2) there is no successful metamorphosis of the glochidia of *A. anatina* on non-native fish species, and (3) the larvae of *A. anatina* are more adaptive and have higher infestation and developmental rates than the larvae of *A. cygnea*.

Due to its ongoing spread in Europe with unknown consequences for native *Anodonta* species, the reproduction of the invasive *Sinanodonta woodiana* was the topic of the **third study**. There, the major objective was not only to analyze the host-parasite relationship between ten native and non-native fish species and the glochidia of *S. woodiana*, but also the comparison between the experiment of *S. woodiana* with the two other infestation experiments with native anodontines to assess possible impacts of the invasive mussel on the native ones in regard to their competition for hosts. Here, it was hypothesized that (1) invasion success of *S. woodiana* is independent from its original hosts and instead depends on its capability to use non-native and indigenous fish species as hosts for its larvae; (2) metamorphosis success and success of juvenile mussel excystment are higher on both co-invasive fish species *Ctenopharyngodon idella*

and *Pseudorasbora parva* than on the native European fish species tested, and (3) *S. woodiana* shows a higher initial infestation of the tested fish species and a higher juvenile mussel excystment compared to *A. cygnea* and *A. anatina*.

2 Materials and methods

2.1 Study design

Three similar standardized laboratory experiments were performed, always using the glochidia of one of the tested mussel species (the native mussels *Anodonta cygnea* and *Anodonta anatina* as well as the invasive species *Sinanodonta woodiana*) and juvenile fish of ten different native and non-native fish species, respectively. The experiments were realized under identical conditions, the same experimental approach and methodology was used throughout, but all three experimental procedures were performed separately at different times and in different years (study with the glochidia of *A. cygnea*: May until July 2015, study with *A. anatina*: January until April 2015, study with *S. woodiana* May until July 2016) (Huber and Geist, 2017, 2019a, b). These standardized host suitability experiments were carried out following the experimental procedures also described by Taeubert et al. (2012a, 2013a).

The experiments were performed to analyze the host-parasite relationship between different mussel larvae and their host fish species to get information about host suitability of different fish species for native and invasive mussel species. Especially the host preference of the mussels' larvae for single fish species is an essential information for further conservation of the endangered bivalves. Moreover, non-native fish were included in the experiments to analyze their suitability as hosts and their impact on future mussel conservation. Due to the standardized laboratory experiments, the results of all studies can be compared with each other.

As described in Huber and Geist (2017, 2019a, b), fish of ten different species were first infested with the mussel larvae and then separated per species and kept in special funnel-shaped holding units to start the second phase of the experiments, the metamorphosis of glochidial larvae on the fish as well as the excystment of fully developed juvenile mussels per fish species.

Individuals of ten fish species were infested all together in one common infestation bath with the larvae of one mussel species per experiment, respectively. The performance of the infestation in one infestation bath is important to guarantee that the glochidia have the possibility to infest all fish species, in regard to their preferences. If all fish would have been infested separately (as described before in Douda et al., 2013) the preferences of the larvae for single fish species as hosts would not be detectable.

In the second phase of the experiments, fish were separated per species in three replicates of infested individuals per fish species, respectively. The three replicates per fish species are important for statistical purposes since data in ecological experiments can show a high variability within the same group (Potvin and Roff, 1993). Moreover, a group of non-infested control fish per species was included. This group was treated in the same way as the infested individuals. The control group also passed through the infestation process (infestation bath without glochidia) and was kept in the same holding units as the infested fish during the whole phase of glochidial metamorphosis and development. This control group was important to indicate whether handling or holding conditions influenced the experiment and the development of juvenile mussels on the fish.

Regarding the conditions during the experiments, the light-dark cycle in the laboratory was identical with the natural conditions of the environment, daylight could enter the laboratory rooms through large windows. Since fish were adjusted to this normal rhythm, no artificial light and no artificial light-dark cycle was produced. The water of the holding units was not heated or cooled during the experiments. Water temperatures always conformed to the current temperatures of the environment.

Moreover, fish were not fed during the studies. Feeding times of the fish were slightly reduced before the start to accustom the individuals on the feeding-free phase during the study. The fish were able to handle this period without food easily due to their very good condition. The condition of the fish during the analyzes was also controlled by an expert staff regularly. The collection of juvenile mussels after drop-off from the hosts is more complicated if there are a lot of fish droppings and can also take more time. Juvenile mussels should be sampled and transferred to special tanks immediately after excystment. In addition, feeding of the fish could result in high ammonia concentrations within the holding units and could thus lead to fish kills.

After their complete metamorphosis, excysted juvenile mussels were as fast as possible transferred to special tanks and flow-through systems (Feiner et al., 2016) to ensure their further development. The experiments were terminated three days after the last excysted juvenile mussel was detected.

2.2 Experimental setup

The experiments were performed at the Laboratory of the Aquatic Systems Biology Unit at the Technical University of Munich. After the infestation of the fish with glochidia of the mussel

species, fish were transferred to funnel-shaped holding units (three units per species with infested fish, one unit per species with non-infested control fish). In total, 40 funnel-shaped holding units were specifically designed for these kinds of experiments and placed randomly in the laboratory (Fig. 2.1). Figure 3.1 shows the schematic construction of a single holding unit (height: 0.50 m, diameter above: 0.60 m, content: maximum of 45 L, material: polyethylene black, Schubert Kunststoff GmbH, Munich, Germany). The black color and the non-transparent material guaranteed that fish were not stressed by environmental circumstances in the laboratory.

Because of the shape of the holding units, fish had enough space to swim at the top and dropped-off glochidia and juvenile mussels could accumulate at the bottom end of the holding units. A valve at the bottom end (Fig. 2.1) also allowed an easy water outlet and a collection of the glochidia and juvenile mussels without interrupting the fish. Due to special translucent and air-permeable coverings of the holding units (Fig. 2.1), fish were able to realize the light-dark cycle despite the black color and non-transparency of the units themselves. Moreover, special designed funnel-shaped nets (Renate Heberle Netzfabrikation, Altusried, Germany) were placed inside of every holding unit, around 10 cm above the bottom of each unit to protect dropped-off glochidia and juvenile mussels from predation by the fish. The funnel-shaped holding units were then placed in special racks (2.00 x 0.36 x 0.93 m, material: stainless steel, Technical University of Munich, Munich, Germany, suitable for 3-4 holding units per rack, Fig. 2).

All holding units were filled with around 40 L of water (bank filtrate, river Moosach). The water within the holding units was the same as in the infestation bath as well as in the basins containing the fish before the experiment. Every unit was equipped with constant aeration of the water due to pumps (Hiblow Air Pump HP 40, Techno Takatsuki CO., Osaka). Besides, a special constant flow of water at colder temperatures was provided for holding units containing brown trout (*Salmo trutta*, Linnaeus 1758) since this species has special requirements on temperature and oxygen supply.

The water within the holding units was not heated or cooled during the experiments, the temperature of the water corresponded to the temperature of the environment (except holding units containing brown trout with constant flow of cooler water). Temperature loggers (Lascar Electronics Limited, Salisbury, UK) were placed in every holding unit, respectively, to measure the water temperature during the experiment every 30 min.

To avoid the transmission of for example pathogens, materials like hand nets were only used for one single holding unit, without contact to other basins. Material was also disinfected every day after use.

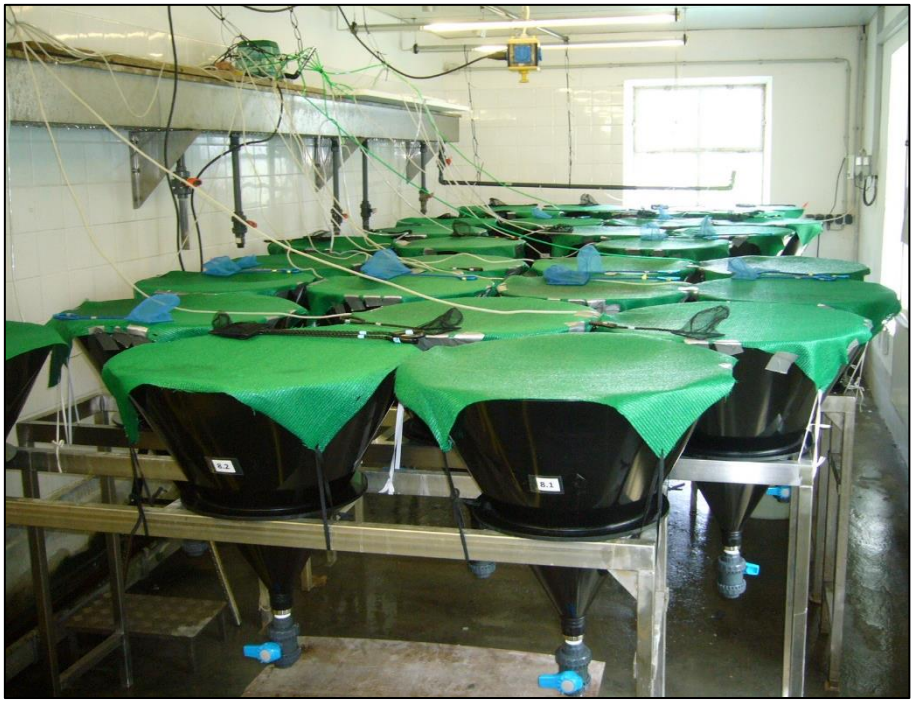

Figure 2.1: Experimental setup post-infestation. Funnel-shaped holding units with covering containing fish (infested and control individuals) after the infestation process. Holding units were randomly placed in special racks for a better handling, aerated by pump or provided by a constant flow of water.

2.3 Experimental species

Adult individuals of the three mussel species used for the experiments were sampled from wild populations. Adult *A. cygnea* came from the Neusee near Bernried, Bavaria, Germany (47° 51’27.2” N, 11° 16’02.0” E), adult *A. anatina* inhabited a private pond in Rehau village, Bavaria, Germany (50° 16’13.6” N, 12° 03’57.1” E) and adult *S. woodiana* were sampled from the wildlife reserve Öberauer Donauschleife (backwater of the Danube near Straubing, Bavaria, Germany, 48° 54’18.6” N, 12° 32’10.9” E), a self-recruiting population. This sampling was approved by license of the responsible nature conservation authority (license number: 55.1-8642.10 U 12). Gravid mussels were detected by opening the valves of the individuals carefully with a special tong and pregnancy was visible due to swollen marsupia (outer gills, Bauer, 2001b; Lefevre and Curtis, 1911). Gravid individuals were then transferred to the laboratory

and held in aquaria until glochidia release. The species of all sampled mussels were genetically confirmed according to the molecular identification key developed by Zieritz at al. (2012).

Fish species were selected according to their co-occurrence with the three mussel species in the same habitats in Central Europe. Since the three bivalves predominantly prefer standing water bodies, especially fish species with this habitat preferences were chosen for the experiments. Populations of the mussel *A. anatina* also occur in fast-flowing streams and rivers. Thus, also rheophilic fish species with different habitat requirements were included in the experiments. Juvenile fish only with no previous contact to mussels were selected to avoid immunity reactions of the fish to glochidial infestation due to previous infestations with mussel larvae.
In total, ten different fish species from four different fish families were chosen for the infestation experiments:

(1) *Salmo trutta* (Linnaeus 1758), brown trout, family Salmonidae. Specimens came from an own offspring of the Aquatic Systems Biology Unit in Freising, Germany.
(2) *Perca fluviatilis* (Linnaeus 1758), perch, family Percidae. Origin of the specimens: Aquaculture Michael Rösch, Bärnau, Germany.
(3) *Gasterosteus aculeatus* (Linnaeus 1758), stickleback, family Gasterosteidae, Origin of the specimens: Aquaculture Michael Rösch, Bärnau, Germany.
(4) *Leuciscus idus* (Linnaeus 1758), ide, family Cyprinidae. Origin of the specimens: Aquaculture Michael Rösch, Bärnau, Germany.
(5) *Gobio gobio* (Linnaeus 1758), gudgeon, family Cyprinidae. Origin of the specimens: Aquaculture Michael Rösch, Bärnau, Germany.
(6) *Rutilus rutilus* (Linnaeus 1758), roach, family Cyprinidae. Origin of the specimens: Aquaculture Michael Rösch, Bärnau, Germany.
(7) *Rhodeus amarus* (Bloch 1782), bitterling, family Cyprinidae. Origin of the specimens: Aquaculture Michael Rösch, Bärnau, Germany.
(8) *Leucaspius delineatus* (Heckel 1843), moderlieschen/sunbleak, family Cyprinidae. Origin of the specimens differed. Experiments with the larvae of *A. cygnea* and *A. anatina*: fish specimens came from Aquaculture Michael Rösch, Bärnau, Germany. Experiment with the larvae of *S. woodiana*: fish specimens came from an Aquaculture near Rheine, Germany. The reason for this change in origin was that the Aquaculture M. Rösch could not get juvenile *L. delineatus* in the year of the last experiment with *S. woodiana*. Therefore, another origin had to be found that fulfilled the same criteria as

mentioned for the other origins (especially juvenile fish with no previous contact to mussels).

(9) *Ctenopharyngodon idella* (Valenciennes 1844), grass carp, non-native species, family Cyprinidae, Origin of the specimens: Bavarian State Office for Environment, Wielenbach, Germany.

(10) *Pseudorasbora parva* (Temminck and Schlegel 1846), topmouth gudgeon, non-native species, family Cyprinidae. Origin of the specimens differed. Experiment with the larvae of *A. anatina*: fish specimens came from Aquaculture Michael Rösch, Bärnau, Germany. Experiments with the larvae of *A. cygnea* and *S. woodiana*: specimens came from a private fish pond in Roth, Germany. The reason for this change in origin was that the Aquaculture M. Rösch could not get juvenile *P. parva* for these experiments as mentioned before for the origin of *L. delineatus*.

Both non-native fish species were chosen for the experiment because of their ongoing spread in native European mussel habitats. All fish species, their status, place and date of origin are also mentioned in Tables 3.1, 4.1 and 5.1 and in Huber and Geist (2017, 2019a, b).

It was not possible to get ten different fish species with different habitat requirements from one origin. Thus, origins were chosen, where juvenile fish with no previous contact to mussels could be guaranteed, moreover, the time of transport of the fish from their origin to the laboratory should be as short and easy as possible. All fish origins had different water constitutions. Therefore, the fish had to adapt to the new water conditions slowly after transferring them to the laboratory and before starting the experiments.

2.4 Glochidial collection

After the transfer of the adult mussels to the laboratory, maturity of the glochidia within the marsupia was checked daily by opening the valves with a special tong. As soon as the glochidia were mature, they were flushed out of the marsupia with a squirt bottle in different 1 L glass beakers and stored at 4.0°C until the start of the experiments (glochidia of every adult mussel were stored separately). Due to the cooling of the larvae it could ensure, that they obtained viable until the infestation of the fish. All larvae were stored at 4.0°C for less than 24 hours.

For assessing the viability of the glochidia, a sample of 10 ml was removed from every beaker and then analyzed under a stereomicroscope (Stemi DV4, Zeiss, Munich, Germany, 12.8x amplification). Due to the addition of NaCl, the viability was visible because of an active clamping mechanism of the glochidia in reaction to the NaCl (Taeubert et al., 2012a). Viability of the glochidia from all mussel species in the three experiments always reached more than 95%. This high value of viability guaranteed that the larvae are high infectious and convenient for the infestation of fish. After checking the viability, the total amount of glochidia has to be determined. The number of larvae in every 10 ml sample was counted and extrapolated to the number of larvae per 1 L beaker (per adult mussel) and then to the total amount of larvae for the infestation bath. Glochidia of every adult mussel were pooled for the infestation to get a larvae concentration of 8000-9000 glochidia per liter in the infestation bath and to avoid bias during the infestation due to the application of larvae of one single adult mussel.
Before using the glochidia suspension for the infestation, larvae had to be gently acclimatized to the water temperature of the infestation bath (~ 12.0°C, bank filtrate, river Moosach) over a few hours.

2.5 Infestation procedure

Fish were caught from their individual ponds and first collected in a common water bath (water without glochidia) to avoid infestation delays due to different catchment times of the fish. After that all individuals from all fish species were transferred synchronously from the water bath to the infestation bath, filled with mussel larvae (concentration of 8000-9000 larvae per liter). The fish stood in the infestation bath for 30-45 minutes. During that time, water was aerated and mixed softly and continuously to guarantee that the glochidia are in motion during the whole infestation process (glochidia are not able to swim actively; Jansen et al., 2001), and that the larvae have the possibility to infest all individual fish independent from the natural behavior of the fish species (for example some species like the gudgeon (*Gobio gobio*) are benthic species, laying on the ground of the basin during the infestation, some species like the perch (*Perca fluviatilis*) are pelagic species, swimming around during the infestation process).

The amount of water in the infestation bath differed in all three experiments and depended on the amount of fish per species that were infested and the total amount of larvae to reach the quoted glochidial concentrations. Therefore, also the number of fish per species in the infestation bath differed between the three experiments. The number of fish was dependent on the

different weights and sizes of the fish of different species at different time points and dependent on the fact that the funnel-shaped holding units for keeping the fish after the infestation had always the same size and number of fish per species had to be adjusted to the size of these holding units (for example from smaller fish species like *L. delineatus* more individuals could be included in the infestation process because more individuals could be put together in one holding unit after the infestation until the end of the experiments. In contrast, from bigger species like *S. trutta* that also had higher requirements on water temperature, space and oxygen supply, a smaller number of specimens could be infested because only 2-4 individuals could be put together in one holding unit after the infestation). This was very important to ensure optimal holding conditions for every fish species during the experiments.

The temperatures of the infestation baths were always the same in all three experiments. Temperatures had around 12.0°C (bank filtrate, river Moosach) and guaranteed the same temperature conditions during infestation of the fish in all experiments and thus a better comparability of the results at the end. After 30-45 min of infestation, fish were transferred to a water bath without glochidia for another 10-15 min to remove non-attached larvae before transferring the fish to the holding units. After the water bath, infested fish were separated per species and divided into three replicates of infested individuals per species (three funnel-shaped holding units with infested fish per species, 30 holding units of infested fish in total) until the end of the experiment. Figure 2.2 shows the schematic procedure of the infestation process until the transfer of the fish to the holding units.

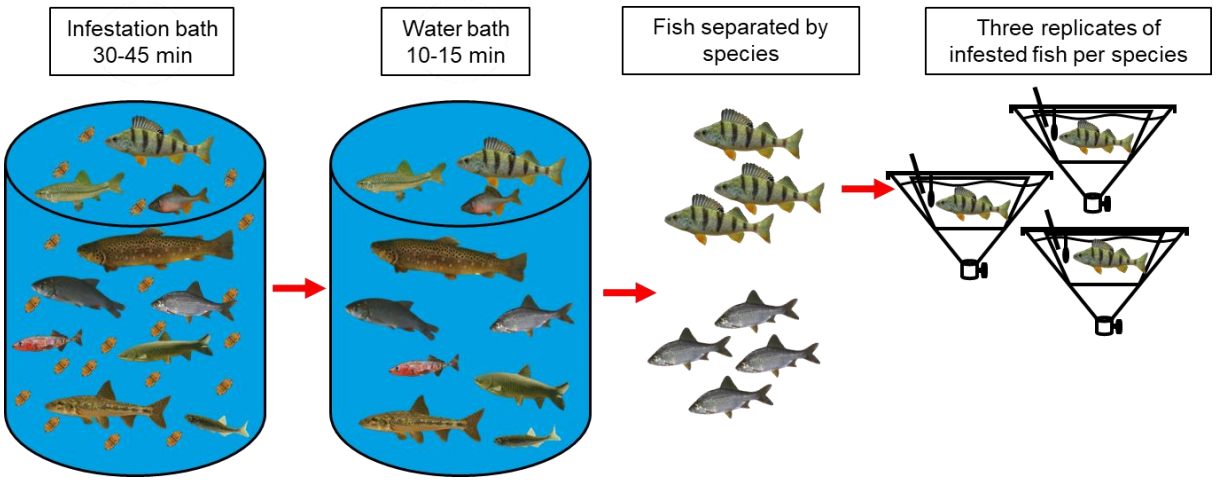

Figure 2.2: Schematic procedure of the infestation of the fish in all three experiments.

The same procedure was also performed for a group of non-infested control fish. These specimens were treated in the same way as the infested ones (they were also transferred to a water bath (without glochidia) for 30-45 min, then to another water bath for 10-15 min before also transferring them to the holding units), to check for bias due to handling or holding conditions. There was one group of control fish per species per experiment (one holding unit per fish species with non-infested/control fish, ten holding units of non-infested fish per experiment in total). Thus, per experiment 40 holding units for the fish of ten different species were needed in total.

2.6 Post-infestation procedure

After the infestation of the fish it was important to get information about the attachment success by the attachment rate of the glochidia on different fish species as well as to determine the success of larval metamorphosis and juvenile mussel excystment developed on different fish species. Without these results, there is no possibility to determine the suitability of fish species as hosts for the mussels.

For the determination of the metamorphosis success the initial attachment rate of glochidia on the fish species had to be calculated. Thus, 2 days after the infestation (2 days post-infestation, pi) the attachment rate of larvae on fins, gills and skins of some individuals per species was determined. The timepoint 2 days pi was chosen because after two days it could be ensured that only completely encysted glochidia are evaluated (Taeubert et al., 2012a). Glochidia that did not attach to the fish or did not encyst completely on the fish are rejected within two days. As mentioned before, a different number of specimens per fish species was infested depending on their weights and sizes. Therefore, also a different number of infested individuals per fish species was sacrificed for the analyses of the attachment rate 2 days pi. The number of infested specimens per species was sacrificed to calculate the glochidial attachment rate also depended on the number of fish per species and holding unit that were still alive at this time point. The number of fish used for the calculation of the glochidial attachment rate differed between fish species but also between the three experiments.
Besides, there was a second time point for the analysis of the glochidial attachment. This second analysis took place at the time point when the first excysted living juvenile mussels could be detected. This time point differed between the three experiments because duration of the metamorphosis phase of the glochidia also differed. The number of sacrificed fish per species at that

time point also differed due to the same criteria as mentioned for the first time point of analysis 2 days pi. This second time point was an additional time point for the analysis of the attachment rate only, in case no fish would have survived until the end of the experiment (in case that the endpoint would not have been measurable). This was not the case for all three experiments. Thus, at the end of the excystment of juvenile mussels, again a number of fish could be analyzed for attached glochidia (at the end no encysted glochidia could be detect on the tested fish in all three experiments).

The success of larval metamorphosis was determined by detecting the number of completely developed, living juvenile mussels per fish species. To detect dropped-off living juveniles as well as not encysted, rejected glochidia, 5 L of water were released from every holding unit containing infested fish and were filtered through a sieve (mesh size 200 µm). Due to their size (glochidia as well as juveniles of the three mussel species tested had an average size from 300-400 µm), dropped-off individuals could be collected within the sieve, flushed out of the sieve into a Petri dish and counted under a stereomicroscope (Stemi DV4, Zeiss, Munich, Germany). Completely developed, living juvenile mussels were detected by an active pedal movement as well as an active contraction of the adductor muscle (Taeubert et al., 2013b). In contrast, dead juveniles could be easily identified by wide gaping of the valves and by the absence of any reaction (Taeubert et al., 2013b). Living juveniles were directly transferred to other special holding systems to guarantee their survival.
The removal of 5 L water per holding unit for the daily water check simultaneously represented a daily renewal of water of 12.5%. After the removal, 5 L fresh water was added to every holding unit (fresh water was also adjusted to the temperature of the environment before adding to the holding units). Water from holding units with non-infested/control fish was also changed daily.

2.7 Data analyses

The number of attached glochidia as well as number of excysted juvenile mussels were calculated per gram fish weight (g^{-1}). This was important to account for differences in weight between different fish species and individuals and to ensure the comparability of the results within one experiment as well as between the three experiments.
Besides, for the comparability of the duration of the metamorphosis phase and the duration of juvenile mussel excystment on different fish species as well as at different temperatures, the

concept of degree-days (dd, sum of daily water temperatures) was applied (Hruska, 1992; Taeubert et al., 2014) in the analyses of all experiments.

All statistical analyses were performed in R version 2.15.3 and version 3.4.3 (R Core Team, 2013, 2017). Data were tested for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test). Since the data were not normally distributed, the non-parametric Kruskal-Wallis sum of rank test and post hoc pairwise Wilcoxon rank sum test with Bonferroni correction were applied. Significance in all tests was accepted at $p \leq 0.05$ (after Bonferroni correction). Named tests were used: to calculate differences between all tested fish species regarding the initial weight-normalized glochidial attachment rate 2 days pi as well as the rate of weight-normalized juvenile mussel excystment; moreover, to calculate differences between good and poor hosts as well as between native and non-native fish species; to calculate differences between different infested tissues of the fish at different time points; to calculate differences between the fish species regarding the duration of metamorphosis phase and duration of juvenile mussel excystment; to calculate differences between the three mussel species.
Moreover, Pearson's product-moment correlation and Spearman's rank correlation were used to calculate for the following correlations: between initial infestation rate and duration of metamorphosis and juvenile mussel excystment; between the total weight of applied fish in the infestation bath and the initial infestation rate of glochidia per gram fish weight 2 days pi; between the number of excysted juvenile mussels per gram fish weight and the mortality rate of the fish during juvenile mussel excystment.

Figures showing the duration of metamorphosis and juvenile mussel excystment and the number of excysted juvenile mussels or percentage of excysted juveniles were performed in Microsoft Excel (2013 and 2016). Boxplots were plotted using R version 2.15.3. Figures showing comparisons of host fish suitability between the three mussel species were also plotted using R version 3.4.3.

3 Glochidial development of the freshwater swan mussel (*Anodonta cygnea*, Linnaeus, 1758) on native and invasive fish species

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Author contributions:

Verena Huber (VH) and Prof. Dr. Jürgen Geist (JG) conceived and designed the study. VH sampled the mussels, performed the genetic analysis and arranged and performed the laboratory experiment. VH and JG discussed the data analysis and manuscript structure. VH analyzed the data and wrote the manuscript. JG improved and edited the manuscript.

3.1 Abstract

The declining mussel *Anodonta cygnea* is an important keystone species in European freshwater systems. Information on the complex life cycle of *A. cygnea* regarding the attachment and metamorphosis of their larvae on suitable host fish species is lacking, yet important as a basis for conservation and fisheries management. Ten different fish species, including eight native and two non-native species from four different families, were simultaneously infested with the glochidia of *A. cygnea* in a standardized laboratory experiment. The results of this study confirmed the hypothesis that *A. cygnea* can be considered a host generalist, as nine out of the ten tested fish species were suitable hosts, and different body parts were infested. Due to the observed differences in initial infestation rates and metamorphosis success, hosts were classed into "good hosts" (*Perca fluviatilis*, *Leuciscus idus*, *Salmo trutta*, *Gasterosteus aculeatus*, *Ctenopharyngodon idella*), "poor hosts" (*Leucaspius delineatus*, *Gobio gobio*, *Rutilus rutilus*, *Pseudorasbora parva*) and "not hosts" (*Rhodeus amarus*). The larval development differed strongly between the single host fish species with regard to success and duration of metamorphosis, as well as timing and synchronization in larval drop-off, suggesting evolutionary consequences of the use of different host fish species. The finding that two non-native fish species were identified as suitable hosts for the glochidia of *A. cygnea* illustrates that the generalizations that non-native species are a threat to native mussel communities and that co-evolutionary patterns between hosts and mussels determine host suitability do not always hold true.

3.2 Introduction

Freshwater bivalves play a key role in the functioning of the ecosystems in which they occur (Boeker et al., 2016; Lummer et al., 2016; Vaughn and Hakenkamp, 2001). As ecosystem engineers they act as connective link between the pelagic and benthic zones of a water body (Gutiérrez et al., 2003; Lopes-Lima et al., 2017; Nobles and Zhang, 2011). Their ecosystem functions include the transfer of matter and energy from the water column to the benthos with strong effects on primary and secondary production, biogeochemical cycles, sedimentation rates and water clarity (Lummer et al., 2016; Strayer et al., 1999). Despite their important roles in freshwater ecosystems, there is a worldwide decline of freshwater mussel populations (Bogan, 2008; Geist, 2010; Lopes-Lima et al., 2017; Lydeard et al., 2004). Currently, 12 out of 16 European freshwater mussel species are listed as threatened or near-threatened on the IUCN Red List (Lopes-Lima et al., 2017).

The swan mussel (*Anodonta cygnea*) was originally considered a widespread species throughout Europe, Russia and the Middle East (Lopes-Lima, 2014b). It occurs in a diversity of habitats with slow or no flow current, including small ponds as well as lakes and lowland rivers (Zettler et al., 2006). Despite the swan mussel still being listed as a species of Least Concern in the IUCN Red List of Threatened Species, global population trends are still unknown and there is a strong indication of currently underestimated declines in abundance on local scales (Lopes-Lima, 2014b). *Anodonta cygnea* in Europe has a conservation status of Near Threatened according to the IUCN European Red List of non-marine molluscs (Cuttelod et al., 2011). In the study region of this work, Germany, the species is considered “threatened” (Jungbluth and von Knorre, 2009). It is even listed as Highly Endangered according to federal German legislation (Bundesartenschutzverordnung (BArtSchV), 2005; Lopes-Lima, 2014b; Zettler et al., 2006) as well as on state-specific lists such as the Bavarian Red List of Endangered species (*A. cygnea* with a status of Endangered) (StMUV, 2005).

Like all other mussel species of the order Unionoida, *A. cygnea* has a complex lifecycle with an obligate parasitic phase of the larvae on a suitable host fish (e.g. Bauer, 1994; Lefevre and Curtis, 1910; Lopes-Lima et al., 2017; Weber, 2005). Gravid mussels eject mature glochidia into the water column usually during the time period between late winter and spring (Lopes-Lima et al., 2017; Niemeyer, 1993). During the parasitic phase on the fish, glochidia metamorphose into juvenile mussels. The larvae of the swan mussel usually attach to multiple body parts of a fish host, for example fins, opercula and gills, which is in contrast to other mussel species of the order Unionoida which often exclusively attach to the fish gills (e.g., *Margaritifera*

margaritifera: Bauer, 2001b; Blažek and Gelnar, 2006; Wächtler et al., 2001). After completion of the parasitic phase, the fully developed juvenile mussels of *A. cygnea* drop off the fish host and bury into the lake bed substratum until they start their adult life as filter feeders (Wächtler et al., 2001).

Since the attachment to, and metamorphosis on a suitable host, can be an important bottleneck in the life cycle of endangered freshwater mussel species (Stoeckl et al., 2015; Taeubert et al., 2012a, b), such information is urgently needed as a basis for conservation, fisheries management and supportive breeding (Gum et al., 2011). In contrast to the very host-specialized species *M. margaritifera*, the larvae of *A. cygnea* are considered to be host fish generalists with a wide range of host fish species (Bauer, 2001b; Wächtler et al., 2001). However, to date, research and conservation strategies into host suitabilities of European freshwater mussels have mostly focused on the thick-shelled river mussel (*Unio crassus*) and the freshwater pearl mussel (*M. margaritifera*). A recent review on the conservation of European freshwater mussels has thus suggested the need of identifying of host fishes for other species (Lopes-Lima et al., 2017), including *A. cygnea*.

The general aim of this study was to characterize the host relationships of *A. cygnea*. Specific objectives included (1) identifying suitable host fish species for *A. cygnea* from the native fish community in central Europe, (2) assessing host suitability and thus possible competition with non-native fish species, (3) characterizing and comparing the duration of the larval phase on different fish hosts, and (4) determining glochidial infestation at different body parts of a host. Specifically, the following hypotheses were tested: (1) Glochidia of *A. cygnea* are generalists concerning their host choice (following the suggestion by Bauer (2001a) and Wächtler et al. (2001)) and suitable hosts are equally infested, showing similar metamorphosis success. (2) Non-native fish species are non-suitable hosts for the glochidia of *A. cygnea* and thus reduce overall metamorphosis success by competing with native hosts. (3) Development times of *A. cygnea* larvae differ between host fish species as previously found in other mussel-host fish relationships (Taeubert et al., 2012b). (4) The numbers of attached glochidia strongly vary between different types of tissue of a single host fish, with gills showing highest infestation rates as suggested by Jansen et al. (2001) and Schneidt (1998).

3.3 Materials and methods

3.3.1 Collection of glochidia

Seven gravid *A. cygnea* individuals from the Neusee near Bernried (Bavaria, Germany) were collected at the 23th October 2014 and brought to the laboratories of the Aquatic Systems Biology Unit at Technical University of Munich, Germany where they were held until glochidia release. Because of the high morphological plasticity of the anodontines, all specimens were genetically validated following the method in Zieritz et al. (2012). The seven adult mussels were kept in aquaria and maturity status of the glochidia inside the marsupia was checked once a week from February to the beginning of May 2015 in order to identify the ideal time point for glochidial collection. On the 13th May 2015 fully developed glochidia were detected in all seven specimens. Thus, marsupia of the seven specimens were flushed with a squirt bottle to collect the glochidia for the following infestation process. Glochidia from each specimen were individually stored in 1 L beakers at 4.0°C for < 24 h. Before host fish infestation, the viability of the larvae was assessed by checking for an active clamping mechanism after addition of NaCl to a small amount of glochidia (Taeubert et al., 2012a). In total, a number of ~ 300,000 larvae from all seven adult *A. cygnea* was harvested.

3.3.2 Infestation

Ten different fish species were infested with the larvae of seven adult *A. cygnea* on 14th May 2015. The selection of tested fish species was based on broad taxonomic representation of different fish families and species that naturally co-occur with *A. cygnea*. In addition, two non-native species (*Ctenopharyngodon idella*, Valenciennes, 1844; *Pseudorasbora parva*, Temminck and Schlegel, 1846), which currently spread within the *A. cygnea*-distribution area, were also included to test their suitability as hosts. Native fish species from the families Salmonidae (*Salmo trutta*, Linnaeus, 1758), Cyprinidae (*Leuciscus idus*, Linnaeus, 1758; *Gobio gobio*, Linnaeus, 1758; *Rhodeus amarus*, Bloch, 1782; *Rutilus rutilus*, Linnaeus, 1758; *Leucaspius delineatus*, Heckel, 1843), Percidae (*Perca fluviatilis*, Linnaeus, 1758) and Gasterosteidae (*Gasterosteus aculeatus*, Linnaeus, 1758) as well as the introduced Cyprinidae *C. idella* and *P. parva* were tested. *C. idella* was primarily introduced to European waterbodies for weed control (Cross, 1969), whereas *P. parva* was primarily introduced accidentally (Copp et al., 2010). Juvenile fish (< 1 year, trout < 2 years) with no previous contact to unionid mussels were used to

exclude a possible immune-response due to previous contact with glochidia. Information about the tested fish species, their origin and number of individuals per species used for the experiment are given in Table 3.1.

Table 3.1: Tested fish species (order based on Table 2: from fish species with the highest number of excysted juvenile mussels after weight-normalization to species with the lowest number of excysted juveniles after weight-normalization), their status, place and date of origin: RO = Aquaculture M. Rösch, Bärnau, 04.11.2014, AS = Chair of Aquatic Systems Biology, 27.11.2012, LU = Bavarian State Office for Environment, Wielenbach, 12.11.2014, PR = Private fish pond, Roth, 11.05.2015; mean size (total length) and weight of applied fish, number of fish in the infestation bath per species, number per tank after the infestation, number of control fish per species; $\overline{X}$ = arithmetric mean

Species	Status	Source	Size $\overline{X}$ ± 1 mm	Weight $\overline{X}$ ± 0.1 g	Nb. of fish infestation bath	Nb. of fish per tank	Nb. of control fish
Perca fluviatilis (perch)	Native	RO	101	8.7	21	7	7
Leuciscus idus (ide)	Native	RO	107	7.8	24	8	8
Salmo trutta (brown trout)	Native	AS	139	21.1	15	5	5
Gasterosteus aculeatus (stickleback)	Native	RO	55	1.3	60	20	20
Ctenopharyngodon idella (grass carp)	Introduced	LU	77	3.8	47	~ 15	14
Leucaspius delineates (moderlieschen/sunbleak)	Native	RO	42	0.4	77	~ 25	23
Gobio gobio (gudgeon)	Native	RO	123	15.0	21	7	7
Rutilus rutilus (roach)	Native	RO	87	6.3	42	~ 14	15
Pseudorasbora parva (topmouth gudgeon)	Introduced	PR	92	6.2	41	~ 13	13
Rhodeus amarus (bitterling)	Native	RO	55	1.8	49	~ 16	15

Before starting the infestation process, the glochidia from all seven parents were gently mixed to a homogenous suspension and acclimatized over 2 h to the temperature of the laboratory water (bank filtrate, river Moosach, ~ 12.0°C) used for the infestation bath. Infestation procedures followed the previously described standard protocol by Taeubert et al. (2012a). The glochidial concentration in the infestation bath was ~ 8500 larvae per liter. Overall, 397 specimens of the ten different fish species were simultaneously infested in the same infestation bath with the larvae of *A. cygnea* to ensure identical starting conditions (Taeubert et al., 2013a). During the infestation, the water was mixed continuously to ensure a homogenous suspension of glochidia and equal attachment conditions. After 30-45 min within the infestation bath, the fish were transferred into a second water bath without glochidia for 15 min to remove non-attached larvae. In addition, 127 individuals from all species not exposed to the glochidia were used as a control. The control fish were treated in the same way as the infested ones to check if influences like stress due to handling or holding conditions affect the mortality during the experiment.

3.3.3 Post-infestation procedure

After the common infestation bath, specimens were sorted, and different species kept in separate holding units (three replicates per species). In addition, in every species, one tank with control fish was maintained under identical conditions. The custom-made, funnel-shaped holding units (Fig. 3.1) contained a volume of ~ 40 L water at a mean temperature of 15.8°C. This system was applied to ensure that all dropped-off glochidia accumulate at the bottom end where they could be collected by opening a lid. Within each holding unit, nets were placed ~ 10 cm above the bottom of the tanks to protect the glochidia and excysted juvenile mussels from possible predation by the fish (Fig. 3.1). To account for differences in fish sizes among different species and to ensure optimal holding conditions, the number of specimens per tank was adjusted accordingly. Holding units containing brown trout were provided with constant flow of water at a colder temperature (12.8°C) due to requirements of this species. The water temperature in the fish holding units was measured with temperature loggers (Lascar Electronics Limited, Salisbury, UK) every 30 min. Fish were not fed during the experiment.

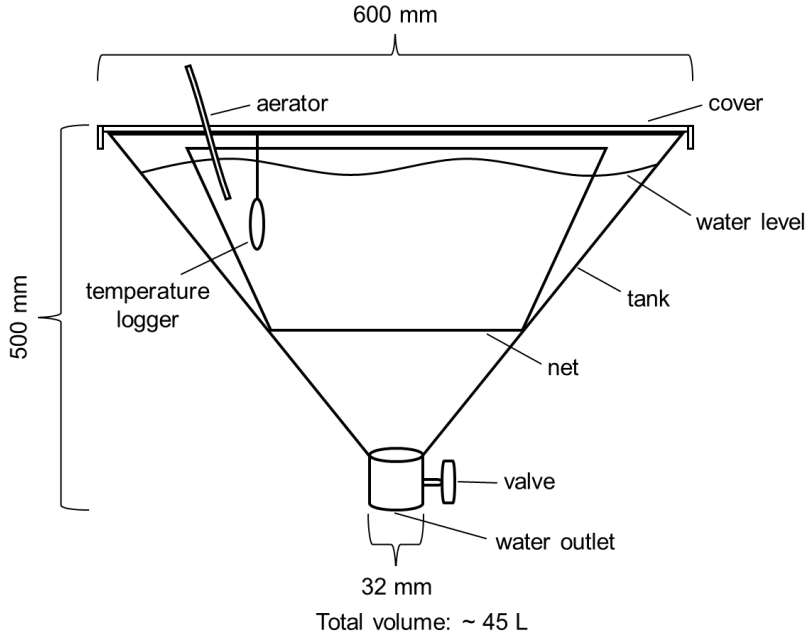

Figure 3.1: Schematic of the custom-made, funnel-shaped holding units. Dropped-off mussel larvae can be collected from the bottom valve. The bottom net prevents predation by fish. Total height: 500 mm, total width: 600 mm, maximum volume: 45 L.

3.3.4 Glochididal development and excystment

Five liters of water from every tank (12.5%) were changed daily. The 5 L water-outflow from all holding units with infested fish were afterwards checked for excysted glochidia and juvenile mussels under a stereomicroscope (Stemi DV4, Zeiss, Munich, Germany, 12.8 x amplification) by filtering the water through a sieve (mesh size 200 µm). Criteria for the occurrence of living juvenile mussels were active pedal movement and active contraction of the adductor muscle (Taeubert et al., 2013b). Living juvenile mussels were counted and directly transferred to special flow-through systems containing sediment according to Feiner et al. (2016).

Infestation success was determined by counting the attached glochidia on the fins, gills and skins of individuals from every fish species at different time points. This sampling was carried out after 2 days (16th of May), 14 days (first time when dropped off living juvenile mussels were found, 28th of May) and at the end of the excystment (until the 31st of July). In total, 28 individuals (18 infested and 10 non-infested/control individuals) were sacrificed 2 days pi and 14 days pi, respectively. At each of the two time points, two infested ide, stickleback, grass carp, moderlieschen, gudgeon, roach, topmouth gudgeon and bitterling were sampled. One infested individual was sampled from the species perch and brown trout due to the small number of animals of both species. At the end of the excystment period, 45 fish were sacrificed in total, 32 infested and 13 non-infested/control fish. At that time point four infested individuals were sampled of the species moderlieschen and topmouth gudgeon, and three individuals were sampled of every other species. Attached glochidia on the fish bodies were identified and counted under the stereomicroscope. Because of high differences in size and weight between different fish species, total length (± 1 mm) and weight (± 0.1 g) of each single individual was determined to calculate the size- and weight-normalized numbers of glochidia and juvenile mussels per fish (Taeubert et al., 2010). The experiment was terminated three days after the last drop-off of a juvenile mussel from the last fish species was detected, i.e. after 11 weeks at the 31st July 2015.

3.3.5 Statistical analyses

Statistical analyses were performed in R version 2.15.3 (R Core Team, 2013). To calculate differences in total glochidial infestation rates and weight-normalized glochidial infestation rates, as well as differences in total juvenile mussel excystment and weight-normalized mussel excystment between all fish species, non-parametric Kruskal-Wallis sum of rank tests and post hoc pairwise Wilcoxon rank sum tests were used since ANOVA assumptions were not fulfilled.

Bonferroni correction was applied to correct for multiple testings. Differences in the duration of the metamorphosis phase between different host fish species, as well as differences between infested fish tissues at different time points were plotted and also tested by using non-parametric Kruskal-Wallis sum of rank tests and post hoc pairwise Wilcoxon rank sum tests with Bonferroni correction. To compare the duration of the metamorphosis phase at different temperatures, the concept of degree-days (the sum of daily water temperatures, dd) was applied (Taeubert et al., 2014).

3.4 Results

A successful encystment of glochidia and successful development resulted in a total of 6029 dropped-off living juvenile *A. cygnea* mussels. Full development occurred in nine out of ten fish species, supporting the hypothesis of a wide spectrum of suitable hosts. Only the bitterling had no larval attachment or development of juvenile mussels. Different host species revealed a great variability in terms of initial glochidial attachment, number of successfully excysted juvenile mussels as well as duration of the metamorphosis phase.

3.4.1 Infestation success

The tested fish species could be divided into three groups of hosts according to the successful excystment of juvenile mussels:

1. Good hosts (perch, ide, brown trout, stickleback, grass carp), with an average of 8.3 completely developed juvenile mussels per gram fish weight.
2. Poor hosts (moderlieschen/sunbleak, gudgeon, roach, topmouth gudgeon), with an average of 0.4 completely developed juvenile mussels per gram fish weight.
3. Not hosts (bitterling), with no juvenile mussel development.

The most successful development of juvenile mussels could be detected on *P. fluviatilis* with a mean number of 15.3 mussels g^{-1} (Table 3.2). Despite the highest mean number of viable juvenile mussels developed on the species *S. trutta* (174.7), this species did not have the highest number of juveniles per gram fish weight (8.3 mussels g^{-1}), compared to *P. fluviatilis*. The best five host fish species for larvae of *A. cygnea*, according to the fish-weight-normalized number of glochidia, were perch, ide, brown trout, stickleback and grass carp (Table 3.2). The five best fish species differed significantly from the other fish species in the number of excysted juveniles

per gram fish weight (overall differences between both groups, non-parametric Kruskal-Wallis sum of rank test, $p < 0.001$).

The differences in host fish suitability were already evident at the stage of initial larval attachment 2 days after the infestation (post infestation, pi). At this time point, there was a highly significant difference between the species in relation to the number of attached glochidia per fish and per gram fish weight (post hoc pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$) (Table 3.2). Mean glochidial load 2 days pi ranged between zero (bitterling) and 704.0 (brown trout) larvae per fish, and between zero (bitterling) and 29.8 (perch) glochidia per gram fish weight (Table 3.2). *P. fluviatilis* had the most successful initial larval attachment of all tested species after weight normalization. Since no glochidia were present on the tissue of *R. amarus* 2 days pi, the mean glochidial load of the bitterling was not assessed any longer (Table 3.2).

On average, 59% of the initially attached glochidia 2 days pi did not develop into juvenile mussels and were lost before completion of metamorphosis. The highest weight-normalized glochidial loss was found at gudgeon (87%) and moderlieschen (80%), the lowest on stickleback (14%) (Table 3.2). A loss of glochidia could already be detected 14 days pi (first juvenile mussel detection) over all species. At this point the mean number of encysted larvae ranged between zero (moderlieschen) and 486.0 (brown trout) glochidia and between zero (moderlieschen) and 26.7 (brown trout) glochidia g^{-1} (Table 3.2). Compared with the initial infestation rates, the average glochidial load over all fish species was reduced 14 days pi (around 45% fish weight-normalized glochidial loss between 2 days pi and 14 days pi) (Table 3.2). The number of attached glochidia and the weight-normalized glochidial load varied significantly between the host fish species (post hoc pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$), with a consistent ranking of fish species at different time points. No difference in mortality between non-infested control fish and infested ones was detected in any of the species, suggesting that the relatively high mortality rates were not caused by the infestation.

Table 3.2: Results of the host infestation with glochidia of *Anodonta cygnea*: host status, mean number of glochidia and glochidia g $^{-1}$ per fish species 2 days and 14 days pi, as well as excystment rate of juvenile mussels per species, mean temperature and host fish mortality (fish that died naturally) during the time span of juvenile mussel excystment per species (mortality is given in percentage and total number of dead fish in brackets); n fish in total = number of infested individuals per species that is still alive at the respective time point (for example: in total 21 perch were infested, 2 days pi: 21 perch were still alive, 14 days pi: 16 perch were still alive, excystment: 13 perch were still alive); 2 days pi and 14 days pi: 1 infested individual of the fish species perch and brown trout was sacrificed, two infested individuals of all other fish species were sacrificed to determine the infestation rate; end of excystment: 4 infested individuals of the species moderlieschen and topmouth gudgeon were sacrificed, three infested individuals of all other species were sacrificed to determine the amount of excysted juvenile mussels; species order: from fish species with the highest number of excysted juvenile mussels after weight-normalization to species with the lowest number of excysted juveniles after weight-normalization; NA = not assessed because no infestation was found 2 days pi; * = introduced, non-native fish species; $\bar{X}$ = arithmetric mean.

Fish species	Host status	2 days pi			14 days pi				Excystment						
		Glo. per fish	Glo. per g fish weight	*n* fish in total	Glo. per fish	Glo. per g fish weight	*n* fish in total	Weight-normalized glo. loss (%)	Mussels per fish $\bar{X}$ ± SD	Mus. per g fish weight	*n* fish in total	Weight-normalized glo. loss (%)	Start - end of excystment (dd)	Temperature $\bar{X}$ ± SD (°C)	Fish mortality (% and total values)
P. fluviatilis	Good	186.0	29.8	21	262.0	26.3	16	12	132.3 ± 8.5	15.3	13	49	264-636	15.5 ± 1.2	10 (2)
L. idus	Good	156.0	19.4	24	25.0	3.2	22	84	67.5 ± 4.1	8.6	20	56	220-628	15.7 ± 1.3	0 (0)
S. trutta	Good	704.0	23.5	15	486.0	26.7	14	0	174.7 ± 3.2	8.3	11	65	243-998	12.8 ± 0.5	13 (2)
G. aculeatus	Good	13.2	8.3	59	14.0	10.0	43	0	9.5 ± 0.3	7.1	7	14	277-799	16.3 ± 1.1	57 (34)
*C. idella**	Good	26.0	5.1	47	5.5	1.6	45	69	9.1 ± 0.4	2.4	40	53	265-686	15.6 ± 1.2	6 (3)
L. delineatus	Poor	2.0	5.4	75	0	0	49	100	0.4 ± 0.03	1.1	3	80	259-421	16.2 ± 1.4	44 (34)
G. gobio	Poor	15.5	0.8	21	1.0	0.1	19	88	1.8 ± 0.2	0.1	17	87	216-447	15.4 ± 1.3	0 (0)
R. rutilus	Poor	2.5	0.4	42	3.5	0.6	40	0	0.6 ± 0.04	0.1	38	75	270-445	15.9 ± 1.2	0 (0)
*P. parva**	Poor	2.0	0.2	41	0.5	0.1	39	50	0.5 ± 0.04	0.1	37	50	296-468	15.6 ± 1.4	2 (1)
R. amarus	Not host	0	0	49	NA	NA									

3.4.2 Suitability of non-native fish species

Surprisingly, the invasive fish species *C. idella* turned out to be one of the best five host species for *A. cygnea* larvae. On this non-native species, a mean number of 9.1 excysted juvenile mussels per fish and 2.4 excysted mussels g^{-1} developed successfully until the end of the experiment. Thus, *C. idella* had significantly higher numbers of dropped-off living mussels than some of the native host species (e.g. *G. gobio*; post hoc pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.05$). The initial infestation of the grass carp revealed an average of 5.1 glochidia g^{-1}. This resulted in a weight-normalized glochidial loss of 53% (Table 3.2). Only 6% of the grass carp died before the end of the metamorphosis phase of the juvenile mussels. In contrast, the second invasive species in the experiment (*P. parva*) was a highly unsuitable host for the glochidia of *A. cygnea* (average number of 0.5 excysted mussels per fish and 0.1 mussels g^{-1}) (Table 3.2). The topmouth gudgeon had the poorest initial infestation rate, with only 0.2 larvae per gram fish weight (Table 3.2).

3.4.3 Duration of larval development

The duration of the metamorphosis phase varied significantly between all host fish species (post hoc pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$). The number and percentage of excysted juvenile mussels over time (in degree-days) of the five best host fish species is given in Fig. 3.2. The first completely metamorphosed and viable juvenile mussels were detected in the tanks of *L. idus* after 14 days. The shortest developmental time from larvae to juvenile mussels, regarding the time and the temperature of the water, was observed on gudgeon (first juvenile mussels after 216 degree-days) and ide (after 220 degree-days). The most synchronized metamorphosis (i.e. the shortest period between the drop-off of the first fully metamorphosed juvenile mussel and the last one) occurred in tanks of the moderlieschen (162 dd). In contrast, the longest time span of excystment (755 dd) was observed on brown trout, exceeding the time period of the shortest drop-off period by a factor of 4.6 (Fig. 3.2), being significantly different from all other fish species (post hoc pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.05$). Despite of the lowest mean water temperature in the *S. trutta* treatment (minimum: 12.0°C, maximum: 14.0°C, average: 12.8°C), drop-off in relation to degree-days started earlier than in six other hosts which excludes that the extended drop-off period can be attributed to a delayed development at colder temperatures.

The release patterns of juvenile mussels over time were highly variable between the host species. Some of them lost a high number of fully metamorphosed juveniles within one day (e.g. perch 58 juveniles per fish on one day), whereas some had several smaller peaks of juvenile mussel excystment over a longer time span (e.g. brown trout) (Fig. 3.2A). Regarding the percentage of excysted juvenile mussels over the time, 50% of fully developed juveniles on the ide were already released after around 250 dd (Fig. 3.2B). In contrast, perch, grass carp, stickleback and brown trout reached the value of 50% completely developed juvenile mussels after around 350 dd, 359 dd, 400 dd and 410 dd (Fig. 3.2B), respectively. No significant correlation was found between the time point (in degree-days) and the number of the first excysted juvenile mussels (per gram fish weight), the time point when 50% of juvenile mussels where excysted and the respective number of juveniles, as well as the time point when 100% of juveniles where excysted and the number of juveniles (always regarding the data of the best five host fish species, data not shown).

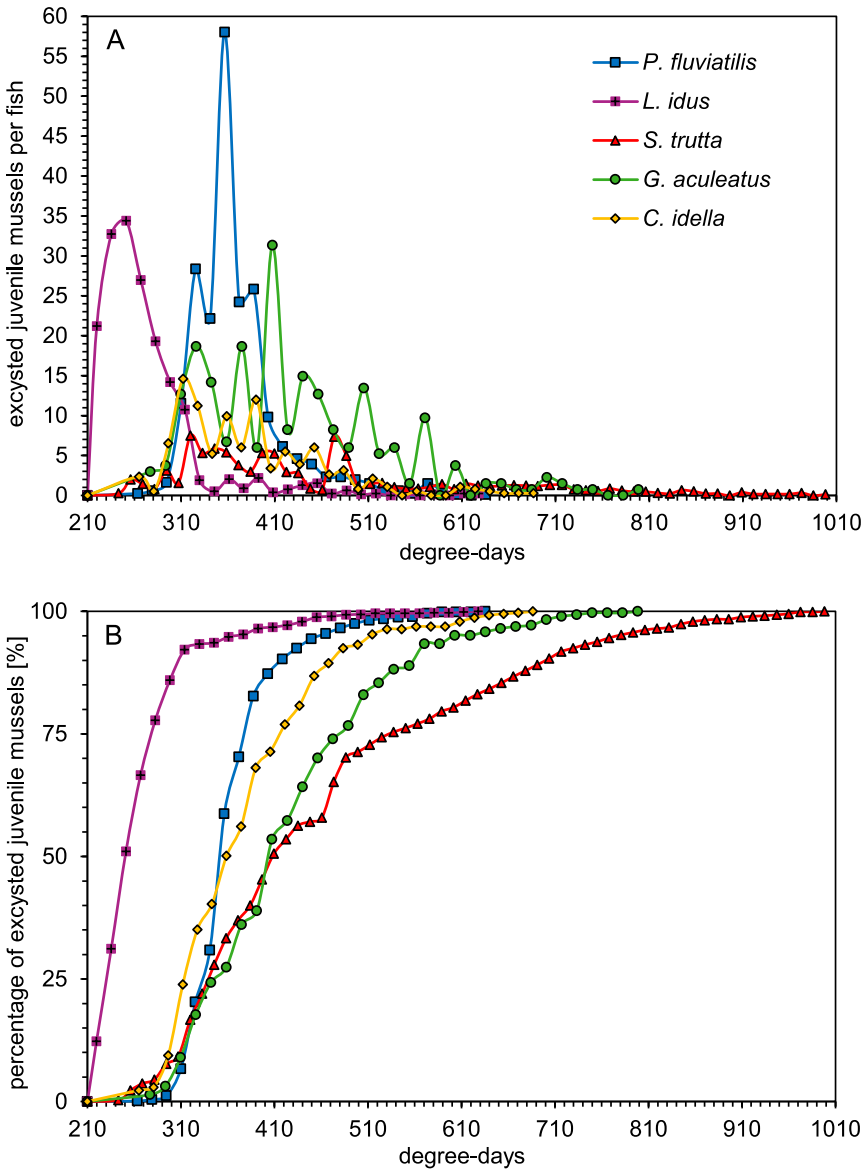

Figure 3.2: Duration of the metamorphosis phase of the best five host fish species: (A) Number of excysted juvenile mussels per fish over the time in degree-days (sum of daily water temperatures, dd). (B) Cumulative percentage of excysted juvenile mussels over the time in degree-days.

3.4.4 Attachment of glochidia

Attached glochidia could be found on the gills, all fins, the area of the mouth (inside and outside) and the skin (whole body surface including eyes, excluding fins). Regarding the five best host fish species for *A. cygnea* glochidia, the total number of attached glochidia differed significantly between all tissues 2 days pi (post hoc pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$) as well as 14 days pi (non-parametric Kruskal-Wallis sum of rank test,

$p < 0.05$) (Fig. 3.3). After 2 days, the gills had a significantly higher number of infested glochidia than all other body areas (post hoc pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.05$). In total, 1174 larvae were detected on the gills of perch, ide, brown trout, stickleback and grass carp 2 days pi (Fig. 3.3A). The second most common tissue for larval attachment after 2 days was the mouth (lips and pharynx).

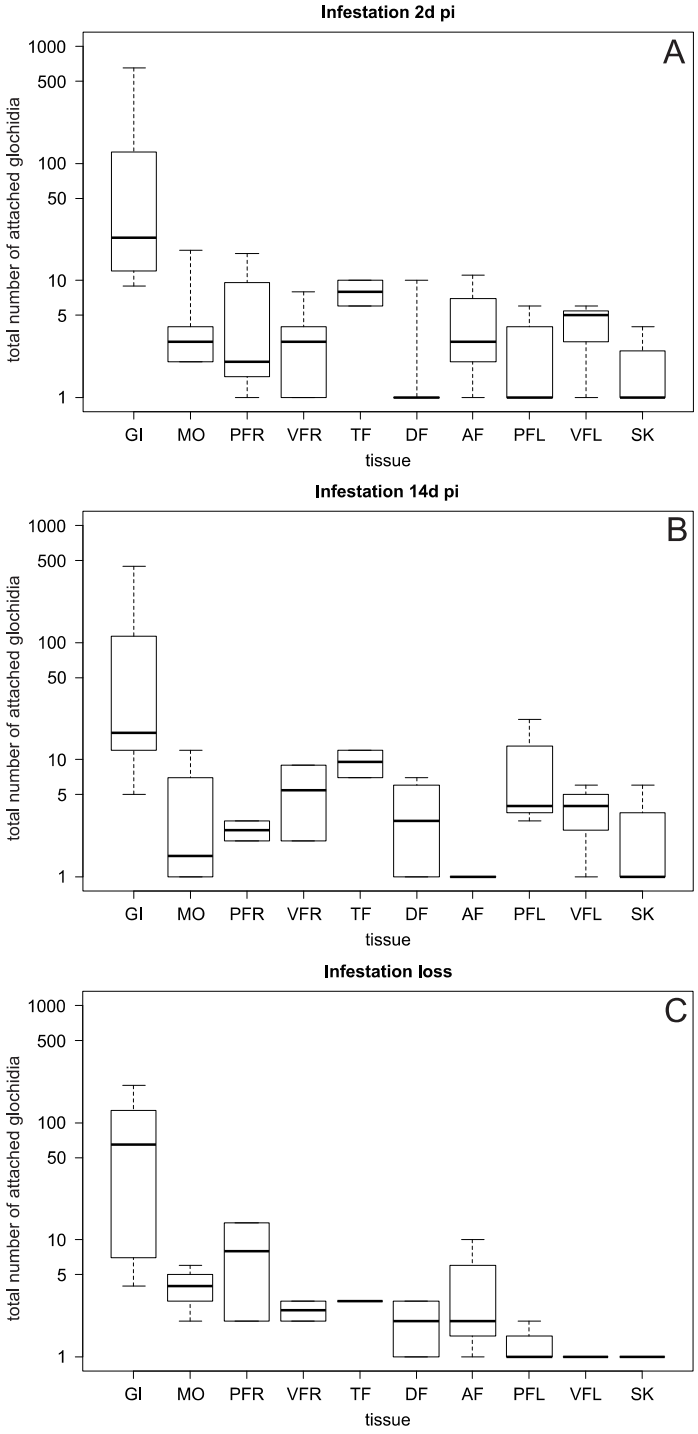

Figure 3.3: Glochidial attachment on tissues of the best five host fish species at different time points: (A) Total number of attached glochidia (logarithmic scale) per tissue 2 days pi; (B) total number of attached glochidia (logarithmic scale) per tissue 14 days pi; (C) total number of attached glochidia (logarithmic scale) per tissue, infestation loss between 2 and 14 days pi. GI = gills, MO = mouth (outside = lips and inside = pharynx), PFR = right pectoral fin, VFR = right ventral fin, TF = tail fin, DF = dorsal fin, AF = anal fin, PFL = left pectoral fin, VFL = left ventral fin, SK = skin. Boxes are 0.75 and 0.25 percentiles and medians.

A number of 29 attached glochidia could be found on the lips and the pharynx over the five best fish species. In contrast, the skin was the surface with the lowest total number of attached larvae

2 days pi (only seven glochidia in total) (Fig. 3.3A). These findings were confirmed after 14 days, when the gills were again the most infested area. At that point of sampling, 722 glochidia were encysted on the tissue of the gills of the five best host fish species (Fig. 3.3B). The lowest number of encysted larvae 14 days pi was detected at the anal fin (only two encysted larvae in total) (Fig. 3.3B). Comparing the larval attachment at the single tissues of the best five host fish species between 2 days pi and 14 days pi, there was a loss of encysted glochidia at almost all body areas (Fig. 3.3C). The gills and the right pectoral fin lost most attached larvae between 2 and 14 days pi with an average loss of 14.5% (gills) and 2.1% (right pectoral fin) encysted larvae (Fig. 3.3C). The two tissues with the lowest number of attached glochidia (skin and left ventral fin), also had the lowest numbers of lost glochidia between 2 and 14 days pi (only one (skin) and two (left ventral fin) larvae were lost from the best five host fish species) (Fig. 3.3C).

3.5 Discussion

This study provides first insight into the host-larvae relationship between *A. cygnea* glochidia and host fish species by simultaneous infestation of ten different fish species including native and non-native species, comparing the duration of the larval phase and determining glochidial infestation at different body parts of a fish. The results of this study confirm our hypothesis that *Anodonta cygnea* can clearly be considered a host generalist since nine out of the ten tested fish species were suitable hosts. However, the highly different timelines of metamorphosis and the different degrees of synchronization in larval drop-off indicate a high degree of complexity and evolutionary consequences in the use of different host species. The consistently observed differences both in initial infestation rates as well as in metamorphosis success suggest a classification of hosts into “good hosts” (five tested host fish species), “poor hosts” (four tested host fish species) and “not hosts” (one tested host fish species), similar to the host classification system in *U. crassus* (Douda et al., 2012a; Stoeckl et al., 2015; Taeubert et al., 2012a, b). The finding that two non-native fish species were identified as suitable hosts for the glochidia of *A. cygnea*, one of them even as a “good host” (*C. idella*) illustrates that the generalization that non-native species are a threat to native mussel communities does not always hold true. The development times of the larvae differ extremely between the single host fish species concerning duration of the metamorphosis phase and the time span of juvenile mussel excystment.

In line with the findings of this study, *A. cygnea* was formerly proposed as host fish generalist by Bauer (2001b) and Wächtler et al. (2001). This result was expected since the entire genus *Anodonta* is described to generally have a very broad host range (Bauer, 2001b; Lopes-Lima et

al., 2017; Trdan and Hoeh, 1982). The most likely explanation for this host generalism is the broad occurrence of *A. cygnea* in diverse habitats (natural lakes and artificial fish ponds as well as slow-flowing rivers) where completion of the life cycle is only possible if different hosts with adaptations to different flow and temperature conditions can be used. As demonstrated in our study, even species from different families with entirely different temperature and flow preferences such as salmonids (high flow preference, cold water species), cyprinids (warm water species) and different spawning requirements (percids as vegetation-spawners, salmonids as gravel-spawners) were found to be suitable hosts. The chance of glochidia attaching to a suitable host increases with a broad host range and only requires the production of a relatively small number of larvae (Bauer, 1994). On the other extreme, the larvae of the freshwater pearl mussel are very specialized according to their host range, using only two different host fish species in Europe (*Salmo trutta* and *S. salar* L.) (Salonen et al., 2016; Taeubert et al., 2010; Young and Williams, 1984) with a preference for either one species in one area (Geist et al., 2006; Ieshko et al., 2016; Karlsson et al., 2014; Taeubert and Geist, 2017). These observed differences in host fish specialization between *A. cygnea* and *M. margaritifera* are logical, given that the latter species exclusively occurs in oligotrophic streams where fish species richness is generally very low and where rheophilic salmonids typically comprise the majority of individuals and biomass (Bauer, 1992; Geist et al., 2006). Since the chances of glochidia attaching to a suitable host are extremely low in these habitats, *M. margaritifera* produces a high number of small glochidia with a very specific adaption to its host fish species (Bauer, 1994; Bauer, 2001b). The larvae then parasitize on a host fish for a long time using a maximum of its resources for growing and metamorphosing (Bauer, 1994). *Anodonta cygnea* reveals the opposite strategy with a broad host use and a comparatively short development time on the host.

Although the larvae of *A. cygnea* use a broad range of hosts, highly different initial infestation rates and different metamorphosis success were evident as previously also described for *U. crassus* (Douda et al., 2012a; Stoeckl et al., 2015; Taeubert et al., 2012a, b). According to these authors, *Phoxinus phoxinus* (Linnaeus 1758) and *Squalius cephalus* (Linnaeus 1758), which sympatrically inhabit the same habitat as *U. crassus*, were mentioned as best host fish species for the thick-shelled river mussels in artificial infestation experiments. Interestingly, in the present study, host suitability assessments were consistent at the different time points, i.e. the same host suitability order was already detectable 2 days after the infestation which can greatly facilitate the future assessment of yet untested fish species. The finding that *P. fluviatilis* was the best host fish species for *A. cygnea* was expected from unpublished reports and theses by Claes (1987), Franke (1993) and Niemeyer (1993), but had to date and to the best of our knowledge

not been published in the peer-reviewed literature. Like the swan mussel, the perch occurs in a wide range of habitats colonizing rivers as well as standing water bodies and thus often co-occurs with mussel populations (Freyhof and Kottelat, 2008). The perch is considered to be a pioneer species that often appears among the first fish species in newly established waterbodies (Nesbø et al., 1999). From an evolutionary perspective, the use of ubiquitous perch by *A. cygnea* as a primary host fish may result in the greatest chances for dispersal between different habitats whereas more specialized fish species would not easily adapt or survive such transitions. On the other hand, specialized species can locally form much higher population densities, and the ability to use such hosts can thus also be beneficial for establishment and population growth. The observation that bitterling was completely unsuitable for the development of *A. cygnea* glochidia was surprising since the bitterling itself parasitizes the mussels by placing its eggs onto the gills of the mussel (Reichard et al., 2007; van Damme et al., 2007), resulting in a population overlap of both species. However, since the bitterling uses a wider range of unionids, the co-evolutionary contact likely resulted in effective defense mechanisms against encysted glochidia governed by the fish immune system (Mills and Reynolds, 2004; Smith et al., 2004). On the contrary, the mussels may not have been able to develop immunity against the bitterling despite its adverse effects on water filtration since the intrusion of bitterling does not result in any cellular harm that would be able to trigger an immune response (Aldridge, 1999).

The two non-native fish species *P. parva* and *C. idella* were integrated in the study due to their increasing expansion in many water bodies and their co-occurrence with populations of *A. cygnea* in the same habitat (Britton et al., 2008; Milardi et al., 2015). Previous studies suggested that invasive fish species have a lower suitability as hosts for freshwater mussels than native ones as confirmed in *M. margaritifera* (Salonen et al., 2016), *U. crassus* (Taeubert et al., 2012a) and *A. anatina* (Linnaeus 1758) (Douda et al., 2013). Such results were often generalized and lead to the assumption that the reproductive success of native freshwater mussels could be reduced if glochidia attach to less-suitable, invasive hosts (Lopes-Lima et al., 2017; Stoeckl et al., 2015). Instead, our data suggest that this generalization does not always hold true since the non-native fish species *C. idella* turned out to be one of the five best host fish species for *A. cygnea*. Similarly, the non-native river blenny was found to be an important host for endangered *Margaritifera auricularia* (Araujo and Ramos, 2000). Thus, a co-evolutionary mechanism of host compatibility between mussels and host fish species, as mentioned in Douda et al. (2013) for *A. anatina* and in Taeubert et al. (2012a) for *U. crassus*, can be excluded for *A. cygnea* and *M. auricularia*. Instead, the development of the glochidia on *C. idella* may be successful due to the fact that large, hooked larvae (in contrast to the hookless, very small glochidia of *M.*

margaritifera), which are already highly developed at the beginning of parasitism, probably already have left the host before its specific innate immune response is initiated (Bauer, 1994; Bauer, 2001b). Then only little selective pressure to adapt to a particular host fish species would be expected (Bauer, 2001b). An acquired cross-resistance of *C. idella* individuals to glochidia (described in Dodd et al., 2005) can be excluded for our dataset since only juvenile fish without previous mussel contact were used. From the perspective of *A. cygnea* conservation removal of non-native species such as *C. idella* is not essential from places where they co-occur with mussel populations, particularly if they do not naturally reproduce. Instead, it appears essential to include them and other non-native species into further host-suitability testings. A sampling of wild *C. idella* individuals in habitats, where they co-occur with *A. cygnea* populations, would be the next step to check for infestation with glochidia and thus to verify our results under natural conditions.

The duration of the larval development (metamorphosis phase and juvenile mussel excystment) differed strongly between the host fish species considering synchrony, degree-days, beginning and ending of the excystment. A variation in the developmental period of glochidia between different host fish species and even of different strains of host fish species was also observed in previous studies for the freshwater mussels *M. margaritifera* and *U. crassus* (Hochwald and Bauer, 1990; Taeubert et al., 2012a, b, 2013b, 2014). Under identical temperature conditions, the developmental period of *U. crassus* on the host fish species *S. cephalus* was ~ 25% extended compared to *P. phoxinus* (Taeubert et al., 2012b). In our study, the period of excystment of *A. cygnea* was ~ 69% extended on *G. aculeatus* compared to *L. delineatus* under identical temperature conditions. This variation in the development time occurred due to general differences regarding the host fish species (e.g. because of variations in genetic imprinting, Taeubert et al., 2010). In addition, the fact that glochidia encysted on different individuals of the same host fish species at the same time revealed highly variable excystment periods, can result from a variable suitability of different specimens as well as a variable suitability of encystment sites within one host (Marwaha et al., 2017; Taeubert et al., 2013b). For example, glochidia encysted on the gills might have a prolonged developmental time (as also mentioned for the hookless glochidia of *M. margaritifera*, which exclusively stay on the gills) than larvae encysted on other body parts of a host, due to different nutrition supply (Taeubert et al., 2013b). For the mussel, a variation in the duration of the parasitic phase extends the overall excystment period of the juveniles during one season (Taeubert et al., 2012b) and increases the probability of more widespread dispersal by the host fishes (Taeubert et al., 2014). Thus, a wide distribution enables the

survival of locally unfavorable conditions and reduces the competition for nutrients (Taeubert et al., 2012b).

Freshwater mussels are susceptible to factors that reduce the abundance and distribution of their host fish species (Taeubert and Geist, 2017). Therefore, conservation strategies for mussels such as *A. cygnea* also have to include conservation and management strategies for the hosts. Native host fish species that were identified during this study should be supported within the distribution range of the swan mussel, or at least not actively be removed from a water body if otherwise considered "undesired" or "useless" for eating or fishing. In addition to communicating knowledge on locally important fish hosts to fisheries managers, concrete measures of host support can include the removal of man-made migration barriers for species such as the ide (Kuliskova et al., 2009), or other means of habitat restoration (Geist and Hawkins, 2016). For instance, improving the spawning habitats for species such as the perch is rather easy since the species greatly benefits from provision of tree branches and dead wood during spawning and juvenile development (Treasurer, 1981). Such structures can be introduced into its habitat without great effort and cost. Fish stocking should only be seen as an emergency measure in specific situations, e.g. after a fish die-off, or for the initial colonization of newly established habitats, since possible evolutionary consequences of changing fish communities can exert selective pressures on the mussel population, change the time patterns of metamorphosis and drop-off, and may also affect other species and ecosystem services. The finding that many fish species can act as hosts, and the observation of differences, e.g. in synchrony and timing of metamorphosis and excystment suggest that one host species cannot simply be exchanged by another. Instead, maintaining host availability and diversity should be at the core of conservation.

Generally, possible limitations of translating results on host suitability from the laboratory to natural conditions must be considered (Taeubert et al., 2013a). Standardized laboratory experiments are a very important, but only a first step of determining which fish species can act as host for *A. cygnea*. An evaluation of freshwater mussel-host relationships also requires the integration of host and mussel behavior in the wild, where differences in the suitability of hosts can exist (Taeubert et al., 2013a) as previously described for *M. margaritifera* (Geist et al., 2006; Karlsson et al., 2014; Taeubert and Geist, 2017). For instance, exposure to glochidia can be affected by species-dependent degrees of stress during the bath infestation, particularly since stress influences ventilation rate (Mikheev et al., 2014), and in turn exposure to glochidia. Since the gills were the main site of infestation, as clearly shown by the present study, it is reasonable to assume that ventilation rate has a major influence on the number of glochidia attached.

Additionally, the genetic diversity and differentiation of *A. cygnea* is not yet well understood (Geist et al., 2010), and differences in the host suitability among different evolutionary significant units (ESU) may occur. Different host suitability of different fish strains for the same mussel population has already been described for *M. margaritifera* (Taeubert et al., 2010) and for *U. crassus* (Taeubert et al., 2012b). Similarly, differences in the host use by different ESUs within a mussel species could occur and should be considered before translating laboratory-derived results to the management of wild populations of mussels and fish. Also, it remains unclear if the comparatively high mortality rate, particularly in *G. aculeatus*, *L. delineatus*, *S. trutta*, and *P. fluviatilis* observed in the laboratory is different from a situation in the wild. Consequently, a logical next step would be the evaluation of host suitability in the wild. A combination of such results with the findings of this laboratory study will result in a reliable picture of host suitability of *A. cygnea*.

3.6 Conclusions

Although the freshwater mussel *A. cygnea* is confirmed a host generalist, the results of our study indicate that there are strong differences in the suitability of different host fish species in terms of metamorphosis success, as well as duration and synchrony of excystment. Thus, changes in fish communities, e.g. resulting from habitat change and fisheries management, are still likely to affect the recruitment success of *A. cygnea*. The previously postulated co-evolutionary distribution patterns of mussels with their primary host fishes was only confirmed for *P. fluviatilis* which co-occurs in most of the diverse mussel habitats. However, the finding that also non-native species with originally non-overlapping distribution ranges such as *C. idella* were found to be good hosts suggests that the current generalization that non-native fish species pose a threat to native mussel species does not hold true in this specific case. Although an evaluation of the results of the laboratory experiments in the wild is not yet performed, this study generates important information about the host-glochidia relationship of *A. cygnea*. In summary, species-specific host assessments are a key to both understanding the ecology and conservation of endangered populations and species of freshwater mussels.

4 Host fish status of native and invasive species for the freshwater mussel *Anodonta anatina* (Linnaeus, 1758)

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Author contributions:

VH and JG conceived and designed the study. VH sampled the mussels, performed the genetic analysis and arranged and performed the laboratory experiment. VH and JG discussed the data analysis and manuscript structure. VH analyzed the data and wrote the manuscript. JG improved and edited the manuscript.

4.1 Abstract

The worldwide extinction of species especially affects freshwater ecosystems. Even widespread species like the European freshwater duck mussel *Anodonta anatina* face population declines in many countries and regions. Due to an obligate parasitic phase in its life cycle, knowledge on host fish use is essential for effective conservation of *A. anatina*. Therefore, in this study host suitability of ten different fish species (native and invasive to Europe) from four different fish families was tested by simultaneously infesting them with the glochidia of *A. anatina*. Nine out of ten fish species were identified as suitable hosts, but infestation rates, duration of metamorphosis phase as well as duration and rate of juvenile mussel excystment differed significantly between all host species. The bitterling (*Rhodeus amarus*) was the only fish species with no juvenile mussel excystment. Surprisingly, one of the tested invasive fish (*Ctenopharyngodon idella*) turned out to be the second best host for the larvae of *A. anatina*, suggesting that the general assumption that non-native fishes would be a threat to native mussel populations no longer holds true. Compared to the second native *Anodonta* species in Europe (*Anodonta cygnea*), this study revealed that *A. anatina* had higher infestation rates and rates of juvenile mussels excystment as well as a different host compatibility than *A. cygnea*. These findings illustrate that species-specific assessments of host suitability form an urgent basis for evidence-based conservation and restoration of freshwater mussel populations and the ecosystem services they provide.

4.2 Introduction

Freshwater mussels can constitute > 90% of the benthic biomass of some rivers (Lopes-Lima et al., 2017; Negus, 1966) and are important components of water ecosystems (Vaughn, 2018; Vaughn and Hakenkamp, 2001). Because of their various impacts on fresh waters for example due to filter-feeding, as well as their effects on substrate properties due to bioturbation (Boeker et al., 2016; Geist, 2011; Lummer et al., 2016; Richter et al., 2016; Vaughn and Hakenkamp, 2001), mussels are often described as ecosystem engineers (Gutiérrez et al., 2003; Lopes-Lima et al., 2017; Nobles and Zhang, 2011). Their key role in aquatic ecosystems is also supported by observations of higher diversity of macroinvertebrates associated with higher bivalve densities (Aldridge et al., 2007; Vaughn and Spooner, 2006), although such effects can be less pronounced under anthropogenic impact (Richter et al., 2016).

Given that 44% of the freshwater mussel species worldwide are classified as Near Threatened or Threatened in the 2015 IUCN Red List of Threatened Species (Lopes-Lima et al., 2017), knowledge on their habitat requirements including host fish use are essential prerequisites for their sustainable and effective conservation management. In Europe, conservation has focused mostly on the freshwater pearl mussel *Margaritifera margaritifera* and the thick-shelled river mussel *Unio crassus*, with much less attention having been paid to the other European species, like the duck mussel *Anodonta anatina*, for which little information on its ecological requirements is available (Lopes-Lima et al., 2017).

Anodonta anatina has a large geographical distribution range which includes almost all parts of Europe and even part of Asia (Lopes-Lima, 2014a). It can be found from fast-flowing streams to lentic habitats, often in co-occurrence with its sister species *Anodonta cygnea* (Niemeyer, 1993). *Anodonta anatina* is considered a species with relatively high plasticity and tolerance to different abiotic conditions (Zettler et al., 2006; Zieritz and Aldridge, 2011). Like other *Anodonta* species, the duck mussel is a long-term brooder, keeping the glochidia larvae over winter and releasing them from late winter/early spring (Graf and Cummings, 2007; Hinzmann et al., 2013). Because of their large and strong hook at the shell, the glochidia of the duck mussel are able to attach not only on the gills, but also on the fins and other body parts of a host fish, which is in stark contrast to other freshwater mussel species like *M. margaritifera* (Bauer, 2001b; Jansen et al., 2001). Although *A. anatina* has been assessed as Least Concern in Europe (IUCN Red List of Threatened species, Lopes-Lima, 2014a), the current population trend is decreasing and there have been local declines in several countries and regions over the last decades (Lopes-Lima, 2014a). The species is already considered Vulnerable in Austria (Reischütz and

Reischütz, 2007) and Romania (Sárkány-Kiss, 2003) and Near Threatened in Spain (Verdú et al., 2006). In Germany the duck mussel is protected under the BArtSchV scheme (Bundesartenschutzverordnung (BArtSchV, 2005). In the German region of Bavaria, *A. anatina* is listed as threatened in the Bavarian Red List of Endangered species (StMUV, 2005).

One of the major threats for freshwater mussel species is the loss of suitable host fish populations (Geist, 2010; Lopes-Lima et al., 2017; Modesto et al., 2018; Stoeckl et al., 2015; Taeubert et al., 2012a), but it is not known whether this also holds true for *A. anatina*. Thus, to suspend the ongoing decline of freshwater mussels, knowledge about the host fish preference of mussel species is important for management and conservation of existing populations as well as for artificial breeding projects (O'Dee and Watters, 1998; Taeubert et al., 2013a). There is detailed knowledge about host fish preference on the highly endangered European freshwater mussels *Margaritifera margaritifera*, *Margaritifera auricularia* and *Unio crassus* due to standardized analyses in peer reviewed literature (Araujo and Ramos, 2000; Geist et al., 2006; Lopez et al., 2007; Taeubert et al., 2010, 2012b), but only little information about the endangered Anodontines, except for a standardized experiment in *A. cygnea* (Huber and Geist, 2017).

The wide geographical distribution of *A. anatina* suggests that it is capable of using a wide range of fish species as hosts for larval metamorphosis (Wächtler et al., 2001). Recent studies in *A. anatina* pointed that only native fish species were good hosts indicating that the changes in the fish fauna are additional threats for local populations (Douda et al., 2013). In general, invasive fish species are almost always being mentioned as a threat for native freshwater mussels due to the displacement of native fish and their unsuitability as hosts for the mussels (Douda et al., 2013; Geist, 2011; Lopes-Lima et al., 2017; Modesto et al., 2018; Strayer, 2006). However, some invasive fish species turned out to be very good hosts for native freshwater mussels as mentioned for *Margaritifera auricularia* and its important host fish the invasive river blenny (*Salaria fluviatilis*) (Bragado Alvarez et al., 2001). Huber and Geist (2017) provided the first indications that non-native species can also be very suitable hosts for native mussels (for example the tested species *Ctenopharyngodon idella* for the glochidia of *A. cygnea*) and others can be very unsuitable ones (for example the tested species *Pseudorasbora parva* for the larvae of *A. cygnea*).

The major aim of this study was to clarify the suitability of different host fish species, native and invasive ones, for glochidial attachment and development of the duck mussel in a standardized laboratory experiment. In particular, the following hypotheses were tested: (1) The larvae of the mussel species *Anodonta anatina* have a very broad host range (Bauer, 2001b; Niemeyer, 1993; Lopes-Lima et al., 2017), but suitable host fish species are not equally infested

and differ in duration and success of metamorphosis as well as in duration and success of juvenile mussel excystment as previously shown for the related species *Anodonta cygnea* (Huber and Geist, 2017). (2) There is no successful metamorphosis of the glochidia of *A. anatina* on non-native fish species and thus the presence of invasive fish reduces the availability of the mussel's hosts as suggested by Douda et al. (2013) for *A. anatina*. (3) The larvae of the freshwater mussel *A. anatina* are more adaptive and have higher infestation and developmental rates than the larvae of *A. cygnea*. This is because *A. anatina* has a much wider distribution and can occupy a wider variety of habitats compared to its closely related species *A. cygnea* (Lopes-Lima et al., 2017).

4.3 Materials and methods

The standardized host suitability experiment was carried out following formerly described methodologies used for the freshwater mussel *Anodonta cygnea* in Huber and Geist (2017), following the experimental standards proposed by Taeubert et al. (2012b). Because of the similarity of both *Anodonta* species and to ensure comparability of results, the same experimental approach and methodology was used throughout, but both studies were performed separately at different times. Glochidia for the experiment with the duck mussel were obtained from five adult *A. anatina* from a private pond in Rehau village (Bavaria, Germany) on the 27th September 2014. The adult specimens were genetically validated as *A. anatina* following the species determination key in Zieritz et al. (2012) before using their glochidia for the experiment. Fully developed larvae were detected on the 19th January 2015 and flushed out of the marsupia with a squirt bottle. Collected glochidia were stored at 4.0°C until the start of the infestation experiment on 20th January 2015. A total amount of ~ 500 000 larvae were harvested with a viability (measured as clamping response to NaCl stimulus) of > 95%.

The selection of the ten different fish species used for the infestation procedure as well as the whole infestation procedure itself closely followed the descriptions in Huber and Geist (2017). The tested fish included eight native fish species from the families Salmonidae (*Salmo trutta*, Linnaeus, 1758), Cyprinidae (*Leuciscus idus*, Linnaeus, 1758; *Gobio gobio*, Linnaeus, 1758; *Rhodeus amarus*, Bloch, 1782; *Rutilus rutilus*, Linnaeus, 1758; *Leucaspius delineatus*, Heckel, 1843), Percidae (*Perca fluviatilis*, Linnaeus, 1758) and Gasterosteidae (*Gasterosteus aculeatus*, Linnaeus, 1758) and the two non-native species *Ctenopharyngodon idella* (Valenciennes, 1844) and *Pseudorasbora parva* (Temminck and Schlegel, 1846) (Huber and Geist, 2017). Information about the tested fish species, their origin and number of individuals per species used for

the experiment are given in Table 4.1. Only juvenile fish (< 1 year, trout < 2 years) with no previous contact to unionid mussels were used. A total number of 467 fish from ten different species were simultaneously infested with the glochidia of five adult *A. anatina* for 30-45 min. The concentration of glochidia in the infestation bath add up to 8500-9000 larvae per liter at a temperature of ~ 12.0°C (laboratory water: bank filtrate, river Moosach). In total 149 individuals of all fish species formed the group of control-specimens (not exposed to glochidia) to analyze if influences like handling or holding conditions or stress are responsible for the mortality during the experiment. Every fish species had one separate group of control fish that was treated in the same way as the infested one.

Table 4.1: Tested fish species (order from fish species with the highest number of excysted juvenile mussels after weight-normalization to species with the lowest number of excysted juveniles after weight-normalization), their status, place and date of origin: RO = Aquaculture M. Rösch, Bärnau, 04.11.2014, AS = Chair of Aquatic Systems Biology, 27.11.2012, LU = Bavarian State Office for Environment, Wielenbach, 12.11.2014; mean size (total length) and weight of applied fish, number of fish in the infestation bath per species, number of control fish per species; number of excysted juvenile mussels per gram fish weight and species; $\overline{X}$ = arithmetric mean.

Species	Status	Source	Size $\overline{X}$ ± 1 mm	Weight $\overline{X}$ ± 0.1 g	Nb. of fish infestation bath	Nb. of control fish	Mussels per g fish weight
Perca fluviatilis (perch)	Native	RO	100	9.6	24	8	19.9
Ctenopharyngodon idella (grass carp)	Introduced	LU	73	3.1	45	15	14.0
Leuciscus idus (ide)	Native	RO	101	8.2	24	8	11.2
Salmo trutta (brown trout)	Native	AS	126	20.4	18	6	11.2
Leucaspius delineatus (moderlieschen/sunbleak)	Native	RO	37	0.3	103	30	10.6
Gasterosteus aculeatus (stickleback)	Native	RO	53	0.9	60	20	5.3
Gobio gobio (gudgeon)	Native	RO	115	11.8	21	7	1.6
Rutilus rutilus (roach)	Native	RO	87	6.3	47	15	0.9
Pseudorasbora parva (topmouth gudgeon)	Introduced	RO	44	0.6	65	20	0.3
Rhodeus amarus (bitterling)	Native	RO	65	3.1	60	20	0.0

Funnel-shaped holding units with a maximum volume of 45L for keeping the fish after the infestation (separated by species) until the excystment of juvenile mussels, as previously used by Huber and Geist (2017), were applied. After the infestation the individuals of every fish species were separated and divided into three groups to get three replicates of holding units per species. The average water temperature within these holding units was 11.5 ± 0.5°C during the experiment (measured with temperature loggers every 30 min, Lascar Electronics Limited, Salisbury, UK). To account for differences in fish sizes among different species and to ensure optimal holding conditions due to different requirements of the single fish species for example on temperature and oxygen content of the water, the number of specimens per tank was adjusted

accordingly (Huber and Geist, 2017). Thus, from smaller species like *L. delineatus* or *G. aculeatus* naturally living in lakes or artificial ponds a higher number of individuals was applied per holding unit than from species with a bigger size naturally living in fast flowing streams like *S. trutta*. Furthermore, for holding units containing brown trout a constant flow of water was ensured. The average water temperature within the holding units containing brown trout was 11.7°C during the experiment.

To determine the infestation success, attached glochidia on fins, gills and skins of fish specimens were counted after 2 days (22nd of January), 23 days (12th of February) and at the end of the excystment (until the 28th of April) (Huber and Geist, 2017). The second time point for counting attached glochidia (23 days post infestation (pi)) was used as backup for the scenario that no fish would have survived until the end of the experiment. Thus, larval attachment after 2 days and at the end of the excystment was used for calculation of metamorphosis success. The number of sacrificed individuals per fish species to calculate the glochidia attachment depended on the amount of living fish per species and holding unit at each of the three time points. In total, a number of 47 specimens (37 infested and 10 non-infested/control individuals) was sacrificed after 2 days, a number of 26 individuals (17 infested and 9 non-infested/control individuals) was sacrificed after 23 days and a number of 41 specimens (27 infested and 14 non-infested/control individuals) was used for calculation of the infestation success at the end of the experiment. Because of differences in weight between different fish species and individuals, total weight (± 0.1g) of each single specimen was determined to calculate the weight-normalized numbers of glochidia and juvenile mussels per fish (Huber and Geist, 2017; Taeubert et al., 2010).

On 12th February, the first fully developed juvenile mussels were detected in holding units of trout. To determine the status of dropped-off glochidia and juvenile mussels, five liters of every tank were analyzed during daily water change (12.5% daily renewal of water) (Huber and Geist, 2017). Living juveniles were directly transferred to aquaria and special flow-through systems (Feiner et al., 2016). At the 28th April 2015 (after 14 weeks) the experiment was terminated, three days after the last juvenile mussel was detected.

Statistical analyses were performed in R version 3.4.3 (R Core Team, 2017). To calculate differences in weight-normalized glochidial infestation rates, as well as differences in weight-normalized mussel excystment, differences in the duration of the metamorphosis phase and duration of juvenile mussel excystment between all host fish species, non-parametric Kruskal-Wallis sum of rank tests and post hoc pairwise Wilcoxon rank sum tests were used since ANOVA assumptions were not fulfilled. Bonferroni correction was applied to correct for multiple

testings. To compare the duration of the metamorphosis phase at different temperatures, the concept of degree-days (the sum of daily water temperatures, dd) was applied (Taeubert et al., 2014). For testing the differences between the two mussel species *Anodonta anatina* and *Anodonta cygnea* (regarding infestation, mortality rates, excystment and duration of metamorphosis phase) again pairwise Wilcoxon rank sum tests with Bonferroni correction were used because of ANOVA assumptions not being fulfilled. For calculation of the correlation between the initial infestation rate and the duration of metamorphosis and juvenile mussel excystment as well as the correlation between the weight of applied fish (infestation bath) and the initial infestation rate per gram fish weight, Pearson´s product-moment correlation was used. Spearman´s rank correlation was applied for the calculation of the correlation between the number of excysted juvenile mussels per gram fish weight and the mortality rate of the fish.

4.4 Results

The glochidia of *A. anatina* developed successfully on nine out of ten tested fish species, matching the expectation of a broad spectrum of host fish suitability. The only fish species with a low initial larval attachment and no juvenile mussel excystment was the bitterling (*R. amarus*), which in turn parasitizes the mussels to lay its eggs and protect its juveniles. Due to no excystment of juveniles, *R. amarus* was excluded from all following calculations. In total, a number of 7431 juvenile mussels developed successfully on the nine host fish species. Despite of the same infestation conditions, a great variability in initial larval attachment, number of excysted juvenile *A. anatina* following metamorphosis, and in duration of the metamorphosis between host fish species were observed.

4.4.1 Success of infestation and juvenile mussel excystment

The initial larval attachment and the excystment of juvenile mussels differed significantly between all tested fish species (non-parametric Kruskal-Wallis sum of rank tests, $p < 0.001$). The highest larval attachment 2 days pi was found on brown trout with an average number of 43.8 glochidia per gram fish weight. The lowest initial glochidial load was found on bitterling with only 0.1 larvae per gram fish weight (Table 4.2). The most successful development of juvenile mussels was detected on *P. fluviatilis* with a mean number of 19.9 mussels per gram fish weight (Table 4.2). Regarding the suitability of host fish species, there was a highly significant difference between a group of five very good host fish species and a second group of poor hosts for

the glochidia of *A. anatina*. This difference was already visible 2 days pi (pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$). The fish species *P. fluviatilis*, *C. idella*, *L. idus*, *S. trutta* and *L. delineatus* had the highest juvenile mussel excystment with an average of 13.4 mussels per gram fish weight (Table 4.2). In contrast, *G. aculeatus*, *G. gobio*, *R. rutilus* and *P. parva* had a low juvenile mussel drop-off with an average of 2.0 mussels per gram fish weight. The variation in juvenile mussel excystment between the group of the best five hosts and the group of poor hosts was also highly significant (pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$). In total, the weight-normalized glochidial loss between the initial larval attachment and the number of totally developed juvenile mussels was 55%, with *P. parva* showing the highest glochidial loss (96%) and *C. idella* showing the lowest glochidial loss (22%) (Table 4.2).

Table 4.2: Results of the host fish infestation with larvae of *Anodonta anatina*: fish species, mean number of glochidia per gram fish weight 2 days post infestation (pi) as well as excystment rate of juvenile mussels per gram fish weight and fish species and percentage of weight-normalized glochidial loss between initial infestation and excystment, start and end of ecystment per species in degree-days (dd), temperature and host fish mortality (fish that died naturally) during the time span of mussel excystment (mortality is given in percentage and total number of dead fish in brackets); species order: from fish species with the highest number of excysted mussels after weight-normalization to fish species with the lowest number of excysted mussels; * = introduced, non-native fish species; $\overline{X}$ = arithmetric mean.

Fish species	Host status	2 days pi	Excystment				
		Glochidia per g fish weight	Mussels per g fish weight	Weight-normalized glochidial loss (%)	Start - end of excystment (dd)	Temperature $\overline{X}$ ± SD (°C)	Fish mortality (% and total values)
P. fluviatilis	Good	36.4	19.9	45	272-965	11.4 ± 1.4	50 (12)
*C. idella**	Good	18.0	14.0	22	291-1117	11.6 ± 1.6	38 (17)
L. idus	Good	20.7	11.2	46	289-1120	11.6 ± 1.7	29 (7)
S. trutta	Good	43.8	11.2	74	268-955	11.7 ± 0.2	22 (4)
L. delineatus	Good	18.3	10.6	42	358-809	11.6 ± 1.1	62 (64)
G. aculeatus	Poor	19.8	5.3	73	398-1048	12.1 ± 1.3	13 (8)
G. gobio	Poor	2.1	1.6	23	384-936	11.3 ± 1.2	0 (0)
R. rutilus	Poor	3.3	0.9	74	281-912	11.7 ± 0.9	83 (39)
*P. parva**	Poor	7.2	0.3	96	447-530	10.4 ± 0.9	11 (7)
R. amarus	Not host	0.1	0.0	100			

Analogously to the differences in host suitability, there were also pronounced differences in development times of glochidia on different host species (Fig. 4.1). The duration of juvenile mussel excystment ranged between 268 degree-days (first juvenile mussel drop-off 23 days pi, trout) and 1120 degree-days (last juvenile mussel drop-off 97 days pi, ide) (Fig. 4.1A), with *S. trutta* showing the fastest metamorphose phase and duration of juvenile mussel excystment (Fig. 4.1B). There was a highly significant difference between all host fish species and the duration of juvenile mussel drop-off regarding degree-days (non-parametric Kruskal-Wallis

sum of rank tests, $p < 0.001$). The shortest time span of mussel excystment could be found on topmouth gudgeon (8 days), the longest on ide (72 days).

There was a moderate mortality rate of all fish during juvenile mussel excystment of 34.2%, with *R. rutilus* showing the highest mortality of 83%. Because of no differences of mortality rates among non-infested control fish and infested fish, the infestation itself could be excluded as the reason for the observed mortalities.

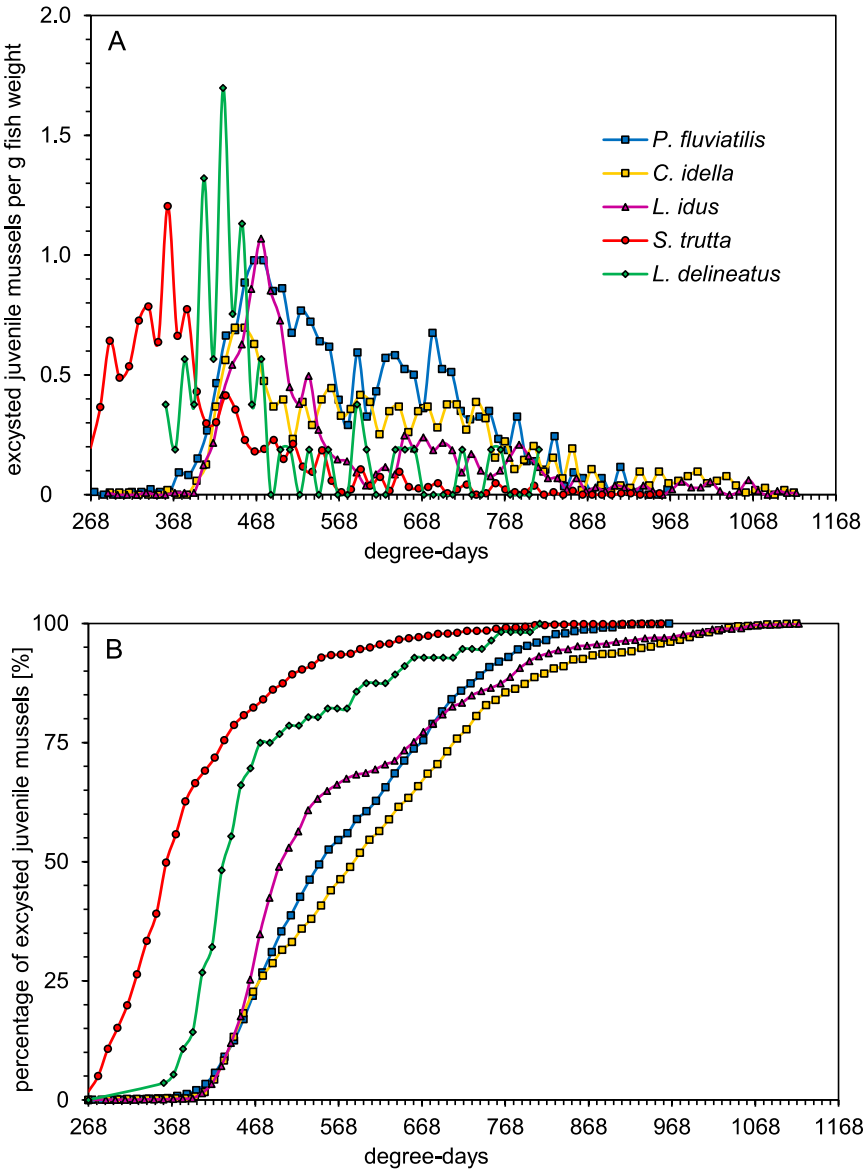

Figure 4.1: Duration of the metamorphosis phase of *A. anatina* on the five best host fish species: A: Number of excysted juvenile mussels per gram fish weight over the time in degree-days. B: Cumulative percentage of excysted juvenile mussels over the time in degree-days.

4.4.2 Glochidial development on non-native fish species

Juvenile *A. anatina* developed successfully on native as well as both tested invasive host fish species. The species *C. idella* had an initial larval attachment of 18.0 glochidia per gram fish weight and a number of 14.0 fully developed juvenile mussels g $^{-1}$. The percentage of weight normalized glochidial loss between the initial infestation 2 days pi and the number of excysted juveniles was the lowest of all tested species (only 22%, Table 4.2). Thus, *C. idella* had a significantly higher number of excysted juveniles per day than some native species like *R. rutilus* or *G. gobio* (post hoc pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$). In fact, non-native grass carp was (after the perch) the second best tested host fish species for the freshwater mussel *A. anatina*.

The second non-native fish species *P. parva* had the highest weight-normalized glochidial loss between 2 days pi and juvenile mussel excystment of 96%. Regarding the initial larval attachment, *P. parva* had a number of 7.2 glochidia per gram fish weight and a higher infestation rate than some native species (Table 4.2). However, only 0.3 juvenile mussels g $^{-1}$ metamorphosed and excysted successfully (Table 4.2). Therefore, topmouth gudgeon was one of the poor hosts for *A. anatina*. Both invasive fish species showed a moderate mortality of 24.5% during juvenile mussel excystment.

4.4.3 Comparison between *A. anatina* and *A. cygnea*

As mentioned before, the same experiment with ten different fish species was also implemented for the second *Anodonta* species in Central Europe *Anodonta cygnea* (Huber and Geist, 2017). In both cases *P. fluviatilis* turned out to be the best host fish species for native *Anodonta*. The only fish species with no development of juvenile *Anodonta* mussels was *R. amarus*.

Compared to *A. cygnea*, the duck mussel (*A. anatina*) had significantly higher initial infestation rates 2 days pi for all tested fish species (pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$). Glochidial infestation of *A. anatina* ranged between 0.1 (bitterling) and 43.8 (trout) larvae g $^{-1}$, glochidial infestation of *A. cygnea* ranged between 0.0 (bitterling) and 29.8 (perch) larvae g $^{-1}$ (Fig. 4.2A). Although there was no statistically significant difference between both mussel species regarding the number of excysted juvenile mussels per gram fish weight, *A. anatina* showed higher rates of fully developed juveniles than *A. cygnea* for all tested fish species, except stickleback (between 0.3 (topmouth gudgeon) and 19.9 (perch) mussels

g $^{-1}$ for *A. anatina*, between 0.1 (topmouth gudgeon) and 15.3 (perch) juvenile mussels g $^{-1}$ for *A. cygnea*) (Fig. 4.2B).

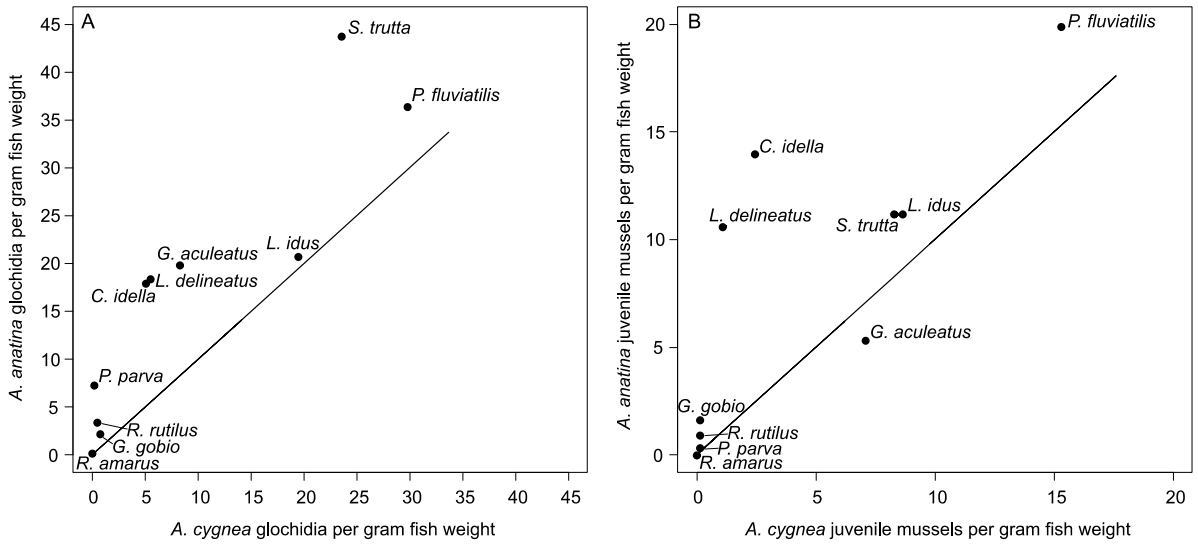

Figure 4.2: Comparison of host fish suitability for the mussel species *A. anatina* and *A. cygnea*: A: Average initial infestation of ten different fish species with glochidia of both mussel species (glochidia per gram fish weight); B: Average excystment of juvenile mussels on ten different fish species (juvenile mussels per gram fish weight); points above the line represent fish species with a higher infestation of *A. anatina* compared to *A. cygnea*.

Surprisingly, *G. aculeatus* revealed a higher initial infestation rate with the larvae of *A. anatina* (19.8 glochidia per gram fish weight), but the weight-normalized glochidial loss was also very high during the experiment (73%). The weight-normalized glochidial loss with larvae of *A. cygnea* on stickleback was only 14%. Thus, stickleback was the only species with higher rates of excysted mussels of *A. cygnea* (7.1 mussels g $^{-1}$, in contrast to 5.3 developed mussels g $^{-1}$ of the species *A. anatina*) (Fig. 4.2B).

The mortality rates of the fish species in both experiments were different, but no statistically significant difference between both mortality rates could be detected (pairwise Wilcoxon rank sum test with Bonferroni correction, $p > 0.05$). In total, 34.2% of the fish, infested with the larvae of *A. anatina*, died during juvenile mussel excystment. The mortality rate of the fish infested with glochidia of *A. cygnea* was much lower (only 14.7%). Nevertheless, the excystment of mussels per gram fish weight was higher in the experiment with the duck mussel. The only species with a higher mortality rate during the infestation with *A. cygnea* was *G. aculeatus* (57%, in contrast 13% of stickleback died with *A. anatina*).

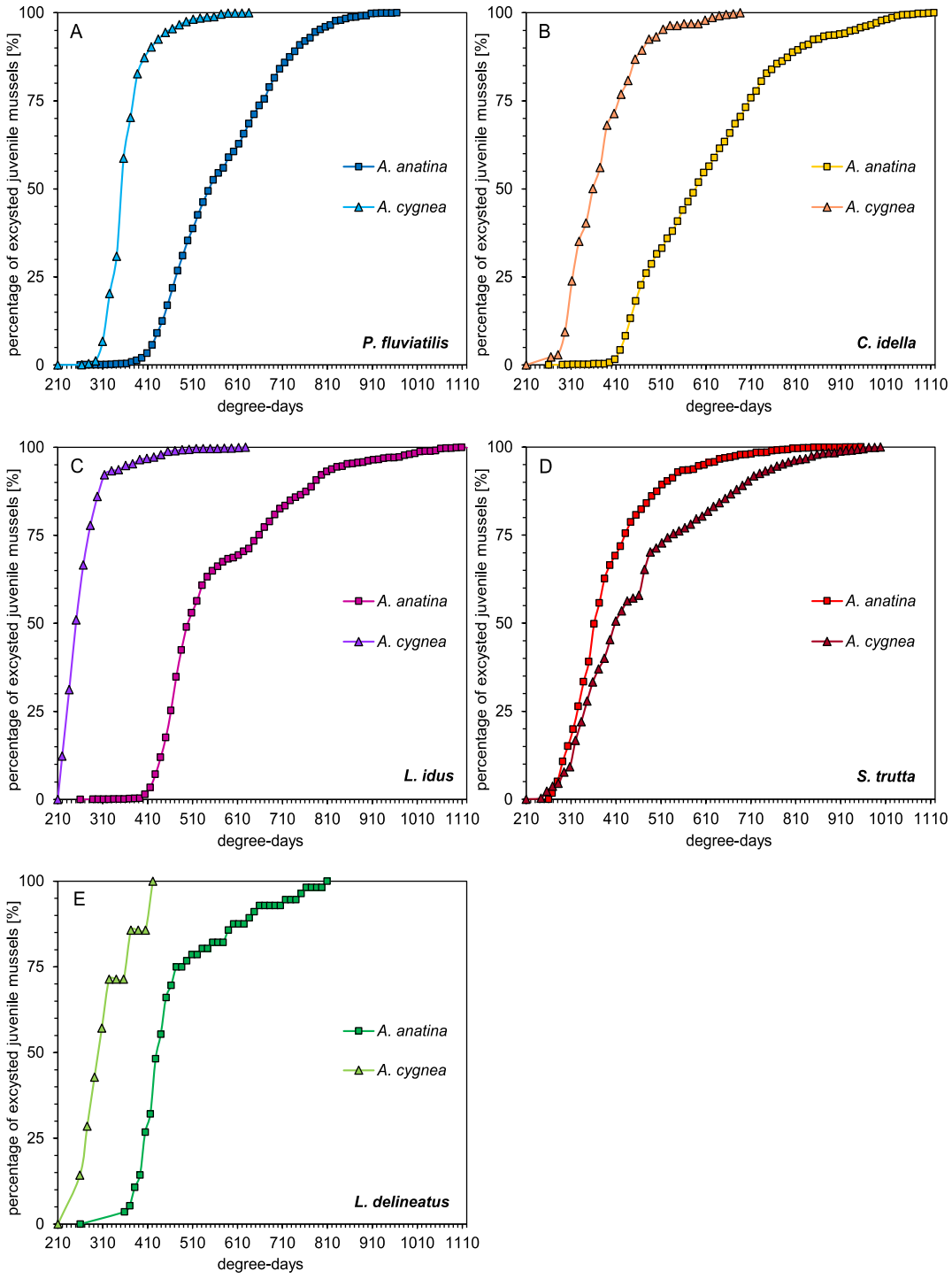

Figure 4.3: Time-series differences in excystment of metamorphosed juvenile mussels between *A. anatina* and *A. cygnea*. A: Cumulative percentage of excysted juvenile mussels on the best host fish species for both mussels, *P. fluviatilis*, over time in degree-days. B: Cumulative percentage of excysted juvenile mussels on the host fish species *C. idella* over time in degree-days. C: Cumulative percentage of excysted juvenile mussels on the host fish species *L. idus* over time in degree-days. D: Cumulative percentage of excysted juvenile mussels on the host fish species *S. trutta* over time in degree-days. E: Cumulative percentage of excysted juvenile mussels on the host fish species *L. delineatus* over time in degree-days.

Highly significant differences between both mussel species were found with regard to the duration of metamorphosis and juvenile mussel excystment (Fig. 4.3). On average, the developmental phase of the glochidia of *A. anatina* took significantly longer than the metamorphosis of the larvae of *A. cygnea* (pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$). Thus, the excystment of juvenile *A. cygnea* started earlier on all tested suitable hosts, except *S. trutta* (Fig. 4.3D). The total duration of the excystment phase of juvenile *A. cygnea* was also shorter than the one of juvenile *A. anatina* (except on *S. trutta*), which is exemplarily shown in Fig. 4.3A-E for the five best identified host fish species of *A. anatina*.

4.5 Discussion

To the best of our knowledge, this study presents for the first time results of a simultaneous infestation of ten different fish species with the glochidia of *A. anatina* to clarify their host suitability. Relative host importance can only be assessed if all tested fish are infested under identical conditions in one common infestation bath (Taeubert et al., 2013a) as realized in this study. Analogously to other studies with freshwater mussels (e.g. *Unio crassus* or *Anodonta cygnea*), host fish species for *A. anatina* can be separated in groups of good, poor and not hosts, depending on their suitability (Huber and Geist, 2017; Stoeckl et al., 2015; Taeubert et al., 2012a). The fact, that these differences in suitability are already detectable immediately after the initial infestation shows that the attachment of glochidia is unlikely a random mechanism as described before (Arey, 1921; Bauer, 2001a; Jansen et al., 2001; Wood, 1974) but instead a very specific and directed process. Taeubert et al. (2012b) found that the initial infestation of fish with glochidia of *U. crassus* as well as the duration of metamorphosis and the timing of excystment also differs between host fish species, even within the group of good hosts. The laboratory infestation of ten different fish species with the larvae of *A. cygnea* confirmed these results (Huber and Geist, 2017). Being in line with the findings in *A. anatina*, freshwater mussels that use more than one fish species as host generally reveal great variation regarding infestation and success of juvenile mussel excystment (Huber and Geist, 2017; Jansen et al., 2001; Stoeckl et al., 2015; Taeubert et al., 2012a). This seems to be a very successful strategy for optimal dispersal and survival of the glochidia and juvenile mussels in species that otherwise have a low degree of specialization since this allows utilization of a broader fish community structure in order to maximize dispersal and fitness (Garnick, 1992; Haag and Stoeckel, 2015; Taeubert et al., 2012a). Additionally, the different and species-specific durations of the metamorphosis phase, along with different host dispersal patterns and habitat preferences, also

contribute to reducing risk of mortality on the level of annual offspring cohorts. This may be particularly crucial in short-lived species such as *A. anatina* only reaching ages of 10 years compared to host specialists such as *M. margaritifera* with life spans of > 80 years (Bauer, 2001a). The extent to which developmental times and excystment of juvenile *A. anatina* also varied between the best tested host fish species, was unexpected, particularly since all species were maintained in the same water at the same temperature. Under natural conditions, variations between hosts regarding the duration of metamorphosis and excystment of mussels are likely to even be greater than in this study due to greater habitat and temperature variabilities and the different preferences of the fish species (Taeubert et al., 2012b). Density-dependent effects due to a high infestation with glochidia as a reason for different developmental times on various fish species can be excluded as evident from the absence of any correlation between the initial infestation rate 2 days pi and the duration of metamorphosis and juvenile mussel excystment (Pearson's product-moment correlation, p-values > 0.05). The high variation in suitability of fish species as hosts as well as the variation in duration of metamorphosis and juvenile mussel excystment can only be explained by species-specific differences in physiology which also need to be considered in case of conservation and artificial breeding measures, e.g. to avoid selection and genetic drift effects (Taeubert and Geist, 2017). From a culturing and captive breeding perspective, it appears reasonable to choose the most suitable host fish species to maximize the output of living juvenile mussels, but host-management strategies should also consider local adaptation and focus on maintaining high quality hosts at a regional scale (Taeubert and Geist, 2017). For management strategies to protect and preserve the genetic variability of *A. anatina* populations, it may also be important to include several different fish species that result in very different metamorphosis times occurring in the natural habitat of the mussel, even if these fish hosts are less suitable.

The best host for the larvae of *A. anatina* was the ubiquitous *P. fluviatilis*. Similar to the duck mussel, the perch is a widespread fish species in Central Europe with habitats in standing waterbodies as well as rivers, often in co-occurrence with mussel populations (Freyhof and Kottelat, 2008). This species was also confirmed as the best host in a previous study from Finland (Jokela et al., 1991) as well as for the second native *Anodonta* species in Europe, *Anodonta cygnea* (Huber and Geist, 2017). Due to the wide distribution of the perch in all types of waterbodies, the mussels' dispersal can be increased by using this host for glochidial development (Huber and Geist, 2017). Nevertheless, the perch can be unsuitable for mussel reproduction if habitat requirements are inappropriate and if the perch is not part of the natural, local fish fauna. Interestingly, there was a minimal initial infestation with glochidia but no juvenile mussel

development on the bitterling. During other studies, e.g. Douda et al. (2013), *R. amarus* was identified as host for *A. anatina* in the Czech Republic. Populations of both species often co-occur because the bitterling parasitizes mussels for its larval development (Reichard et al., 2007; van Damme et al., 2007). Because of this co-evolution, the defense mechanisms of the bitterling's immune system seem to be more effective against encysted glochidia (Mills and Reynolds, 2004). The fact that *R. amarus* appears to be a suitable host for the duck mussel in the Czech Republic (Douda et al., 2013) may be explained by genetic differences of hosts and mussels and possible local adaptations. As confirmed for *U. crassus* before, local adaptations can lead to differences in host suitability within the same species (Taeubert et al., 2012b). Thus, it has been shown that the suitability of hosts for *A. anatina* can also differ between different populations of one host fish species. This has to be considered in further experiments or conservation measures. In the study of Douda et al. (2013), the transformation rate of the larvae on the bitterling was low, with only a small number of glochidia successfully developing. Regarding *R. amarus*, the differences between two different populations were low. In both cases it was a very unsuitable host for the duck mussel. A stocking of bitterling in waters with mussel populations is thus only useful for the conservation of the bitterling, but not for the mussels. In regard to the origin of different populations, there might also be differences in the suitability of host fish species if another origin or population of the duck mussels would have been applied. Due to the fact that the populations of *A. anatina* from central and northern Europe belong to the same evolutionary significant unit (ESU) their genetic differentiation is weak (Lopes-Lima et al., 2016) and differences in host suitability seem to be unlikely. In contrast, populations of southern Europe belong to a completely different ESU (Lopes-Lima et al., 2016). Therefore, results from this study cannot be conferred to these South European populations of the species *A. anatina*.

Due to their fast spreading in Europe in the last decade (Britton et al., 2008; Milardi et al., 2015), the two non-native fish species *C. idella* and *P. parva* were integrated in this study. Introduced fish are often mentioned in literature as a general factor of threat for native freshwater mussels. This is based on conventional wisdom that the mussels would not be able to use them for reproduction (Douda et al., 2013; Modesto et al., 2018; Salonen et al., 2016; Strayer, 2008; Taeubert and Geist, 2017, Watters and O'Dee, 1998). Invasive species native to areas with a high diversity and abundance of parasites may allocate more resources to defense systems (Schmid-Hempel and Ebert, 2003), therefore they seem to be more resistant to novel parasites in their invaded ranges (Douda et al., 2013). Surprisingly, *C. idella* was the second best host fish species for the larvae of *A. anatina* in this study. This result was also confirmed for *A.*

cygnea before (*C. idella* was one of the five best host fish species) (Huber and Geist, 2017). For freshwater mussels that parasitize a high number of hosts, fish introductions can be advantageous, because the presence of new species enhances the availability of potential hosts for the mussels (Garner et al., 1999; Kelly et al., 2009; Modesto et al., 2018; Poulin et al., 2011). Moreover, successful invaders often have great dispersal and adaptabilities to a wide range of habitats, also increasing the chances for attached mussel larvae to colonize new habitats. Conversely, the second non-native fish species of this study, *P. parva*, had the lowest juvenile mussel excystment. Due to the fact that *C. idella* turns out to be a very good host, and *P. parva* a very poor host for *A. anatina* larvae, a general recommendation for the handling of invasive fish species in mussel waters is not possible. On the contrary, it has to be differentiated between single invasive fish species and between the respective waterbody where mussels and non-native fish co-occur. Both tested non-native fish species are very common in Europe, but with regard to the reproduction success for *A. anatina* and *A. cygnea* the occurrence of *C. idella* can be assessed as a positive, the occurrence of *P. parva* as a negative development.

The both *Anodonta* species of Central Europe, *A. anatina* (duck mussel) and *A. cygnea* (swan mussel), often co-occur in the same habitats (Niemeyer, 1993). Therefore, the similarity of both species regarding excystment of juveniles on non-native fish species during laboratory experiment is not surprising. On the contrary, the extreme differences in duration of the metamorphosis as well as initial infestation and number of developed juvenile mussels between both *Anodonta* species are interesting. Possible reasons for these differences could potentially be (1) differences in water temperature of the infestation bath, (2) differences in mortality rate of the infested fish during the experiment, (3) differences in total fish weight in the infestation bath, (4) differences in glochidial concentration in the infestation bath, (5) differences in water temperature within the holding units of the infested fish during the experiment, and (6) differences in glochidial morphology and thus better attachment abilities.

Although the fish were infested and treated in the same way during both experiments, *A. anatina* generates higher infestation rates and a higher number of fully developed juvenile mussels than *A. cygnea* for most of the tested host fish species (except for stickleback). The first possible reason for these differences could be a variation in the temperature of the infestation bath. In both cases, the water during the infestation process was exactly the same with a mean temperature of ~ 12°C. Very cold temperatures during the infestation could possibly suppress the immune system of the fish and lead to a higher rate of infestation and thus to a higher transformation success of juvenile mussels (Roberts and Barnhart, 1999). Due to the same water

temperature in both infestation baths, this cannot be the reason for the higher infestation of hosts with glochidia of *A. anatina* and thus a higher number of excysted juvenile mussels.

The second possible reason for the differences between both anodontines is a higher mortality rate of fish during the experiment with *A. cygnea*. However, there was no correlation between the number of excysted juveniles per gram and the mortality of the fish during the experiments (Spearman´s rank correlation, p-value > 0.05). A high mortality of the host fish reduces the total amount of mussels because glochidia that are still encysted are lost before metamorphosis (Taeubert et al., 2014). The mortality rate of the fish during the excystment phase of the experiment with *A. anatina* was higher than with *A. cygnea* (33.8% compared to 14.7%). Fish mortality can be excluded as reason for a lower number of excysted juvenile mussels of the species *A. cygnea*.

Differences in initial infestation and numbers of excysted juvenile mussels are also not caused by differences in fish weight included in the two infestation baths. A higher weight of fish could lead to a bigger surface for larval attachment and thus to higher infestation rates and a possible higher number of juveniles. No such correlation could be found between the weight of applied fish and the initial infestation rate per gram fish weight (Pearson´s product-moment correlation, p-value > 0.05). Besides, the total weight of fish in the infestation bath was higher with *A. cygnea* than with *A. anatina* (1807.1 g compared to 1598.9 g).

A variation in the glochidial concentration within both infestation baths could also be responsible for the differences in the infestation rate and the number of excysted juveniles between both *Anodonta* species. However, this point can also be excluded because the concentration of larvae in the infestation bath was the same for both experiments (~ 8500-9000 glochidia per liter).

On an average, *A. anatina* had longer developmental times and a longer duration of excystment than *A. cygnea*. The artificial experiment with glochidia of the duck mussel started in January, the one with the swan mussel started in May. Thus, the average water temperature within the holding units of the infested fish during the experiment was lower with *A. anatina* (average water temperature in the study with *A. anatina*: 11.5°C, average with *A. cygnea*: 15.4°C). In general, an extended developmental time and duration of excystment at lower temperatures was also shown for the larval development of *U. crassus* on different host fish species (Taeubert et al., 2014). Compared to *A. cygnea*, *A. anatina* also occurs in rivers with greater current speed (Niemeyer, 1993) and thus lower temperatures during the reproduction phase of the mussel. Extended duration of larval metamorphosis due to lower water temperatures also exists under natural conditions (Roberts and Barnhart, 1999). The longer larval development and excystment

of juveniles might benefit the distribution of the mussel in rivers due to an increased probability of host migration as it was also mentioned in Taeubert et al. (2014) for *U. crassus*. This may be an evolutionary advantage and the reason for the higher dispersal of *A. anatina* populations and on the other hand the reason for the comparatively higher status of threat of *A. cygnea*. However, the colder water temperatures during the experiment with *A. anatina* can only be the explanation for an extended duration of metamorphosis and juvenile mussel excystment and not the explanation for a higher number of excysted juvenile mussels. This can be illustrated using the example of the stickleback which had higher infestation rates but a lower number of excysted juvenile mussels with the duck mussel compared to the swan mussel and thus a higher total glochidial loss (73% with *A. anatina*, 14% with *A. cygnea*) despite of the low water temperature during the experiment.

Another possible reason why there were higher initial infestation rates and more juvenile mussels with the duck mussel than with the swan mussel, is a different morphology of the glochidia of both species. The larvae of *A. anatina* applied in this experiment had an average size of 355µm (length), the mean size of the larvae of *A. cygnea* in the experiment was 323µm (length). In general, the duck mussel produces the biggest glochidia of all native mussel species in Central Europe (Scharsack, 1994). The larvae of both species have large hooks for a better attachment on the host, but these hooks differ in their ending. The one of *A. cygnea* has a more lance-shaped apex, the one of *A. anatina* is rather narrow at the ending (Wächtler et al., 2001; Scharsack, 1994). These differences in the morphology of the glochidia of both species may be the crucial reason for the better infestation of the fish with the larvae of *A. anatina* and therefore for the higher rates of excysted juvenile mussels compared to *A. cygnea*, because all other reasons can be excluded. This more successful excystment rate of juvenile mussels can be one possible explanation for the broad spreading and the higher number of populations of *A. anatina* in comparison to *A. cygnea*. However, this question cannot be answered completely due to the fact that this study was a laboratory experiment that cannot be uncritically translated to the field situation. Under natural conditions other factors like environmental changes or pollution can also increase the further decline of *A. cygnea*. In this experiment it has been shown that under the same conditions *A. anatina* has better chances of reproduction than *A. cygnea* due to its higher infestation rates and the more successful development of juvenile mussels.

In this laboratory study, the host suitability of ten important fish species potentially co-occuring with *A. anatina* was tested. A key finding is that all fish species differed significantly in their suitability, the initial larval attachment and juvenile mussel excystment as well as in the duration of metamorphosis and excystment of juveniles. This can be considered a first step in

understanding the host relationships of this species, with the logical next steps being testing of additional species and comparing the findings of host suitability in the laboratory with the situation in the wild. Thus, regarding the conservation of *A. anatina* populations, suitable host fish species must be identified in every waterbody with mussel populations separately. From a management perspective, knowledge on suitability, density and demography of local fish populations that co-occur with mussel populations is crucial (Bishop et al., 2007; Taeubert and Geist, 2017). In case of fish stocking activities, no general recommendation for waters with *A. anatina* populations can be given. Instead, the results of this laboratory study can be used as a basis to verify the best host fish species for the mussels in specific regions. There is the possibility that a fish species tested as poor host for *A. anatina* (like the stickleback) may be the only available host for the mussel in a special case or that a so called poor host can generate a better developed offspring due to differences in the duration of the metamorphosis phase. In some waterbodies, good and poor hosts together contribute to a strong and healthy population of mussels because of different developmental times and therefore a higher distribution of juveniles. These facts are also important for artificial breeding measures.

In general, the reproduction of the mussel is susceptible to disruption by any factor that reduces the abundance, distribution or mobility of host fish species (Taeubert et al., 2012a). Interventions in a waterbody that harm the fish community also unavoidable harm mussel populations dependent on the fish fauna (Modesto et al., 2018). Efforts to conserve and manage the mussel species are useless if the hosts are not conserved and managed as well (Watters, 2007). Implications for conservation of mussels have to ensure healthy fish populations with a high number of juvenile fish that can be used for glochidial infestation (Modesto et al., 2018). If management activities in regard to invasive fish species in native waterbodies are necessary, this study suggests that a careful and case-specific differentiation between single invasive species has to be made.

With regard to the differences between the two *Anodonta* species, *A. anatina* and *A. cygnea*, conservation measures also have to differentiate more accurately between both species. They differ not only in habitat preferences, but also in suitability of host fish species and thus in status of threat. A genetic differentiation between both anodontines is essential before starting management activities (Zieritz et al., 2012). In general, the results of this study emphasize that the knowledge about host fish suitability cannot be transferred even between sister species. Thus, conservation of freshwater mussel species can be successful only, if species-specific measures are taken based on the results of studies about their host-parasite interaction.

5 Reproduction success of the invasive *Sinanodonta woodiana* (Lea, 1834) in relation to native mussel species

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Author contributions:

VH and JG conceived and designed the study. VH sampled the mussels, performed the genetic analysis and arranged and performed the laboratory experiment. VH and JG discussed the data analysis and manuscript structure. VH analyzed the data and wrote the manuscript. JG improved and edited the manuscript.

5.1 Abstract

Invasions of non-native species are major threats for freshwater ecosystems. One of the most invasive freshwater mussels in Europe is the Asian *Sinanodonta woodiana* (Chinese pond mussel). It occurs in the same waterbodies as the endangered native species *Anodonta cygnea* and *Anodonta anatina* with unknown consequences for them. Thus, the analysis about the host-parasite relationship between the larvae of *S. woodiana* and host fish species in Europe is important to assess impacts on the native mussels regarding their competition for hosts. In this study, host suitability of ten different fish species (native and non-native to Europe) for the glochidia of *S. woodiana* was analyzed by simultaneous infestation of the fish. All fish species were identified as suitable hosts but differed significantly in initial infestation rate as well as duration and rate of juvenile mussel excystment. Surprisingly, the glochidia developed successfully on *Rhodeus amarus* (bitterling), which cannot use *S. woodiana* for its own reproduction, and which is an unsuitable host for native anodontines. Compared to both native *Anodonta*, *S. woodiana* glochidia developed more successfully resulting in a higher number of excysted juvenile mussels at similar larval exposure. Metamorphosis was also faster on all tested fish species. These factors, together with the faster growth and higher number of offspring in *S. woodiana* likely contribute to a competitive advantage over native anodontines. The great likelihood of spreading this mussel on a large number of different possible host fish species deserves attention in fisheries management and stocking programs.

5.2 Introduction

Invasive species represent one of the most important global threats to biodiversity (Carlton and Geller, 1993; Sousa et al., 2014), with freshwater ecosystems being particularly strongly affected (Geist, 2011; Strayer, 2006). The introduction of non-native aquatic biota is mostly related to human activities like trade or aquaculture (Cohen and Carlton, 1998; Sousa et al., 2014). *Corbicula fluminea* (Müller, 1774) or *Dreissena polymorpha* (Pallas, 1771) provide examples of widely distributed and extremely successful aquatic invaders (Sousa et al., 2014). For instance, the invasion of *Dreissena polymorpha*, originally native to the Ponto-Caspian region (Strayer et al., 2011), changed the physical, chemical and biological characteristics of many lakes and rivers in North America (Strayer et al., 1999; Strayer, 2006), resulting in the extirpation of many populations of native unionid mussels (Ricciardi et al., 1998; Strayer, 1999). Invasive freshwater mussels can harm native ones by competition for food and space or by attaching to their shells which hampers their filter feeding (Ferreira-Rodríguez et al., 2018, 2019). The high dispersal ability of the most invasive bivalve species depends not only on their short generation times, rapid growth and early sexual maturity (Sousa et al., 2008), but also on their reproduction strategy itself. They typically have simple life cycles and often produce free-living larvae (Douda et al., 2012b; Karatayev et al., 2007; Stoeckel et al., 1997). However, there are also invasive freshwater mussels with a more complex life cycle including an obligatory parasitic stage like *Sinanodonta woodiana* (Lea, 1834) in which the factors governing invasion success are less well understood.

Sinanodonta woodiana is native to tropical eastern Asia, primarily the Amur and Yangtze basins (Cummings, 2011; Kraszewski and Zdanowski, 2007; Soroka, 2005). It has been introduced around the world, for example to the USA, Costa Rica and many Asian and European countries (Bogan et al., 2011; Cummings, 2011; Konečný et al., 2018). Microsatellite data indicated a single colonization event and an early establishment of two invasive centers serving as sources for further expansion across Europe (Konečný et al., 2018). A commercial import of Asian carps like silver carp, *Hypophthalmichthys molitrix* (Valenciennes, 1844) and grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) from the River Yangtze basin to hatcheries in Romania in the early 1960s was the most likely source of further *S. woodiana* expansion in Europe (Kondakov et al., 2018; Konečný et al., 2018). These fish species were introduced outside their native ranges for food, control of aquatic vegetation or hatchery water quality maintenance (Watters, 1997). *S. woodiana* normally prefers relatively warm and either stagnant or slow flowing waters (Soroka, 2005; Zettler and Jueg, 2006). In Germany, many self-recruiting

populations of *S. woodiana* nowadays exist in all parts of the country and in different kinds of waterbodies (Bahr and Wiese, 2018; Duempelmann, 2012; Pfeifer, 2002; Reichling, 1999). The increased spreading of *S. woodiana* in Germany in the last decades is especially due to the selling of this non-native species in pet shops and garden centers, where it is often erroneously labelled as native *Anodonta* species (Bahr and Wiese, 2018; Schoolmann et al., 2006). The most important factor for the fast spreading of *S. woodiana* is believed to be its ability to use novel host fish species, native to the invading region (Douda et al., 2012b). However, relatively little is known about invasive freshwater mussels with host-parasite interactions like *S. woodiana* and their invasion and threat for native species (Sousa et al., 2014). This holds especially true for invasive freshwater mussels with a life cycle which is similar to native Unionida with possible host competition (Donrovich et al., 2017).

Thus, the major aim of this study was to analyze the host-parasite relationship between the glochidial larvae of a self-recruiting German population of *S. woodiana* and ten different host fish species (native and invasive to Germany) in a controlled infestation experiment. The findings were then compared with the results of two other infestation experiments with the larvae of the native *Anodonta anatina* (Huber and Geist, 2019a) and *Anodonta cygnea* (Huber and Geist, 2017) to assess possible impacts of the invasive freshwater mussel on the native ones regarding their competition for hosts. In detail, the following hypotheses were tested: (1) Invasion success of the non-native freshwater mussel species *S. woodiana* in Europe is independent from its original hosts within its native range and instead depends on its capability to use co-invasive non-native fish species as well as indigenous ones as hosts for its glochidial larvae (Douda et al., 2012b; Dudgeon and Morton, 1984; Watters, 1997). Therefore, glochidia of *S. woodiana* attach to and metamorphose on all tested fish species successfully. (2) Although the larvae of *S. woodiana* are host fish generalists, metamorphosis success and success of juvenile mussels excystment are higher on the both co-invasive fish species *Ctenopharyngodon idella* (confirmed host in the native range of *S. woodiana*, Beran, 2008; Watters, 1997) and *Pseudorasbora parva* (common Asian origin, Britton et al., 2010) than on the native European fish species tested. (3) All three mussel species are host fish generalists and compete for hosts. The invasive *S. woodiana* and two native *Anodonta anatina* and *Anodonta cygnea* have the same habitat preferences and often co-occur in the same water bodies (Beran, 2008; Bössneck and Klingelhöfer, 2011; Wojton et al., 2012). Because *S. woodiana* is a broad generalist in host use (Douda et al., 2012b), has a high competitive potential and produces a higher number of glochidia than native anodontines (Wächtler et al., 2001), *S. woodiana* shows a higher initial

infestation of the tested fish species and a higher juvenile mussel excystment compared to *A. anatina* and *A. cygnea*.

5.3 Materials and methods

The host use experiment started on the 24th of May 2016 with the simultaneous infestation of 481 fish of ten different fish species with the glochidial larvae of the freshwater mussel *S. woodiana* following the experimental standards given in Taeubert et al. (2012a). The infestation procedure as well as the whole implementation of the experiment followed the descriptions of Huber and Geist (2017, 2019) to ensure comparability of the results between the two native species *A. cygnea* and *A. anatina* with the invasive *S. woodiana*. All three experiments were performed separately at different times but at the same place, with the same methodology and the same ten fish species.

Adult *S. woodiana* were sampled on the 18th of May 2016 from the wildlife reserve Öberauer Donauschleife (backwater of the Danube near Straubing, Bavaria, Germany), one of the self-recruiting populations in Germany. The sampling of ten adult mussels of the local population was approved by license of the responsible nature conservation authority (license number: 55.1-8642.10 U 12). The mussels were transferred to the laboratory of the Aquatic Systems Biology Unit at Technical University of Munich and the species was genetically confirmed according to the molecular identification key by Zieritz et al. (2012). Mature glochidia were flushed out of the marsupia of five adult mussels with a squirt bottle on the 23rd of May 2016 and stored at 4.0°C overnight. In total, approximately 500,000 glochidia larvae with a viability of more than 95% were used for the infestation. Larval viability was checked by observing an active valve clamping mechanism after the addition of a NaCl stimulus.

The fish species were chosen according to their co-occurrence with the freshwater mussels *A. cygnea*, *A. anatina* and *S. woodiana* in central Europe. The three mussel species inhabit especially lakes and slow flowing streams, but populations of *A. anatina* also occur in fast flowing streams with colder temperature regimes (Lopes-Lima et al., 2017; Niemeyer, 1993; Soroka, 2005). Therefore, limnophilic fish species as well as rheophilic ones with different habitat requirements representing four different fish families were used for the experiment: Salmonidae, *Salmo trutta* (Linnaeus, 1758); Cyrpinidae, *Leuciscus idus* (Linnaeus, 1758), *Gobio gobio* (Linnaeus, 1758), *Rhodeus amarus* (Bloch, 1782), *Rutilus rutilus* (Linnaeus, 1758), *Leucaspius delineatus* (Heckel, 1843); Percidae, *Perca fluviatilis* (Linnaeus, 1758); and Gasterosteidae, *Gasterosteus aculeatus* (Linnaeus, 1758). Due to the origin of *S. woodiana*, two non-native Asian

fish species, both cyprinids, which also spread throughout Europe, were included: *C. idella* and *Pseudorasbora parva* (Temminck and Schlegel, 1846). Date and place of origin of the different fish species and the number of individuals per species used for the experiment are listed in Table 5.1. Juvenile fish only were used for the experiment. All fish had no previous contact with unionid mussels to exclude possible pre-immunisation.

Table 5.1: Tested fish species (order from fish species with the highest number of excysted juvenile mussels after weight-normalization to species with the lowest number of excysted juveniles after weight-normalization), their status, place and date of origin: RO = Aquaculture M. Rösch, Bärnau, 04.11.2014 (G. aculeatus, L. idus, G. gobio, R. rutilus, P. fluviatilis) and 12.04.2016 (R. amarus), AS = Aquatic Systems Biology Unit, 27.11.2012, LU = Bavarian State Office for Environment, Wielenbach, 12.11.2014, RH = Aquaculture near Rheine, 20.05.2016, PR = Private fish pond, Roth, 17.04.2016; mean size (total length) and weight of applied fish, number of fish in the infestation bath per species, number of control fish per species; average number of excysted juvenile mussels per gram fish weight and species; $\overline{X}$ = arithmetic mean.

Fish species	Status	Source	Size $\overline{X}$ ± 1mm	Weight $\overline{X}$ ± 0.1g	Nb. of fish infestation bath	Nb. of control fish	Mussels per g fish weight
C. idella (grass carp)	Introduced	LU	90	8.3	32	11	26.2
G. aculeatus (stickleback)	Native	RO	76	4.1	48	16	18.2
L. idus (ide)	Native	RO	112	9.6	22	7	15.6
G. gobio (gudgeon)	Native	RO	121	13.2	21	7	14.7
S. trutta (brown trout)	Native	AS	191	58.2	6	2	8.0
L. delineatus (moderlieschen/sunbleak)	Native	RH	44	0.7	177	59	6.2
R. rutilus (roach)	Native	RO	96	6.8	28	9	2.9
P. fluviatilis (perch)	Native	RO	121	16.4	18	6	2.4
P. parva (topmouth gudgeon)	Introduced	PR	58	2.0	78	26	1.0
R. amarus (bitterling)	Native	RO	64	2.9	51	17	0.3

All 481 individual fish were infested for 30-45 min in one common infestation bath with a glochidial concentration of around 8500-9000 larvae per liter (glochidia of the five adult mussels were pooled for the infestation). The infestation bath was filled with water (bank filtrate, river Moosach) with a temperature of 12°C. After the infestation, fish were separated in three replicates per species. Each replicate was then kept in one special funnel-shaped holding unit with a maximum volume of 45 L until the end of the experiment. The number of specimens per replicate and holding unit was adjusted according to the respective weights and sizes of the fish as well as on the different requirements of the fish species to ensure optimal holding conditions during the experiment (Huber and Geist, 2017, 2019). For example, from smaller fish species like *L. delineatus* more individuals per holding unit were included than from the bigger ones like *S. trutta*. Due to their high oxygen need, tanks containing *S. trutta* were set up with constant water flow. This water flow also resulted in slightly lower temperatures in holding units with *S. trutta* during the experiment (average water temperature: 12.5 ± 0.4°C). The average water

temperature in all other tanks was 15.8 ± 0.3°C. Temperatures were measured with temperature loggers (Lascar Electronics Limited, Salisbury, UK) every 30 min. Additional to the three replicates of infested fish per species, one separate group of non-infested control fish per species (not-exposed to glochidia) was included (Table 5.1). This group was treated in the same way as the infested ones to analyze if influences like handling or holding conditions are responsible for the mortality of the fish during the experiment (Huber and Geist, 2019a).

To calculate the infestation success, glochidial attachment rate on gills, fins and skins of the fish had to be determined for every fish species at different time points. Therefore, some individuals per fish species were sacrificed after 2 days (2 days post infestation, pi), 12 days and at the end of the excystment of juvenile mussels. The number of specimens per fish species sacrificed to calculate the glochidial attachment rate depended on the amount of living fish per species and holding unit at each of the three time points. In total, 20 infested specimens (one specimen of *S. trutta*, *P. fluviatilis*, *L. idus* and *G. gobio*, two specimens of *C. idella*, *R. rutilus* and *G. aculeatus*, three specimens of *R. amarus* and *P. parva*, four specimens of *L. delineatus*) were analyzed 2 days pi and glochidial attachment rate per fish species was calculated. Again, on the 5th of June 2016 (12 days pi, time point of the first detected juvenile mussels), in total 15 infested fish were sacrificed. This additional time point was used as backup only, in case no fish would have survived until the end of the experiment (Huber and Geist, 2017, 2019). At the end of the excystment of juvenile mussels, a number of 24 infested individuals (one specimen of *S. trutta*, two specimens of *P. fluviatilis*, *R. rutilus*, *G. aculeatus* and *G. gobio*, and three specimens of *L. idus*, *C. idella*, *R. amarus*, *L. delineatus* and *P. parva*) was analyzed. The number of sacrificed fish per species at each sampling point depended on the number of fish applied in the experiment and the number of fish that were still alive at the respective time point.

The success of metamorphosis was determined by detecting the number of completely developed, living juvenile mussels per fish species. Five liters of water of every holding unit containing infested fish were checked for the presence of excysted juveniles during daily water change (12.5% daily renewal of water) (Huber and Geist, 2017, 2019). The first excysted mussels dropped off after 12 days simultaneously from *P. fluviatilis*, *L. idus*, *C. idella*, *R. rutilus*, *L. delineatus* and *G. gobio*. All dropped-off juvenile *S. woodiana* were then transferred to special holding systems. The last excysted juvenile mussel was counted in the holding unit of *S. trutta*. The whole experiment was terminated on the 27th of July 2016 (64 days pi), three days after the last mussel had dropped off.

To account for differences in weight between different fish species and single specimens and to ensure comparability, number of attached glochidia as well as number of excysted juveniles

were calculated per gram fish weight. To compare the duration of the metamorphosis phase and the duration of the juvenile mussel excystment on different fish species and at different temperatures, the concept of degree-days (dd, sum of daily water temperatures) was applied (Hruska, 1992; Taeubert et al., 2014). Statistical analyses were performed in R version 3.4.3 (R Core Team, 2017). To calculate differences between all tested fish species regarding their initial weight-normalized glochidial infestation 2 days pi as well as their rate of weight-normalized juvenile mussel excystment, non-parametric Kruskal-Wallis sum of rank tests and post hoc pairwise Wilcoxon rank sum tests were used since ANOVA assumptions were not fulfilled. Bonferroni correction was applied to correct for multiple testings. Differences between the two groups of good and poor hosts as well as the two groups of native and invasive fish species regarding the initial infestation rate, rate of juvenile mussel excystment and duration of juvenile excystment respectively were also tested with pairwise Wilcoxon rank sum tests including Bonferroni correction. Non-parametric Kruskal-Wallis sum of rank tests were used to identify differences in the duration of juvenile mussel excystment and the duration of the metamorphosis phase between single fish species. Statistical analysis of differences between the three mussel species (*S. woodiana*, *A. anatina* and *A. cygnea*) were performed using Kruskal-Wallis sum of rank tests and post hoc pairwise Wilcoxon rank sum tests with Bonferroni correction. Here, differences in initial infestation, rate of juvenile mussel excystment, duration of excystment and fish mortality rate of all tested fish per mussel species were calculated. Spearman´s rank correlation was used to explore the link between the total weight of applied fish (infestation bath) and the initial infestation rate (glochidia per gram fish weight) 2 days pi as well as the correlation between the mortality rate of the fish during juvenile mussel excystment and the number of juvenile mussels per gram fish weight.

5.4 Results

In total, 15,495 living juvenile *Sinanodonta woodiana* were detected following the parasitic phase. Number of attached glochidia and dropped-off juvenile mussels strongly varied between the single host fish species. Similarly, duration of juvenile mussel excystment also differed between the host fishes.

5.4.1 Success of infestation and juvenile mussel excystment

The highest initial infestation rate 2 days pi was measured on *G. aculeatus*, native to Europe, with an average number of 70.5 glochidia of *S. woodiana* per gram fish weight (Table 5.2). *Rhodeus amarus* had the lowest infestation after 2 days with only 4.8 glochidia g^{-1} (Table 5.2). There was a statistically significant difference between all fish species in terms of initial infestation per gram fish weight (non-parametric Kruskal-Wallis sum of rank test, $p < 0.01$). The number of excysted juvenile mussels per gram fish weight also differed significantly between all fish species (non-parametric Kruskal-Wallis sum of rank test, $p < 0.001$). In particular, the highest number of juvenile *S. woodiana* per gram fish weight was found on *C. idella*, which is also of Asian origin, with an average of 26.2 juveniles g^{-1} (Table 5.2). In line with the low initial infestation, the lowest number of juvenile mussels excysted on *R. amarus* (only 0.3 juvenile mussels per gram fish weight, Table 5.2). Regarding the weight-normalized glochidial loss during the metamorphosis phase, in total 74% of initial attached glochidia were lost until the start of excystment on all fish species. *Perca fluviatilis* and *R. amarus* showed the highest larval loss of 94%. In contrast, *C. idella* only lost 34% of its initially attached glochidia during the metamorphosis (Table 5.2).

Table 5.2: Results of the host fish infestation with larvae of *S. woodiana*: fish species, their determined host status, mean number of glochidia per gram fish weight 2 days post infestation (pi) as well as excystment rate of juvenile mussels per gram fish weight and fish species and percentage of weight-normalized glochidial loss between initial infestation and excystment, start and end of ecystment per species in degree-days (dd), average water temperature, host fish mortality (fish that died naturally) during the time span of mussel excystment (mortality is given in percentage and total number of dead fish in brackets); species order: from fish species with the highest number of excysted mussels after weight-normalization to fish species with the lowest number of excysted mussels; * = introduced, non-native fish species; $\overline{X}$ = arithmetric mean.

Fish species	Host status	2 days pi	Excystment				
		Glochidia per g fish weight	Mussels per g fish weight	Weight-normalized gloch. loss (%)	Start - end of excystment (dd)	Temperature $\overline{X}$ ± SD (°C)	Fish mortality (% and total values)
*C. idella**	Good	39.8	26.2	34	195-814	16.3 ± 1.1	47 (15)
G. aculeatus	Good	70.5	18.2	74	211-812	16.2 ± 1.1	46 (22)
L. idus	Good	35.1	15.6	56	192-814	16.0 ± 0.9	14 (3)
G. gobio	Good	27.0	14.7	45	193-708	16.1 ± 0.9	14 (3)
S. trutta	Poor	35.5	8.0	77	188-790	12.5 ± 0.4	0 (0)
L. delineatus	Poor	36.7	6.2	83	184-415	15.4 ± 0.6	50 (89)
R. rutilus	Poor	34.9	2.9	92	188-392	15.7 ± 0.7	0 (0)
P. fluviatilis	Poor	43.4	2.4	94	187-406	15.6 ± 0.7	0 (0)
*P. parva**	Poor	13.6	1.0	93	201-417	15.4 ± 0.6	9 (7)
R. amarus	Poor	4.8	0.3	94	206-396	15.8 ± 0.7	4 (2)

Although all tested fish species were found to be possible hosts for the larvae of *S. woodiana*, they could be divided in two groups: good and poor hosts. Four fish species *C. idella*, *G. aculeatus*, *L. idus* and *G. gobio* formed the group of good hosts due to their high number of excysted juveniles (more than 14 mussels per gram fish weight). The other six fish species (*S. trutta*, *L. delineatus*, *R. rutilus*, *P. fluviatilis*, *P. parva* and *R. amarus*) formed the group of poor hosts with numbers of excysted juveniles between 8.0 and 0.3 mussels g^{-1} (Table 5.2). This classification was not evident 2 days pi, because some species with a very high initial glochidial infestation also had a high glochidial loss during the metamorphosis phase. For example, *P. fluviatilis* was the second highest infested fish 2 days pi with 43.4 larvae per gram fish weight. In the end, only an average number of 2.4 juvenile mussels per gram fish weight fully developed on this species. There was a statistically significant difference between the group of good hosts and the group of poor hosts in regard to the initial infestation 2 days pi (pairwise Wilcoxon rank sum test with Boferroni correction, $p < 0.05$) and also in regard to the juvenile mussel excystment (pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$). The group of good hosts had higher rates of initial infestation as well as a higher juvenile mussel excystment (good hosts: average glochidial infestation 43.1 larvae g^{-1}, average number of excysted mussels 18.7 juveniles g^{-1}; poor hosts: average glochidial infestation 28.2 larvae g^{-1}, average number of excysted mussels 3.5 juveniles g^{-1}).

5.4.2 Duration of metamorphosis and juvenile mussel excystment

The difference between the two groups of host fish species was also evident from the differences in duration of juvenile mussel excystment which was significantly longer in the group of good hosts than in the other six species (pairwise Wilcoxon rank sum test with Boferroni correction, $p < 0.001$). The average duration of juvenile mussel excystment on the four best hosts was 589 degree-days (dd), the average duration of excystment on the poor host species was 277 dd only. Regarding single fish species, all hosts also differed significantly regarding the duration of juvenile mussel excystment (non-parametric Kruskal-Wallis sum of rank test, $p < 0.001$). The longest duration of excystment could be detected on *L. idus* and *C. idella* (622 dd and 619 dd), the shortest excystment phase was found on *R. amarus* and *R. rutilus* (190 dd and 204 dd). Duration of juvenile *S. woodiana* excystment on the best four host fish species and number of excysted juveniles per gram fish weight per species over the time in degree-days as well as the cumulative percentage of excysted juveniles over the time in degree-days on the four best hosts is given in Fig. 5.1A and B. In contrast, there was no significant difference between the host

fish species regarding the duration of metamorphosis (time span from the start of the experiment until the beginning of the juvenile mussel excystment, non-parametric Kruskal-Wallis sum of rank test, $p > 0.05$) (average of 172 dd until the first juvenile mussel drop-off).

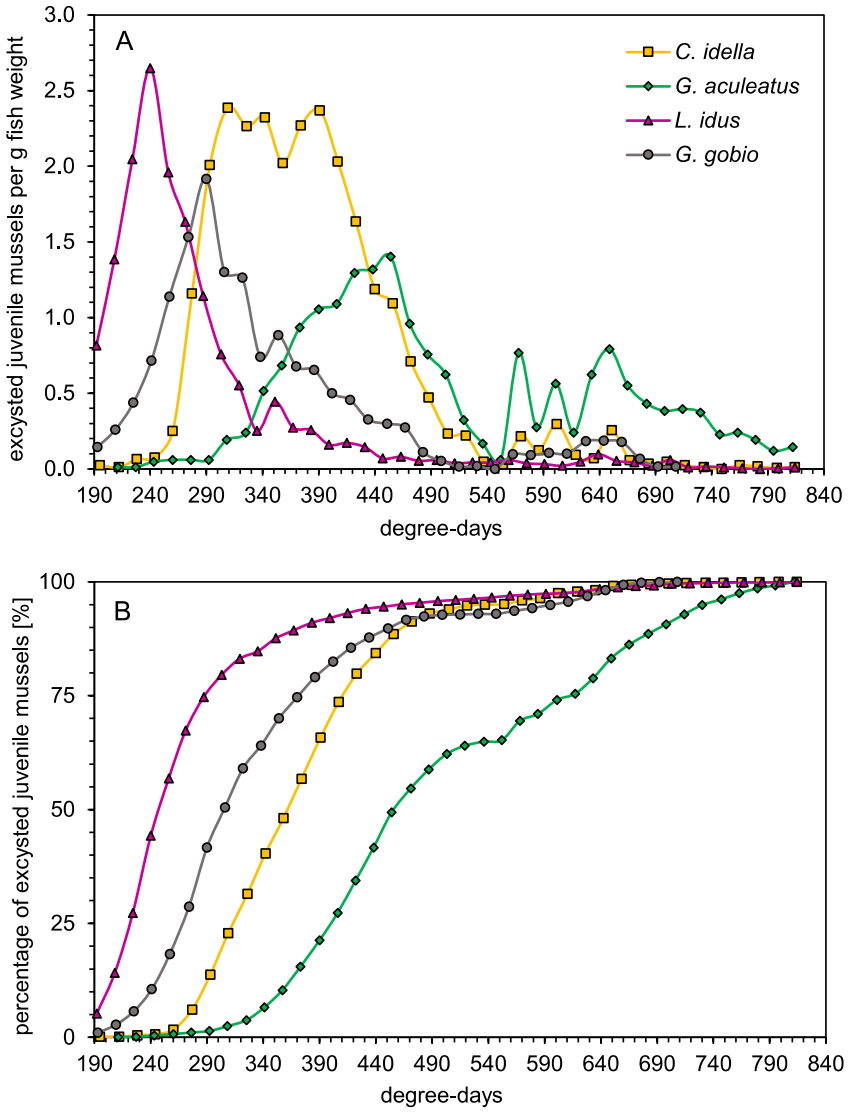

Figure 5.1: Duration of juvenile mussel excystment of *S. woodiana* on the four best host fish species: A: Number of excysted juvenile mussels per gram fish weight and day over the time in degree-days. B: Cumulative percentage of excysted juvenile mussels over the time in degree-days.

5.4.3 Glochidial development on co-invasive fish species

Whilst *C. idella* was the best host fish for *S. woodiana*, the second non-native fish species *P. parva* was one of the least suitable host fish species with an average of 1.0 juvenile mussels g^{-1} only. The glochidia of *S. woodiana* developed more successful on most tested fish species native to Europe (i.e. from outside the original *S. woodiana* distribution range) than on *P. parva*. There were also no significant differences between native and invasive fish species in regard to initial infestation rate 2 days pi, rate of juvenile mussel excystment and duration of juvenile mussel excystment (pairwise Wilcoxon rank sum test with Boferroni correction, $p > 0.05$ in all cases). Both invasive fish showed a mortality rate of average 28% during juvenile mussel excystment, the native fish species had an average mortality rate of 16%. *Leucaspius delineatus* had the highest mortality rate during juvenile mussel excystment with 50%, followed by *C. idella* (47%). The lowest mortality rates were found in *S. trutta*, *R. rutilus* and *P. fluviatilis* (0% for all three species) (Table 5.2). In total, the mortality rate calculated for all fish species during juvenile mussel excystment added up to 18%.

5.4.4 Comparison between *S. woodiana* and native *Anodonta* species

The three mussel species differed significantly regarding the initial infestation rate of all fish species (non-parametric Kruskal-Wallis sum of rank test, $p < 0.001$) (Fig. 5.2A) and the rate of juvenile mussel excystment on all hosts (non-parametric Kruskal-Wallis sum of rank test, $p < 0.001$) (Fig. 5.2B). *Sinanodonta woodiana* had the highest infestation rates 2 days pi with an average number of 34.1 larvae per gram fish weight calculated for all tested fish species as well as the highest numbers of excysted juvenile mussels per gram fish weight with an average of 9.6 juveniles g^{-1}. Combining the initial infestation rates of both native *Anodonta* species (average values of the initial attachment rate of *A. anatina* and *A. cygnea*), *S. woodiana* showed the highest initial infestation rates on all tested fish species (Fig. 5.2A). The experiment with *S. woodiana* also revealed significant differences in the duration of juvenile mussel excystment compared to the native *A. anatina* (pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.001$) and *A. cygnea* (pairwise Wilcoxon rank sum test with Bonferroni correction, $p < 0.05$). Regarding the duration of excystment on all tested fish species, *A. cygnea* had in total the shortest duration of juvenile mussel drop-off with an average of 358 dd, followed by *S. woodiana* with an average of 402 dd. The longest duration of juvenile mussel excystment was observed in *A. anatina* (average duration of 600 dd, calculated for all tested fish species).

Although all three mussel species differed significantly in initial infestation rate, success of juvenile mussel excystment and duration of excystment, there was no significant difference between the mussel species in regard to the fish mortality rate calculated for all fish species (non-parametric Kruskal-Wallis sum of rank test, $p > 0.05$).

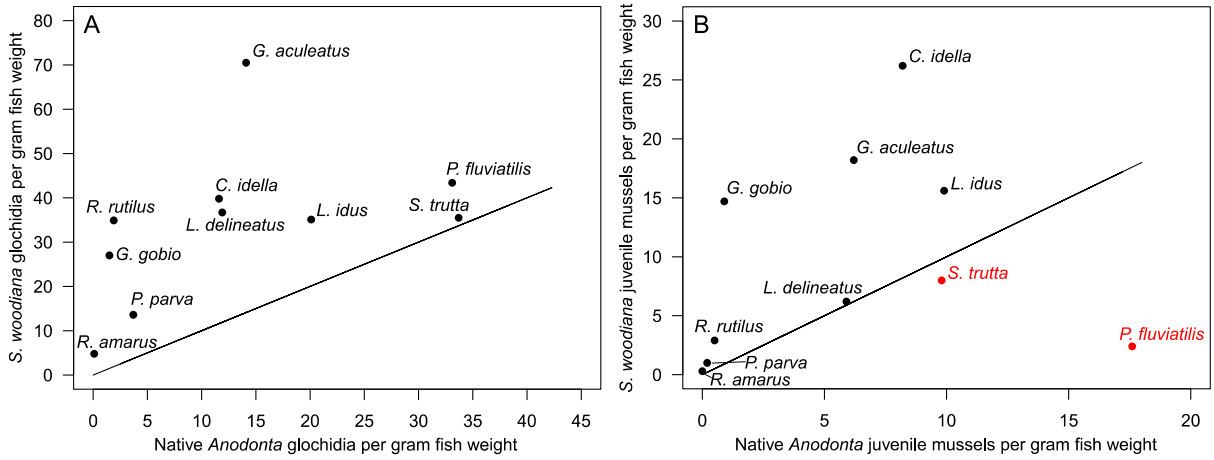

Figure 5.2: Comparison of host fish suitability for the mussel species *S. woodiana* with both native *Anodonta* species (*A. anatina* and *A. cygnea*): A: Average initial infestation of ten different fish species with glochidia of the mussel species (glochidia per gram fish weight); B: Average excystment of juvenile mussels on ten different fish species (juvenile mussels per gram fish weight); points above the line represent fish species with a higher infestation of *S. woodiana* compared to both native *Anodonta*. Marked in red: fish species with a higher rate of native juvenile mussel excystment only.

5.5 Discussion

Ten different fish species (eight native and two invasive) were simultaneously infested in this study with the larvae from an established German population of the invasive *S. woodiana* (Chinese pond mussel). In line with our hypothesis, all ten different fish species were identified as suitable hosts for the larvae of *S. woodiana* including both native and invasive fishes. Due to the fact that the same experiment was also performed previously with the native *A. anatina* and *A. cygnea* (Huber and Geist, 2017, 2019), the results of the infestation success of the three freshwater mussel species allow direct comparisons and conclusions about the impact of the invasive mussel on host competition with native anodontines. Native *Anodonta* populations are already in decline due to diverse reasons and the host competition will increase their decline in light of the ongoing spread of *S. woodiana* in Germany and Europe. The glochidia of *S. woodiana* successfully infested all tested fish species, native and invasive ones, but with marked preferences. This result confirms the findings of Douda et al. (2012b) in the Czech Republic,

where the larvae of *S. woodiana* also infested invasive as well as native fish. As broad host generalist that has the ability to infest also fish species that do not have evolutionary contact, *S. woodiana* has a high invasion potential as host community structure generally influences the establishment and prevalence of parasites (Holt et al., 2003). Together with the fact that *S. woodiana* can tolerate a variety of different environmental conditions (Douda et al., 2012b) and has a higher stress tolerance (Bielen et al., 2016), its fast spreading throughout Europe will likely continue, especially if mussels or infested fish are spread by human activities in fisheries management.

5.5.1 Reproductive success of *S. woodiana*

Whilst the glochidia of *S. woodiana* infested all tested fish species, metamorphosis success as well as success of juvenile mussel excystment and duration of juvenile mussel excystment differed between the hosts, as previously also described for the fish hosts in its natural Asian range (Dudgeon and Morton, 1984). This was also observed for the native *A. anatina* (Huber and Geist, 2019a) and *A. cygnea* (Huber and Geist, 2017) as well as for the native *Unio crassus* (Philipsson, 1788) (Taeubert et al., 2012b) and thus seems to be a general characteristic in host use of generalist freshwater mussel species. The different developmental progress of glochidia encysted on different host fish reveals that host suitability itself has substantial influence on glochidial development (Taeubert et al., 2012b). *Sinanodonta woodiana* is a fast-growing freshwater mussel with a relatively short lifespan (Dudgeon and Morton, 1983). Especially, different excystment times on different hosts enhance the possibility for the juvenile mussels to spread continuously by migrating fish and to generate new populations (Huber and Geist, 2019a; Taeubert et al., 2012b; Taeubert et al., 2014; Watters and O'Dee, 1999).

In total, all tested fish can be separated into a group of good and a group of poor hosts. In contrast to other host fish generalists (like *A. anatina* or *A. cygnea*), this differentiation of the fish in the two groups of hosts according to their suitability was not already evident after the initial infestation 2 days pi. The glochidia of *S. woodiana* showed high infestation rates on most of the tested fish, but also high larval losses during metamorphosis (for example on *P. fluviatilis*). This is in contrast to results from Douda et al. (2012b) where all tested fish species had high rates of transformation and interspecific differences in transformation success among hosts were low. However, Douda et al. (2012b) only used a few individuals per species and single specimens were infested separately whereas differences among fish species in juvenile mussel excystment may only be apparent in a simultaneous infestation. The successful invasion of *S.*

woodiana into new regions of the world perhaps also depends on this infestation strategy: the glochidia unspecifically attach to all fish, including species from outside the mussels' original distribution area. Despite of co-infestation of poor hosts with low transformation success of the mussels, chances of colonization of new habitats are increased.

The assumption that the success of larval metamorphosis as well as the success of juvenile mussel excystment of *S. woodiana* would be higher on both co-invasive fish species *C. idella* and *P. parva* was not confirmed. The best host fish species for the glochidia of *S. woodiana* was the co-invasive *C. idella*. This is not surprising, since co-adaptation in mussel-host relationships has been previously described (Taeubert et al., 2010) and since *C. idella* is also one of the major hosts in the mussels' natural Asian home range (Watters, 1997). In contrast, the second non-native fish *P. parva* was one of the worst hosts for *S. woodiana*. *P. parva* often naturally co-occurs in the same Asian regions and basins like *S. woodiana*, for example the River Amur basin (Pinder et al., 2005). Thus, a differentiation in host fish suitability also appears under hosts that naturally co-occur with *S. woodiana* in its native Asian range and even in mussel species using a broad spectrum of hosts. In any case, the further introduction and spreading of *C. idella* and *P. parva* in European water bodies will increase the spreading of *S. woodiana* and should be prevented. Interestingly, larval attachment and juvenile mussel excystment was also detected on *R. amarus*. It usually parasitizes European freshwater mussels like *A. anatina* or *A. cygnea* for its own larval development (Reichard et al., 2007; van Damme et al., 2007). Reichard et al. (2012) also revealed that *S. woodiana* successfully developed on *R. amarus* whereas the fish was unable to use *S. woodiana* for its own reproduction. Therefore, invasive bivalves may temporarily benefit from a coevolutionary lag by exploiting evolutionary naïve hosts (Sousa et al., 2014). Although *R. amarus* is listed as Least Concern in Europe (Freyhof, 2010), it is recognized as Endangered or Vulnerable in many European Countries (Kozhara et al., 2007). For example, *R. amarus* is listed as Vulnerable in the Austrian Red List (Wolfram and Mikschi, 2007) and it is listed as Endangered in the German Red List of threatened species (Haupt et al., 2009). Thus, an increasing spreading of *S. woodiana* in Germany could also increase the threat of *R. amarus* if *S. woodiana* reaches extremely high population densities, dislodging native mussels, in waterbodies with *R. amarus* populations, as demonstrated for areas in the Czech Republic (Douda et al., 2012b).

Extreme differences in the duration of juvenile *S. woodiana* excystment on different hosts, but no differences in the duration of the metamorphosis were observed. The excystment of juvenile mussels started almost simultaneously on all tested fish, even if the temperatures of the water within the holding units differed from each other. Normally, the developmental time and the

growth rates of invertebrates are dependent on water temperatures (Manoj Nair and Appukuttan, 2003; Taeubert et al., 2014) and increasing water temperatures lead to shorter developmental durations and vice versa (until species-specific temperature limits) (Taeubert et al., 2014). Nevertheless, metamorphosis phase of the glochidia of *S. woodiana* on *S. trutta* was shorter than on other fish species maintained at higher average water temperature (comparing the calculations in degree-days) and no prolongation of metamorphosis at lower water temperatures was observed in this species. Instead, duration of excystment was longer in the group of good hosts, irrespective of warmer water temperatures. Thus, the suitability of the host itself seems to be of crucial importance in determining duration of the development and the excystment as for example also true for the excystment of *Margaritifera margaritifera* (Taeubert et al., 2010, 2013b). This warrants special caution in fisheries management measures if transferring fish from waters with populations of *S. woodiana* to water bodies where it does not yet occur. The variable time span for reproduction and larval development on the fish requires inspection of fish for larval infestation before translocating them into other water bodies.

5.5.2 Comparison to the reproduction of native *Anodonta*

The most important objective in comparatively analyzing the reproductive success of *S. woodiana* on native and invasive fish species is to get information about possible consequences of the increasing invasion of this Asian mussel for the native freshwater mussel species. Thus, the results of this experiment were compared with the results of two methodologically identical experiments with the native anodontines (*A. cygnea* and *A. anatina*). The comparability of the results of all three experiments could be guaranteed due to the same methodology, for example the same parameters of the infestation bath (temperature and glochidial concentration), the use of the same fish species and the same holding conditions of the fish as well as the same evaluation of the results. Moreover, it was ensured that parameters that differed between the experiments had no influence on the procedure and the results (no correlation between the total fish weight in the infestation bath and glochidial infestation rates 2 days pi (Spearman´s rank correlation, $p > 0.05$), no correlation between the mortality rate of the fish during juvenile mussel excystment and the number of juveniles per gram fish weight (Spearman´s rank correlation, $p > 0.05$), colder temperatures within the holding units of the fish did not influence the duration of the metamorphosis phase). Especially different water temperatures in the tanks of the fish could affect the duration of metamorphosis phase and the success of juvenile mussel excystment. Lower temperatures might allow unionids to use some species of hosts that reject

infestations at higher temperatures (Roberts and Barnhart, 1999) and can also be the reason for an extended duration of metamorphosis and juvenile mussel excystment and the reason for higher survival rates of juvenile mussels due to a longer parasitic phase on the fish (Marwaha et al., 2017). However, duration of the metamorphosis phase was not prolonged at colder temperatures for *S. woodiana*. In general, the different water temperatures may influence the duration of the excystment (as shown for the experiment with *A. anatina*), but cannot be the explanation for the differences in the number of excysted juveniles (Huber and Geist, 2019a). For example, *S. woodiana* had a higher rate of juvenile mussel excystment on seven out of ten tested fish species only (*C. idella*, *G. aculeatus*, *L. idus*, *G. gobio*, *R. rutilus*, *P. parva* and *R. amarus*) compared to the native anodontines. Moreover, *A. cygnea* had a higher excystment rate on *G. aculeatus* compared to *A. anatina* and *A. anatina* had higher excystment rates on *S. trutta*, *L. delineatus* and *P. fluviatilis* compared to both other mussel species. These differences cannot be explained by differences in water temperatures during the experiments.

Thus, differences in initial infestation and success of juvenile mussel excystment can be driven by differences between the three mussel species. One possible reason for the higher average initial infestation rate of the larvae of *S. woodiana* is the higher attachment capability of the glochidia due to their bigger size (Wächtler et al., 2001). Interestingly, not all fish species had the highest infestation rates with the glochidia of *S. woodiana* ruling out that glochidial attachment capabilities are not the only reason for a higher juvenile mussel excystment. Instead, fish species themselves have a high influence on glochidial development. For example, larval infestation on *S. trutta* was higher with *A. anatina* than with *S. woodiana*. Therefore, *S. trutta*, a fish species which is a very important host for some native freshwater mussels (in fact the exclusive host for *M. margaritifera* in many central European populations) (Geist et al., 2006; Taeubert and Geist, 2017), is unsuitable for the invasive *S. woodiana*. Due to the unsuitability of *S. trutta* as host and due to the preference of *S. woodiana* for standing and slow flowing waters, we assume that the expansion and competition of *S. woodiana* with native mussels should be low in rivers where *S. trutta* predominates. In the native range of *S. woodiana*, salmonids like *S. trutta* as well as percids like *P. fluviatilis* usually do not co-occur with the mussel. The best host fish in the native Asian range of *S. woodiana* are species from the family of cyprinids (Dudgeon and Morton, 1984; Watters, 1997). Thus, co-evolutionary mechanisms of host compatibility between mussels and fish species may play a role for *S. woodiana*, as previously described for *U. crassus* (Taeubert et al., 2012b) and *M. margaritifera* (Taeubert et al., 2010). In contrast, one of the best host fish species for the native mussel *A. anatina* was the non-native *C. idella* (Huber and Geist, 2019a). Suitability of hosts for mussel larvae may therefore not only

determined by co-evolutionary adaptations but also by the individual fish specimens, their genetic constitution and immune defense.

5.5.3 Invasion of *S. woodiana*: consequences for native mussel species

Regarding the possible consequences for native mussel species due to the increasing invasion of *S. woodiana*, host fish suitability will play a crucial role in the future. It has been shown in this experiment that the glochidia of *S. woodiana* highly infested most of the tested fish species without differentiation between native and non-native fish. Although the success of excystment of *S. woodiana* differed between the tested host fish species, it was on average higher than the excystment success of the native anodontines. Moreover, *S. woodiana* grows faster than the native *Anodonta* and has the ability to produce glochidia two or three times per year (Sárkány-Kiss et al., 2000) in very high numbers (Wächtler et al., 2001). In contrast, native *Anodonta* species produce glochidia only one time per year, between winter and spring (Lopes-Lima et al., 2017; Niemeyer, 1993), and in smaller numbers than *S. woodiana* (Wächtler et al., 2001). In addition, our current experiment also suggests that *S. woodiana* uses more host fish species (for example also *R. amarus*, which had no successful larval development with the glochidia of native anodontines) than the native mussels and has the shortest metamorphosis phase of all three tested mussel species which was also independent from the temperature within the holding units of the fish. Although the experiment with *A. cygnea* also started in May and had the same average water temperatures in the holding units, metamorphosis phase was longer on all tested fish species. Due to a faster metamorphosis and mussel excystment, juvenile *S. woodiana* will start their sessile life earlier and have higher chances to survive during the winter months because of better condition. Moreover, Donrovich et al. (2017) found that the transformation success rate of *A. anatina* was significantly reduced on host fish that were infested before with the larvae of *S. woodiana* compared to naïve hosts. Even if the juvenile mussel excystment of *S. woodiana* is lower on some hosts, the high infestation of the fish with its larvae will likely lead to a decreased second infestation with other native mussel larvae that co-occur in the same waterbody.

The increasing number of self-recruiting populations of *S. woodiana* in Europe suggests that their host-larvae relationship is very efficient, and their spreading is not limited by missing hosts. For example, the best host fish for *S. woodiana* (the non-native *C. idella*) does not occur in the Öberauer Donauschleife (origin of the mussel specimens used for this experiment) (Barnerßoi, 2012), but the population of *S. woodiana* in this habitat is increasing and has

persisted for a long time (Barnerßoi, 2012; Fischereifachberatung Niederbayern, personal comment). A number of 18 native and four non-native species were described for the Öberauer Donauschleife, including *Perca fluviatilis, Rutilus rutilus, Rhodeus amarus* and *Pseudorasbora parva* which were also included in this study (Barnerßoi, 2012; Bezirksfischereiverein Straubing e.V., personal comment). However, these four species were found to be unsuitable hosts for *S. woodiana*. Matching the results of this study, the population of the Öberauer Donauschleife must be adapted to other host fish species, maybe especially native ones. Many of the self-recruiting populations of *S. woodiana* also occur in artificial fish ponds (for example carp ponds). Use of such fish, infested with *S. woodiana* glochidia, in stocking programs will increase the spread of this invasive mussel. Additionally, direct selling of adult *S. woodiana* in pet shops and garden centers will also increase the risk of further spreading in natural lakes and ponds, particularly if staff are not trained and if mussels are declared as native *Anodonta* (Duempelmann, 2012). Unfortunately, the selling of this invasive mussel is not yet forbidden by law, but the spreading of the species in natural habitats, where it does not occur naturally, is forbidden for example by German law (Bundesnaturschutzgesetz §40 (1), Bundesministerium der Justiz und Verbraucherschutz, 2009).

Factors like a low water temperature are unlikely to constrain the ongoing invasion of *S. woodiana* as visible from the population in the Öberauer Donauschleife (with the area sometimes being completely frozen during winter), or populations in Sweden (von Proschwitz, 2008). Due to the same habitat preferences in standing or slow flowing waterbodies, the invasion of *S. woodiana* will especially be an ongoing threat for the native *A. cygnea*, which exclusively occurs in these kind of water bodies (in contrast to *A. anatina* that also lives in faster flowing rivers) (Niemeyer, 1993). *Anodonta cygnea* is endangered in many European countries invaded by *S. woodiana*. For example, *A. cygnea* is listed as Endangered in Poland on the Polish Red List (Zając, 2002), it is also listed as Vulnerable on the Red List of Threatened Species of the Czech Republic (Farkač et al., 2005) or listed as Near Threatened on the Austrian Red List of Molluscs (Reischütz and Reischütz, 2007). In Germany, *A. cygnea* is also endangered (Jungbluth and von Knorre, 2009), and it has to be monitored how the invasion of *S. woodiana* will affect co-occurring populations of *A. cygnea* (and also all other co-occurring native mussel species) in the wild. The experiment conducted herein only gives first insights into host-parasite relationship of the invasive *S. woodiana* by simultaneous host infestation, showing the higher reproductive success of *S. woodiana* compared to native *Anodonta* species. Further experiments have to follow, where self-recruiting populations of *S. woodiana* and their competition for hosts

with native freshwater mussels must be considered and analyzed separately, because host fish suitability can potentially differ among different water bodies and mussel populations.

6 General discussion

The results of this thesis clearly show the differences between the three mussel species *A. cygnea*, *A. anatina* and *S. woodiana* regarding their host fish use and emphasizes the competition between the native anodontines and the invasive Chinese pond mussel. For the first time a simultaneous infestation of fish species was performed and allows deep insights into the differences of host-parasite relationships between the different species. The competitive advantage of the non-native *S. woodiana* compared with the native *A. cygnea* and *A. anatina* regarding its reproductive success is clearly shown. Due to their fast spreading, the analysis of the influence of non-native fish and mussel species on the life cycle of the endangered native anodontines is an important step for future management and conservation activities.

6.1 Differences in the reproductive success of native *Anodonta*

Studies of host-parasite interactions between European freshwater mussel larvae and fish hosts normally focus on the analyses of one single mussel species and the suitability of different fish species or strains as host for the glochidia of this single mussel (Douda et al., 2012a, 2013; Österling and Larsen, 2013; Taeubert et al., 2010, 2012b). This study compared two sister mussel species regarding their host use, timing of excystment and success of metamorphosis by performing identical experiments with the same fish species under the same laboratory conditions. Moreover, the simultaneous infestation of all fish species in one infestation bath enables to analyze, which fish species is preferential attached by the mussels' glochidia and which species is not attached if there is a possibility to choose between different hosts. The design of the funnel-shaped holding units for hosting the fish during the experiment is a completely new and unique development with a lot of advantages for these kind of analyses in mussel-host relationships (Fig. 3.1, chapter 3). It allows an easy water change and check of the water without an extreme intervention in the experiment and without stressing the fish and affecting the results of the analyses. Moreover, fish of one species can stay together in one holding unit and will not get stressed additionally due to separating individual specimens. In total, the results showed that both native *Anodonta* species differ extremely in their host use. Thus, future conservation measures must be developed for *A. cygnea* (swan mussel) and *A. anatina* (duck mussel) separately.

Until the middle of the 20th century the number of *Anodonta* species in Europe was unclear and all species were merged into the single taxon *Anodonta cygnea* (Lopes-Lima et al., 2017). The

visual differentiation between both species is very difficult due to their similar morphology and a clear determination of the species can only be achieved by a genetic identification (Lopes-Lima et al., 2017; Zieritz et al., 2012). In the past, only little attention has been paid to *Anodonta* species in Europe since other freshwater mussels like *M. margaritifera* and *U. crassus* are more threatened and studies about host-parasite relationships mainly focus on these highly endangered species (Lopes-Lima et al., 2017). Therefore, the host-parasite relationship of these endangered species as well as about other highly endangered species from other continents like North American freshwater mussels is well known (Lopes-Lima et al., 2017; Strayer et al., 2004; Williams et al., 1993), but only little information exists about the host-parasite relationship of European *Anodonta*. Especially analyses about *A. cygnea* and its hosts are rare although populations of this freshwater mussel are decreasing and *A. cygnea* is more in decline in many countries than *A. anatina* (Lopes-Lima, 2014a, b). The more important are the results of this study especially the understanding that both anodontines are very different in their host use and that both species cannot simply consider as identical. These differences are shown in chapter 3 and 4. There, not only the differences in host fish suitability, but also the differences in duration of metamorphosis and juvenile mussel excystment as well as success of initial infestation and juvenile mussel excystment are mentioned. Compared to *A. cygnea*, *A. anatina* had higher infestation rates on all tested fish species as well as a lower average weight-normalized glochidial loss from the initial infestation to the excystment of juvenile mussels. Populations of *A. anatina* can be found in lakes and ponds, sympatric with *A. cygnea*, but also in rivers and lotic habitats, often sympatric with mussel species like *U. crassus* (Franke, 1993; Lopes-Lima, 2014a). Therefore, the distribution area of the duck mussel is wider compared to its sister species. Moreover, *A. anatina* produces the biggest glochidia of all native freshwater mussels in Central Europe (Scharsack, 1994) with high attachment capabilities on different body parts of a host fish. This might enable a higher initial infestation rate of a very diverse fish community and its higher distribution in contrast to *A. cygnea*. Moreover, the sister species differ in their sexual strategy. The duck mussel is predominantly dioecious but tend to a more plastic sexual strategy highly dependent on habitat characteristics, populations of the swan mussel are predominantly hermaphroditic (Bauer, 2001b; Hinzmann et al., 2013). Populations of *A. anatina* in river systems may have a better opportunity to get in contact with other populations due to a wider glochidial transport and to have a better genetic exchange than for example populations of *A. cygnea* in isolated lakes and carp ponds. Thus, the duck mussel might have the opportunity to a faster evolutionary adaptation to new hosts and a diverse host community, whereas populations of the swan mussel are at risk of getting into a reproductive isolation in closed systems. If suitable

hosts are missing in these closed systems or if hosts are removed from these water bodies, populations of *A. cygnea* are threatened or will completely go extinct very fast. Therefore, the status of *A. anatina* is more advantageous due to its higher average excystment rate of juvenile mussels on different host fish species as shown in chapter 4.

Interestingly, the only fish species with higher excystment rates of the larvae of *A. cygnea* was *G. aculeatus*. Although *A. anatina* showed a higher glochidial attachment rate on *G. aculeatus* than *A. cygnea*, the stickleback has a better suitability as host for the larvae of the swan mussel. The stickleback might be a better host for *A. cygnea* due to their similar habitat preferences. *Gasterosteus aculeatus* also prefers standing and slow flowing water bodies (NatureServe, 2019). There might be a better co-evolutionary adaptation between the stickleback as host for the swan mussel than between the stickleback and the duck mussel. On the other hand, a very important result of the experiment was that the bitterling is not a suitable host for both native *Anodonta* species. There seems to be another co-evolutionary development between the bitterling and the anodontines since *R. amarus* uses the mussels themselves as hosts for its eggs (Reichard et al., 2007; van Damme et al., 2007). Although both *Anodonta* show similarities in co-evolutionary development to host fish species (for example the best host fish species for both native *Anodonta* was *P. fluviatilis*), they differ significantly in their order from good to poor hosts and the excystment of juvenile mussels on different host fish species. These differences are especially important if both mussel species co-occur in the same water bodies, because acquired immunity of fish species to one mussel species confers immunity to other related species and can especially be stronger between closely related mussel species (Dodd et al., 2005; Haag and Stoeckel, 2015; Shiver, 2002). Therefore, it is logical that both anodontines have to use another composition of suitable hosts. Otherwise, they would always compete for suitable host individuals. A very important result of the studies described in chapter 3 and 4 is, that these closely related freshwater mussel species differ extremely in their host use and should be considered separately regarding management, conservation or artificial breeding measures. Thus, a genetic determination of the species must be the basis of successful management measures and for a successful protection of populations of these threatened native *Anodonta* species.

6.2 Influences of non-native fish species on the host fish use of native *Anodonta*

Introductions of non-native fish species in native freshwater ecosystems increase due to human activities such as aquaculture, recreational and commercial fisheries, biological control, and pet

and ornamental animals' industry (Gozlan, 2008; Kolar and Lodge, 2001; Modesto et al., 2018; Padilla and Williams, 2004). These introduced species can harm total aquatic ecosystems and led to a decrease or extinction of native species (Modesto et al., 2018; Simberloff et al., 2013), as well as to changes in host-parasite relationships between mussels and native host fish species (Modesto et al., 2018). Recent investigations summarized that in most cases, non-native species may not constitute a suitable host to freshwater mussels due to the lack of physiological, ecological and evolutionary adaptations (Salonen et al., 2016; Strayer, 2008; Watters and O'Dee, 1998). For example, Salonen et al. (2016) described the invasive brook trout (*Salvelinus fontinalis*) as unsuitable host for the endangered freshwater pearl mussel (*Margaritifera margaritifera*) in Europe. Therefore, if the main host (brown trout) of *M. margaritifera* will be displaced by the invasive brook trout, the local freshwater pearl mussel population will not survive in these habitats (Salonen et al., 2016). If the glochidia of native freshwater mussels will frequently attach to less-suitable, non-native hosts, the reproductive success of freshwater mussels will be reduced (Lopes-Lima et al., 2017). Therefore, the ongoing expansion of invasive fish species and the introduction of new possible invaders will likely result in a dilution of suitable host resources (Clavero, 2011; Modesto et al., 2018; Villéger et al., 2015).

Recent findings about the native *A. anatina* also demonstrated that this host fish generalist is less successful to develop on some non-native fish species (Douda et al., 2013). It is mentioned that the developmental success of *A. anatina* on non-native fish species was significantly less than the developmental success on native fish species (Douda et al., 2013). As mentioned in chapter 3 and 4, these results could not be confirmed because the non-native fish species *C. idella* (grass carp) turned out to be a suitable host for the native *Anodonta*. It was very surprising that the grass carp was the second-best host for *A. anatina* and one of the five best hosts for *A. cygnea*. The infestation of *C. idella* individuals with glochidial larvae as well as the excystment of juvenile anodontines was successful. This information showed that co-evolutionary mechanisms may not be the crucial aspect in host-parasite relationships and that host fish generalists like native *Anodonta* may profit from the introduction of new fish species because their host fish spectrum may grow. On the other hand, host fish specialists like the freshwater pearl mussel (*M. margaritifera*) may not have the chance to adapt to new hosts because of the fast spread of new fish species and the fast decline of native fish (Salonen et al., 2016).

The results from chapter 3 and 4 also emphasize that it has to be differentiated between non-native fish species and their role as hosts for native freshwater mussels. Another non-native fish *P. parva* was a very unsuitable host for both native anodontines in this study. Even the infestation success on topmouth gudgeon was very low. These results must influence further

management measures and must also be integrated in conservation strategies for other native freshwater mussels like for example *Unio pictorum* or *Unio tumidus* which also often co-occur with these non-native fish species in fish ponds and in standing water bodies in general (van Damme, 2011a, b). Non-native fish and their influence on host-parasite relationships between mussels and fish must be further investigated and studied for every single species separately. Although some non-native fish can be suitable hosts for native mussels, their influences on aquatic ecosystems can be very dramatic (Geist, 2011), and thus their willful introduction in native waters should be prevented.

6.3 Consequences for native freshwater mussels due to the spreading of the invasive *S. woodiana*

Not only non-native fish species, but also non-native mussel species spread extremely in the last decades and threaten native aquatic ecosystems and organisms therein (Geist, 2011; Sousa et al., 2014; Strayer et al., 1999; Strayer, 2006). There are a lot of information about the invasive *Corbicula fluminea* or *Dreissena polymorpha* which are spreading around the world and cause ecological and economical damage in many lakes and rivers (Sousa et al., 2008; Strayer et al., 1999; Strayer, 2006). It is also well known how these invasive mussels harm native ones, for example by competition for food or space (Ferreira-Rodríguez et al., 2018, 2019), but *C. fluminea* and *D. polymorpha* have different life cycles as native freshwater Unionida and cannot harm their host-parasite relationship directly. In contrast, the spreading of non-native mussels with the same life cycle than native Unionids, like the invasive freshwater mussel *Sinanodonta woodiana* (Chinese pond mussel), may be more harmful for native bivalves due to their competition for food, space and host fish species (Douda et al., 2012b).

Chapter 5 clearly reveals that, compared to both native *Anodonta*, *S. woodiana* has a broader spectrum of suitable host fish species and uses native and non-native fish as hosts for its glochidia. On an average, S. *woodiana* had the highest larval attachment rate, the highest rate of juvenile mussel excystment and the shortest metamorphosis phase of all three tested mussel species. The fact that it uses the same host fish species as *A. cygnea* and *A. anatina*, occurs in the same water bodies and produces a higher number of glochidia (Beran, 2008; Wächtler et al., 2001) is quite disturbing. Moreover, *S. woodiana* can reproduce several times per year, and fish that were previously infested with the glochidia of the Chinese pond mussel have a decreased second infestation with the larvae of other mussel species (Donrovich et al., 2017). In general, very similar reproduction strategies of freshwater mussels offered limited possibilities

for coexistence. Mussel species were predicted to coexist when they differed in terms of their success in contacting different fish host species (Rashleigh and DeAngelis, 2007). Furthermore, changes in fish host ratios through selective mortality, fishing pressure, fish stocking could negatively affect coexistence of mussel species (Rashleigh and DeAngelis, 2007). Thus, a further spreading of the invasive *S. woodiana* will have consequences for the reproduction success of native freshwater mussels that co-occur in the same water bodies. Especially *A. cygnea* will be affected because it prefers exactly the same habitats as *S. woodiana* and is already threatened in many countries (Lopes-Lima, 2014b; Zettler et al., 2006). Other native freshwater mussels that will be affected by the spreading of the Chinese pond mussel are the species *Unio pictorum* (painter's mussel) and *Unio tumidus* (swollen river mussel) which also often co-occur in standing and slow flowing water bodies (van Damme, 2011a, b). There is hardly any information about the host preference of both species. Like the anodontines, *U. pictorum* and *U. tumidus* received less attention in the past because they are not as threatened as other mussel species like *M. margaritifera* or *U. crassus* in Europe (Lopes-Lima et al., 2017). For both species, population trends are unknown and exact population data are not available throughout Europe (van Damme, 2011a, b). Although the status of both species is Least Concern, especially *U. tumidus* is listed as Critically Endangered (for example in Austria) or Endangered (for example in Germany) in some countries and a declining population trend is still visible for both species (van Damme, 2011a, b). Research about both species and conservation actions are needed to stop the ongoing decline of populations of these Unionids in Europe. Although the reproductive cycles of many *Unio* species like *U. tumidus* and *U. pictorum* are poorly known (Lopes-Lima et al., 2017), both Unionids are mentioned to use a wide range of host fish species (Lopes-Lima et al., 2017). Due to their co-occurrence with *S. woodiana* in the same habitats, these freshwater mussel species will most likely be affected as well by the invasion of the Chinese pond mussel and their reproductive success may also decline in the future. Research about *U. tumidus* and *U. pictorum* and their host-parasite relationships is urgently needed to get also information about the influence of the spreading of *S. woodiana* on other native freshwater mussel species than *Anodonta*.

6.4 Key topics for conservation and management efforts

With the ongoing decline of freshwater mussel populations, also the ecosystem functions they provide will be drastically reduced (Vaughn et al., 2015). Therefore, conservation of all European freshwater mussels is essential to maintain the resulting ecosystem services (Lopes-Lima

et al., 2017). Future management efforts should include all European freshwater mussel species, not only the most threatened ones (*M. margaritifera* and *U. crassus*), because all of them are in decline. Basic data on distribution, population size, accurate identification of threats and basic life history traits are still lacking for many species (Hinzmann et al., 2013; Lopes-Lima et al., 2018). Conservation measures for both native *Anodonta* species are rare since global population trends are still unknown (Lopes-Lima, 2014a, b). *Anodonta anatina* benefits from conservation actions established for *U. crassus* since both species often co-occur in the same river habitats (Bayerisches Landesamt für Umwelt, 2013).

Specific management measures for *A. cygnea* are rare, especially because the number of existing populations is mostly unknown. Due to the common occurrence of populations of the swan mussel in larger lakes, where they are simply not detectable, and their occurrence in carp cultivations, where they are still ignored and not resettled during winter drain, it is not easy to determine the actual distribution of the species (Lopes-Lima, 2014b). Despite, conservation measures for populations inhabiting private ponds are only achievable with the collaboration of the owners.

In general, increasing awareness of the general public about the importance of conserving freshwater bivalves is essential (Lopes-Lima et al., 2018). The involvement of local stakeholders, policy makers, local authorities, and others responsible for freshwater management is a major challenge in achieving successful freshwater mussel conservation (e.g., Linehan, 2007). In addition, collaborative efforts will help to harness public interest and knowledge (Ferreira-Rodríguez et al., 2019). Until today, most people do not even know about the existence of freshwater mussels and they are not aware of their key roles in freshwater ecosystems (Strayer, 2017). Education of all kinds can increase an understanding why freshwater mussels might reasonably be included in decision making about environmental management (Strayer, 2017). The ignorance of the public regarding freshwater mussels is especially shown when invasive species like *S. woodiana* are sold as native *Anodonta* in pet shops or garden centers (Duempelmann, 2012). Knowledge about mussels must be spread not only for conservation efforts of native mussel species, but also for stopping the ongoing expansion of invasive freshwater mussels. As shown in chapter 5, *S. woodiana* will be a threat for the reproduction of native freshwater mussels in the same habitats. Because of its high shell plasticity, there is often a high misidentification of *S. woodiana* with native *Anodonta* (Guarneri et al., 2014) and the consequences of this misidentification are potentially negative for native species. Thus, knowledge and training of all relevant stakeholders is important in the future for conservation and

management of freshwater mussel species and for stopping the spread of invasive mussels like the Chinese pond mussel.

Nevertheless, conservation actions for mussels must always include their host fish (Modesto et al., 2018; Watters, 2007). Since the successful recruitment of freshwater bivalves is highly dependent on the availability of suitable host, the integration of fisheries management in rivers with known mussel populations should also be part of the conservation plans (Lopes-Lima et al., 2017). The attachment of the glochidia on suitable host fish species and the metamorphosis on the host is the crucial phase in the life cycle of freshwater mussels (Stoeckl et al., 2015; Taeubert et al., 2012a, b). Without a suitable host, no juvenile mussel development is possible. Changes in fish community will also affect the recruitment success of the mussels. Thus, in waters with mussel populations, the composition of fish species does not have to be changed during stocking activities. Moreover, relative host abundance should be considered in the selection of sites for relocation and reintroduction of mussel species (Rashleigh and DeAngelis, 2007). Another very important point is that the suitability of fish as hosts for freshwater mussel larvae may not only differ between fish species, but also between different populations and strains of one fish species (Taeubert et al., 2010; Taeubert et al., 2012b). In case of stocking activities or artificial reproduction of mussels, the common origin of the populations of fish and mussels can be crucial for a better reproduction success (Taeubert and Geist, 2017). Thus, all kinds of conservation efforts must be assessed species-specifically and at the regional population level, because even in co-occurring mussel species different factors may limit their recruitment (Lopes-Lima et al., 2017).

Conservation measures must also focus on the quality of mussel habitats. Especially the juvenile mussels that live buried in the riverbed substratum for the first months or years in their life need oxygen-rich interstitial zones for their survival (Denic and Geist, 2015; Taeubert et al., 2012b; Wächtler et al., 2001). Clogged interstitial gaps due to the input of fine sediments would kill the juveniles and prevent the recruitment of the population (Denic and Geist, 2015). Management efforts in regard to mussel habitats must guarantee the survival of the juvenile mussels but also stop the ongoing threats for adult mussels due to habitat fragmentation and pollution of water with mussel populations (Lopes-Lima et al., 2017). All these aspects must be taken into account for future conservation and management efforts regarding freshwater mussel species. A conservation of the mussels can only be successful, if the protection of freshwater ecosystems in total will come into focus of policymakers.

6.5 Outlook

Unionids are keystone species in freshwater ecosystems but knowledge about their relationship with host fish species is still lacking for many European species although this phase on the host fish is crucial for the development of juvenile mussels. Based on the results of this thesis, there is a new level of knowledge in the host-fish use of native *A. cygnea* and *A. anatina* as well as on the invasion success of *S. woodiana*. This knowledge will be an important basis for future conservation of native anodontines as well as for managing the ongoing spread of the invasive Chinese pond mussel. Future research on other native freshwater bivalves should also focus on host-parasite interactioins because missing host fish species is one of the main reasons for the ongoing decline of mussel populations. The presented standardized laboratory experiments based on a system of funnel-shaped holding units can easily be used for studies with other mussel species and facilitate the comparability.

The shown differences in host fish use between the three tested mussel species as well as the different suitability of fish species as hosts emphasize the importance of these analyses for further management measures. However, the results of this thesis should be tested in the wild since a fish species tested as poor host in the laboratory might be the only available host for mussel populations in their natural habitat. Therefore, fish stocking activities must base on the knowledge about the host use of the respective mussel population inhabiting the waterbody.

To get a continuous overview about population trends of the different freshwater mussels, information about newly detected populations should be collected and stored. Information about the population trends would especially be important for freshwater mussels like *A. cygnea* that occur in lakes and ponds sometimes in great depths, and therefore populations are often not known. The swan mussel had the poorest reproductive success compared to the duck mussel and the Chinese pond mussel as described in this thesis. Therefore, conservation has especially be focused on this species. Without the knowledge about existing mussel populations it will be hard to control if management efforts are efficient for a permanent conservation of the mussels. In this case, it would also be very important and helpful if stakeholders are more informed about freshwater mussels, their morphology, life cycle and importance for aquatic ecosystems. Then, volunteers could actively report existing mussel populations and contribute to their conservation.

An improved knowledge about population trends of anodontines can also support the control of the ongoing spread of *S. woodiana*. The presented results emphasize that the Chinese pond mussel competes for hosts with native anodontines and has better reproductive chances than *A. cygnea* and *A. anatina*. Right now, existing and self-recruiting populations of *S. woodiana* are mostly detected accidentally. Detailed knowledge about existing populations of this invasive species would help to avoid the dispersal of glochidia in new waterbodies by transfer of infested host fish. Moreover, the spread could be stopped by informing the people, for example the owners of ponds with *S. woodiana* populations, about the threat of this invasive mussel for native freshwater mussels.

In general, an interdisciplinary cooperation between fisheries management, mussel conservation and water management will be more and more important in the future. The conservation of freshwater ecosystems and the organisms therein as well as an efficient control of the spreading of invasive species will only be successful by this cooperation.

7 Publications and oral presentations

7.1 Publications related to the thesis

Huber, V., Geist, J., 2017. Glochidial development of the freshwater swan mussel (*Anodonta cygnea*, Linnaeus 1758) on native and invasive fish species. Biol. Conserv. 209, 230-238.

Huber, V., Geist, J., 2019a. Host fish status of native and invasive species for the freshwater mussel *Anodonta anatina* (Linnaeus, 1758). Biol. Conserv. 230, 48-57.

Huber, V., Geist, J., 2019b. Reproduction success of the invasive *Sinanodonta woodiana* (Lea 1834) in relation to native mussel species. Biol. Invasions 21, 3451-3465.

7.2 Further publications

Mainzer, K., Huber, V., Schütter, J., 2016. Communication, Mobility and Logistics. In: Wilderer, P.A., Grambow, M. (Eds.), Global Stability through Decentralization? In Search for the Right Balance between Central and Decentral Solutions. Springer International Publishing, Switzerland, pp. 165-180.

7.3 Oral presentations

Huber, V., 2014. Entwicklung von Schutz- und Managementmaßnahmen für heimische Teichmuschel-Arten durch Untersuchung der Interaktion mit Wirtsfischen. 123. Stipendiatenseminar der Deutschen Bundesstiftung Umwelt, Roggenburg.

Huber, V., Geist, J., 2015. Entwicklung von Schutz- und Managementmaßnahmen für heimische Teichmuschel-Arten durch Untersuchung der Interaktion mit Wirtsfischen. Jahrestagung Muschelschutz in Bayern, Freising.

Huber, V., 2015. Entwicklung von Schutz- und Managementmaßnahmen für heimische Teichmuschel-Arten durch Untersuchung der Interaktion mit Wirtsfischen – Zwischenstand des Promotionsprojekts. 132. Stipendiatenseminar der Deutschen Bundesstiftung Umwelt, Ostritz.

Huber, V., 2016. Interaktion zwischen heimischen Teichmuschellarven und Wirtsfischen. 134. Stipendiatenseminar der Deutschen Bundesstiftung Umwelt, Bad Bederkesa.

Huber, V. Geist, J., 2017. Glochidial development of the freshwater swan mussel (*Anodonta cygnea* L.) on native and invasive fish species. Symposium of the Freshwater Mollusk Conservation Society (FMCS), Cleveland (Ohio).

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