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Host-parasite interactions in aquatic ecosystems – The relationship between fishes and endangered freshwater mussels

Jens-Eike Täubert

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Brown trout (*Salmo trutta* L.) with encysted larvae of the freshwater pearl mussel (*Margaritifera margaritifera* L.).

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Preface

This work is intended to contribute to the conservation of large freshwater mussels by studying the relationship between these mussels and their host fishes using the example of *Unio crassus* and *Margaritifera margaritifera*, two endangered mussel species in central Europe.

Following an introduction providing essential background information on the importance of freshwater mussels for aquatic ecosystems, their life history, phylogeny, distribution, and the current population status of the two investigated mussel species, five specific research topics concerning the host-parasite interaction between fishes and the two mussel species are presented. Each specific topic was published as an autonomous research paper in a slightly modified form (according to the different journal requirements). These specific aspects merge into a general discussion of the interaction between mussels and their hosts, the positive and negative effects on both interaction partners as well as the different levels of host-specificity. Finally, the general discussion deduces implications for conservation of large freshwater mussels and identifies promising directions for future research.

Summary

Freshwater mussels of the order Unionoida are one of the most imperiled groups of animals worldwide. Central European species, such as the thick-shelled river mussel (*Unio crassus*, Philipsson 1788) and the freshwater pearl mussel (*Margaritifera margaritifera* L.), have strongly declined in the last decades and become priority species in aquatic conservation. The complex life cycle of unionoid species include an obligatory development phase on a suitable host fish implying a strong dependency of their hosts. Consequently, there is an urgent need to understand the interactions of freshwater mussels and their hosts in order to develop effective conservation strategies including management of wild fish stocks, as well as to improve the efficiency of supportive breeding methods.

This work is intended to contribute to the conservation of large freshwater mussels by systematically assessing the relationship of fishes and freshwater mussels. In a first step, the suitability of different fish species and populations were tested for hosting larvae of *U. crassus* and *M. margaritifera.* Different infestation and / or metamorphosis rates of mussel larvae in addition to different development times of encysted larvae indicate that host suitability can vary across different host species, populations and individuals. From an evolutionary point of view, the observed differences in the development speed and the timing of excystment on different host species and populations are likely to increase the chances of successful dispersal and survival of adverse environmental conditions. For *U. crassus*, the fish species *Chondrostoma nasus*, *Cottus gobio*, *Gasterosteus aculeatus*, *Leuciscus idus*, *Phoxinus phoxinus*, *Scardinius erythrophthalmus*, and *Squalius cephalus* were found to be the most suitable hosts, while *Salmo trutta* and *Alburnoides bipunctatus* were less suitable. In contrast, *Acipenser ruthenus*, *Alburnus alburnus*, invasive *Neogobius melanostomus*, introduced *Oncorhynchus mykiss* and *Rutilus rutilus* were considered to be unsuitable for hosting larvae of *U. crassus*. Although data gained from laboratory and field investigations strongly suggest that *S. cephalus* is the most important host for *U. crassus* in the Danube drainage, some mussel populations exclusively depend on other hosts. The wide host range of *U. crassus* indicates that natural recruitment in functional *U. crassus* populations can be secured by highly different fish community structures, which might explain the wide distribution of the mussel species and increases the probability of successful restoration of priority populations. For *M. margaritifera*, host suitability analyses of *Hucho hucho* and different *Salmo trutta* populations confirmed that *S. trutta* can be considered as the most important host for *M. margaritifera* in central Europe and that various populations within one host species reveal significant differences in host-specificity. Studying excystment of *M. margaritifera*, after maintaining the infested hosts under constant water temperatures

between 11 and 12 °C, indicate a highly variable development time of encysted *M. margaritifera* larvae on the same host population, ranging between 1,700 and 3,400 day degrees. In addition, the absence of a previously postulated threshold temperature of ≥ 15 °C for successful excystment of *M. margaritifera* was found. Consequently, the obligatory host dependent phase does not seem to limit the current distribution range of the species, its culturing under constant water temperature conditions in typical salmonid fish hatchery setups as well as reintroductions of the species into cool headwater areas. Successful development of *M. margaritifera* larvae to juvenile mussels in calcareous water indicate that neither the infestation process nor the metamorphosis of the freshwater pearl mussel depend on low lime concentrations, which is typical for the native streams of the species. The study of the relationship between *M. margaritifera* and its hosts suggest a parasitic character of this interaction as evident from the high mortality rates of heavy infested hosts. On a sublethal level, swimming performance of heavy infested hosts was significantly reduced by ~ 20 % compared with the hosts with the lowest infestation rates.

The sustainable conservation management of *U. crassus* and *M. margaritifera* populations is closely linked to the effective management of their host fish populations and conservation efforts should focus on integrative approaches considering these relationships. In streams of *U. crassus* and *M. margaritifera*, suitable host species should be actively supported by improvement of environmental conditions. In addition to this long term conservation efforts, there are also some host-specific conservation strategies - like stocking of infested host fishes and the increase of host densities to facilitate natural recruitment - which can help to bridge deficient recruitment on a short term scale. Since detailed knowledge on the host-specificity of several central European mussel species is still rare, future research should focus on the host-use of species like *Unio tumidus, Unio pictorum, Anodonta anatina, Anodonta cygnea* and *Pseudanodonta complanata*.

Zusammenfassung

Süßwassermuscheln der Ordnung Unionoida gehören zu den am stärksten gefährdeten Tieren weltweit. Unter den mitteleuropäischen Arten sind besonders die Bestände der Bachmuschel (*Unio crassus*, Philipsson 1788) und der Flussperlmuschel (*Margaritifera margaritifera* L.) in den letzten Jahrzehnten stark zurückgegangen, weshalb diese Arten einen hohen Stellenwert im aquatischen Artenschutz genießen. Der komplexe Lebenszyklus der Unionoiden enthält eine obligate Entwicklungsphase auf einem geeigneten Wirtsfisch, wodurch die Muscheln von ihren Wirten abhängig sind. Demzufolge ist ein grundlegendes Verständnis der Interaktion von Süßwassermuscheln und ihren Wirten notwendig, um effektive Schutzstrategien zu entwickeln, die sowohl auf dem Management der vorkommenden Fischbestände als auch auf bestandsstützenden Nachzuchtprogrammen basieren können.

Mithilfe einer systematischen Analyse der Interaktion von Fischen und Muscheln soll diese Arbeit zum Schutz der großen Süßwassermuscheln beitragen. In einem ersten Schritt wurden verschiedene Fischarten und Populationen einer Fischart hinsichtlich ihrer Eignung als Wirt für die Larven von *U. crassus* und *M. margaritifera* getestet. Aufgrund unterschiedlicher Infektions- und / oder Metamorphoseraten und verschiedenen Entwicklungszeiten von enzystierten Muschelarven wurde ersichtlich, dass die Eignung als Wirt sowohl zwischen verschiedenen Fischarten als auch zwischen verschiedenen Populationen und Individuen variiert. Aus evolutionärer Sicht erhöhen die beobachteten Unterschiede in der Entwicklungszeit wahrscheinlich die Chancen auf eine erfolgreiche Verbreitung der Jungmuscheln und das Überleben von ungünstigen Umweltbedingungen. Geeignete Wirte für *U. crassus* waren *Chondrostoma nasus*, *Cottus gobio*, *Gasterosteus aculeatus*, *Leuciscus idus*, *Phoxinus phoxinus*, *Scardinius erythrophthalmus* und *Squalius cephalus*, während *Salmo trutta* und *Alburnoides bipunctatus* weniger gut geeignet waren. Die Fischarten *Acipenser ruthenus*, *Alburnus alburnus*, *Neogobius melanostomus*, *Oncorhynchus mykiss* und *Rutilus rutilus* waren nicht als Wirt für *U. crassus* geeignet. Obwohl die Daten aus Labor- und Freilanduntersuchungen darauf hindeuten, dass *S. cephalus* der wichtigste Wirt für *U. crassus* im Donaueinzugsgebiet ist, sind einige Muschelpopulationen vollständig von anderen Wirtsarten abhängig. Das große Wirtsfischspektrum von *U. crassus* weist darauf hin, dass die Reproduktion in funktionellen *U. crassus* Populationen durch verschiedene Fischzönosen gewährleistet werden kann. Dies könnte die weite Verbreitung von *U. crassus* erklären und die Erfolgsaussichten bestandsstützender Maßnahmen bei prioritären Populationen erhöhen. Am Beispiel von *M. margaritifera* bestätigten die Untersuchungen zur Wirtsspezifität an *Hucho hucho* und

verschiedenen *Salmo trutta* Populationen, dass *S. trutta* der Hauptwirt von *M. margaritifera* in Mitteleuropa ist und verschiedene Populationen innerhalb einer Wirtsart signifikante Unterschiede in der Wirtsspezifität aufweisen. Die Analyse des Exzystments von *M. margaritifera*, nachdem die infizierten Wirtsfische unter konstanten Wassertemperaturen zwischen 11 und 12 °C gehalten wurden, weist auf eine sehr variable Entwicklungszeit zwischen 1700 und 3400 Tagessummengraden der enzystierten Larven hin. Zusätzlich konnte durch diese Experimente die Notwendigkeit einer vorher postulierten Schwellenwerttemperatur ≥ 15 °C als Grundvoraussetzung für ein erfolgreiches Excystment von *M. margaritifera* widerlegt werden. Infolgedessen scheint die wirtsabhängige Phase von *M. margaritifera* weder die derzeitige Verbreitung zu limitieren, noch die Zucht von *M. margaritifera* unter konstanten Wassertemperaturen, wie sie in einer typischen Forellenzucht üblich sind. Auch die Wiederansiedelung *M. margaritifera* in kühleren Oberläufen der Gewässer scheint nicht durch die wirtsabhängige Phase limitiert. Eine erfolgreiche Entwicklung von *M. margaritifera* Larven zu jungen Muscheln in kalkreichem Wasser zeigt, dass weder der Infektionsprozess noch die Metamorphose von geringen Kalkkonzentrationen abhängen, die typisch für das natürliche Verbreitungsgebiet der Art sind. Anhand der hohen Mortalitätsraten stark infizierter Wirtsfische wird eine parasitäre Beziehung zwischen *M. margaritifera* und den Wirten deutlich. Verglichen mit den am schwächsten infizierten Wirtsfischen konnte auf subletaler Ebene eine signifikante ca. 20 %ige Reduktion der Schwimmleistung der am stärksten infizierten Wirte nachgewiesen werden.

Ein nachhaltiges Management von *U. crassus* und *M. margaritifera* Populationen ist eng mit einem effektiven Management ihrer Wirtsfische verbunden und die Schutzstrategien sollten integrative Ansätze beinhalten, die diese Beziehungen berücksichtigen. In Gewässern, in denen *U. crassus* und *M. margaritifera* vorkommen, sollten die geeigneten Wirtsarten aktiv durch die Verbesserung der Umweltbedingungen unterstützt werden. Zusätzlich zu diesen, auf längere Zeit angelegten Maßnahmen, gibt es einige wirtsspezifische Schutzstrategien wie z. B. den Besatz mit infizierten Wirtsfischen und die Erhöhung der Wirtsfischdichte, um die natürliche Reproduktion der Muscheln zu fördern bzw. Störungen in der natürlichen Reproduktion kurzfristig auszugleichen. Da der Kenntnisstand über die Wirtsspezifität der europäischen Großmuschelarten sehr begrenzt ist, sollten sich zukünftige Studien auf die Wirtsspezifität von Arten wie *Unio tumidus, Unio pictorum, Anodonta anatina, Anodonta cygnea* und *Pseudanodonta complanata* fokussieren.

1. General introduction

1. 1. The importance of freshwater mussels in aquatic ecosystems

Freshwater mussels are important elements of aquatic ecosystems, because they filter out large quantities of algae, diatoms, bacteria, organic particles, silt and they absorb heavy metals (Strayer et al., 1999; Bogan, 2008). In addition, freshwater mussels increase nutrient cycling rates and create sediment microhabitats by excretion and biodeposition of faeces and pseudofaeces (mucous packaged sediment which has not passed through the gut of the mollusk) (Vaughn and Hakenkamp, 2001; Vaughn et al., 2008). The physical presence of their shells generates microhabitats for a number of benthic invertebrates and juvenile fishes (e.g. Ziuganov et al., 1994). While the shells of the mussels stabilizes the substrate, their movement (bioturbation) increases the water and oxygen supply of the interstitial zones and thereby enhances the release of nutrients from the sediment to the water column (Vaughn and Hakenkamp, 2001). Some freshwater mussels are economically valuable for their shells (pearl button industry) and pearls or serve as an important source of food for humans (Lasiak and Dye, 1989; Ziuganov et al., 1994; Strayer et al., 1999; 2004; Bogan, 2008). In contrast, other species such as the introduced zebra mussel *Dreissena polymorpha* (Pallas 1771) are economically important pests (e.g. by clogging water intakes) and can have dramatic ecological effects by competing with other suspension feeders for food as well as changing energy and nutrient flows in aquatic systems (Richardson and Bartsch, 1997; Ricciardi et al., 1998; Strayer et al., 1999; 2004).

The ecological and economic value of a species is closely linked to its life history which controls its abundances and the mode of dispersal. In addition, the life history of a species affects its vulnerability to anthropogenic activities, which is especially true for species with complex life cycles such as the large freshwater mussels (Unionoida).

1. 2. Life cycle of unionoid mussels

Although the physical appearance of large freshwater mussels is often unimpressively, they are interesting from a biological perspective (Bauer and Wächtler, 2001; Strayer et al., 2004). Most of the large freshwater mussels have a unique life cycle including both parental care (i.e. brooding) and larval parasitism on freshwater fishes and occasionally other vertebrates (Wächtler et al., 2001 and references therein; Taeubert and Geist, 2013). After fertilization, the embryonic development occurs within the gills of the parent mussel, which are modified

to brooding chambers, so called marsupia (Tankersley and Dimock, 1993; Schwartz and Dimock, 2001; Zimmermann and Neves, 2002). For most species, the fully developed larvae are released and must attach to a suitable host for further development (Figure 1-1).

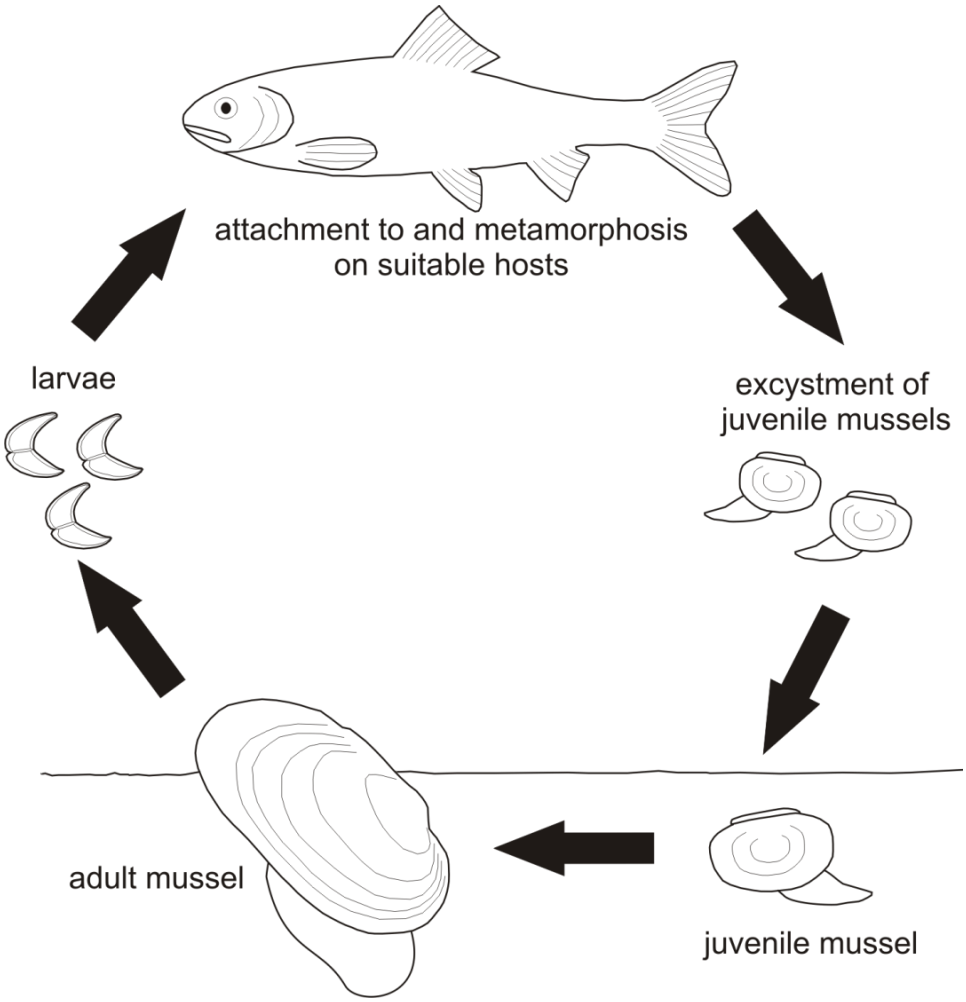

Figure 1-1: Life cycle of unionoid mussels according to Hastie et al. (2003).

The reproductive strategies, especially the host infection strategies, vary considerably among the Unionoida. These host infection strategies range from a random encounter of the released larvae and the host to a wide variety of strategies by actively attracting the hosts, including behavioural and morphological adaptations of the female mussel (Strayer et al., 2004; Barnhart et al., 2008). In the case of a random encounter, the released larvae or fragile conglutinates are passively distributed by the water current, and the attachment to the host occurs randomly (Barnhart et al., 2008). The low probability of an encounter of larvae and hosts favours the broadcast of a high number of very small larvae to increase the number of offspring that can be produced (Bauer, 1994). The advantage of using small instead of big larvae is that a higher number of larvae can be produced at the same energetic costs. In addition, a small size of larvae enhances the suspension in the water column and facilitates the encounter with pelagic hosts (Barnhart et al., 2008). The respiratory current across the

host gills might enhance the probability of larvae attachment for mussel larvae which preferentially encyst on the hosts' gill tissue.

In the case of active host attraction strategies, some mussels species use lures (often modifications of the mantle margin) or conglutinates (aggregates of larvae) that mimic prey items of the host fishes in form of fish eggs, insect larvae and pupae or swimming minnows (Haag et al., 1995; Barnhart et al., 2008). When host fish try to feed on these lures or conglutinates, they bring themselves into contact with the mussel larvae resulting in an increased probability of a successful infection (Strayer et al., 2004; Barnhart et al., 2008). Mussels using active host attraction strategies typically produce fewer larvae than species broadcasting free larvae, indicating that these strategies enhance the success of host infection (Haag and Staton, 2003; Barnhart et al., 2008).

On suitable hosts, larvae metamorphose successfully into juvenile mussels before they excyst and fall off their hosts. The juvenile mussels bury themselves into the substratum and live for up to five years (depending on the species) as pedal feeders. Once they have developed into adult indiviuals they come up to the substratum surface where they live as filter feeding mussels.

1. 3. Larval types of freshwater mussels in relation to phylogeny and distribution

Unionoida is a monophyletic order of large freshwater mussels (also known as naiads), which are members of the class Bivalvia within the phylum Mollusca. The large freshwater mussels are found in freshwater rivers and lakes on all continents except Antarctica (Bauer and Wächtler, 2001; Graf and Cummings, 2006). To date, the global estimate of freshwater mussel diversity is about 840 species (Graf and Cummings, 2007). Although reproductive characters such as larval form and female brooding morphology have been used to classify the Unionoida, these characters should be evaluated for homoplasy and only used with caution (Barnhart et al., 2008). The diverse group of the Unionoida is divided into two superfamilies, the Etherioidea and the Unionoidea which partially use different larvae forms (Graf and Cummings, 2006; 2007). The Etherioidea consists of 154 species, which belong to the Hyriidae, the Etheriidae, the Mycetopodidae and the Iridinidae, and are generally distributed among the southern continents (Africa, Australasia, South America). The Etheriidae, Mycetopodidae and Iridinidae use lasidia larvae which are small (85–150 µm) uncalcified larvae with a univalved shell and a trilobed body (Graf and Cummings, 2006). Lasidia of some species attach to the host epithelium and form a cyst for metamorphosis (Graf and Cummings, 2006). In other species like *Mutela bourguignati* (Bourguignat 1885), a modified lasidium known as "haustorium" attaches to the host via tubular appendages that penetrate the host and forms a connection between the developing mussel and the host

(Wächtler et al., 2001; Graf and Cummings, 2006). The phylogeny of the Unionoida and the larval forms are summarized in Figure 1-2.

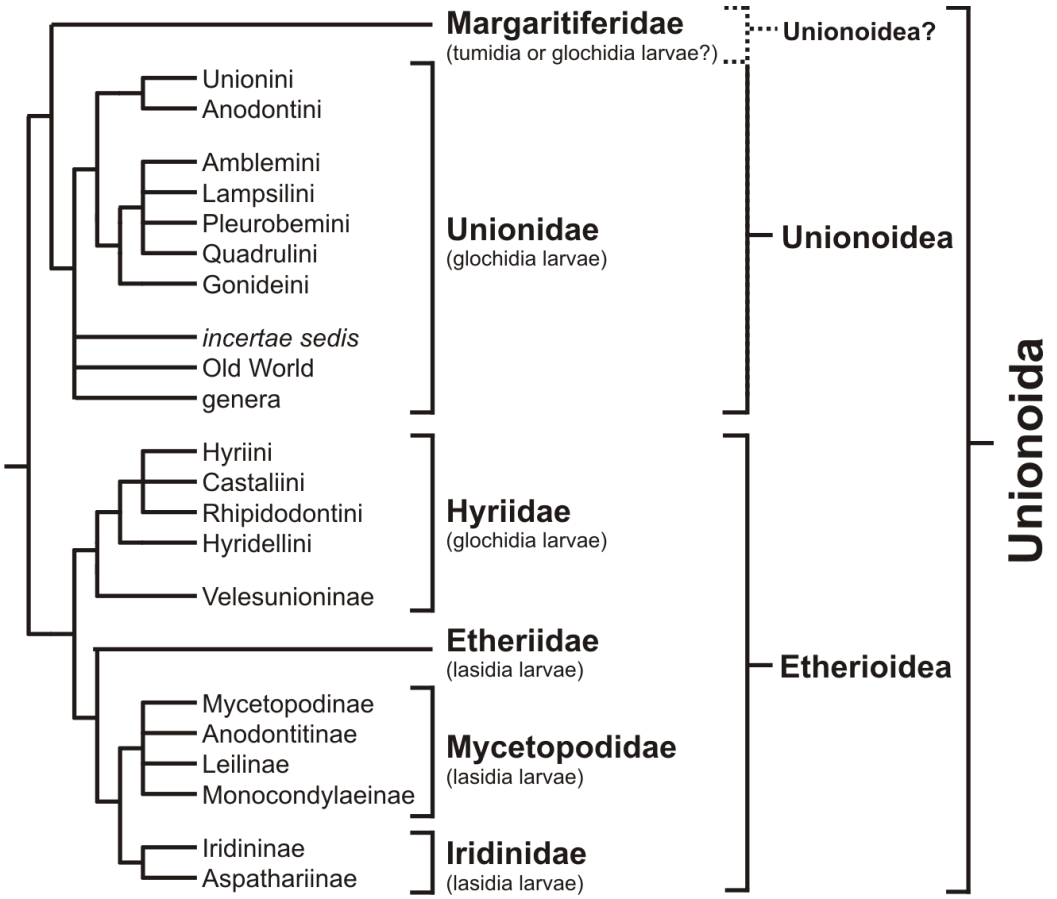

Figure 1-2: Cladogram of freshwater mussel taxa, modified from Graf and Cummings (2007).

The Unionoidea consists of about 700 species (Graf and Cummings, 2007), which are mainly distributed over the northern continents and include the Unionidae (Africa, Eurasia, India, North America) and the family of the Margaritiferidae (Eurasia, North America). While the Unionidae (674 species) is the largest and most widespread family of the Unionoidea, the Margaritiferidae (12 species) was thought to be only a small and primitive family (Graf and Cummings, 2007). According to the present literature, the Unionoidea and the Hyriidae produce glochidia larvae which resemble small bivalves with an elastic hinge and a well developed adductor muscle (Wächtler et al., 2001; Graf and Cummings, 2006; 2007). After their release, glochidia attach to the host surface epithelium (gill filaments, fins or skin) by clamping the valves following encapsulation of the larvae by migration of host epithelial cells (Rogers-Lowery and Dimock, 2006). Except for a few species like *M. margaritifera*, glochidia larvae have a hook at their apical edge, which facilitates attachment. The hook can vary in size, shape and inclination between different mussel species (Wächtler et al., 2001). Very recent research proposed an entirely new larval form - the so called tumidium - for the Margaritiferidae which developed from a glochidium by transition to uncalcified larvae

(Barnhart and Bogan, pers. comm.). Since this needs further investigation, the more widely accepted term “glochidia” is used throughout this thesis.
Seven native species from the superfamily Unionoidea are found in central Europe. This includes six members of the Unionidae (*Unio crassus, Unio tumidus, Unio pictorum, Anodonta anatina, Anodonta cygnea, Pseudanodonta complanata)* and one member of the Margaritiferidae (*Margaritifera margaritifera*).

1. 4. Study species of this work - *Unio crassus* and *Margaritifera margaritifera*, two endangered central European unionoid species

1. 4. 1. *Unio crassus* (Phillipsson, 1788)

The thick-shelled river mussel (*Unio crassus*, Philipsson 1788) is listed as endangered in IUCN Red list of threatened species (Van Damme, 2011) and in annexes II and IV of the European fauna and flora habitat´s (FFH) directive (Council of the European Communities, 1992). Up to the first half of the 20th century, *U. crassus* was the most abundant unionid species in Europe, but today only a few intact populations remain (Engel and Wächtler, 1989; Zettler and Jueg, 2007). In central Europe, *U. crassus* have a relatively short life-span of usually 8 - 23 years (Figure 1-3) (Hochwald 1997; 2001), which makes the populations susceptible for short term deficits in recruitment. In contrast, a life span of up to 75 years was observed at more northern latitude (Timm, 1994). During summer, *U. crassus* females release ~ 100,000 relatively big (~ 220 µm) glochidia larvae per spawning event into the free-flowing water (Bednarczuk, 1986; Hochwald, 1997, Taeubert et al., 2012a). *U. crassus* is able to spawn several times within one spawning period, which typically ranges from April to August. Glochidia of *U. crassus* are either passively distributed by the water current or actively by the so called “spurting behaviour“, which is an interesting modification of a simple broadcast of glochidia and was suggested to facilitate the probability of infecting host fishes (Vicentini, 2005). During spurting behaviour, the female mussel moves into shallow water at the bank sites and spurts a stream of glochidia containing water to the centre of the stream. By disrupting the water, the “spurts” are intended to resemble insects and attract potential host fishes (Vicentini, 2005). In addition, the spurting behaviour actively enhances the dispersal of glochidia in the main stream (Vicentini, 2005).

Characteristics

Species:	***Unio crassus* (Phillipsson, 1788)**
Genus:	Unio
Tribe:	Unionini
Subfamily:	Unioninae
Family:	Unionidae
Superfamily:	Unionoidea
Order:	Unionoida
Class:	Bivalvia
Phylum:	Mollusca
max. age:	8 - 75 years
max. size:	50 - 80 mm
larval size:	~ 220 µm

Figure 1-3: Species characteristics of *Unio crassus*.

The attachment of *U. crassus* glochidia to the host tissue is facilitated by hooks. Once attached to a suitable host, the glochidia become encysted by host epithelial cells and develop into juvenile mussels (Hochwald and Bauer, 1990). *U. crassus* larvae are considered to complete their development preferentially in the gill epithelium of host fishes (Engel and Wächtler, 1989; Hochwald, 1997). Juvenile *U. crassus* excyst within the same summer of encystment, fall off their hosts and bury themselves into river bed substratum (Bednarczuk, 1986; Hochwald and Bauer, 1990). After two or three years, the mussels emerge to the stream bed surface, where they filter-feed on particles in the free flowing water. In contrast to other mussel species like *M. margaritifera*, where the suitability of different host fish species is well-known (Young and Williams, 1984a; Bauer, 1987a; Geist et al., 2006), the host-specificity of *U. crassus* is less clear. Most Unionids are considered to have a relatively broad host range (Bauer et al., 1991). This might be also true for *U. crassus*, because the former wide distribution all over Europe and the heterogeneity of habitats that are colonized by the species indicate a high plasticity in the host use.

1. 4. 2. *Margaritifera margaritifera* (Linnaeus, 1758)

Among the European unionoid species, the freshwater pearl mussel (*Margaritifera margaritifera*) is a highly threatened long-lived bivalve occuring in cool running waters of the Holarctic region (Geist, 2010). *Margaritifera margaritifera* is listed as endangered in IUCN Red list of threatened species (Mollusc Specialist Group, 1996) and in annexes II and V of the European fauna and flora habitat´s (FFH) directive (Council of the European Communities, 1992). While in the early 20th century, some Bavarian *M. margaritifera* populations had reached extremely high densities, with hundreds of mussels per square metre over a stream length of 20 – 30 km (Bauer, 1991); today successful juvenile recruitment was not found for decades. *M. margaritifera* is considered one of the long lived animals worldwide and can reach an age of up to 200 years in the very northern regions (Figure 1-4) (Ziuganov et al., 2000; Mutvei and Westermark, 2001). This longevity is the only reason why most of the remnant *M. margaritifera* populations still exist in central Europe.

Within its distribution area, *M. margaritifera* reach a maximum shell length of 80 to 145 mm (Figure 1-4) (Bauer, 1992). The phylogenetically ancient freshwater pearl mussel represents a typical member of a mussel that uses the “random encounter strategy” between glochidia and hosts. In a well synchronized spawning event, *M. margaritifera* release 2 - 4 million of relatively small (40 – 70 µm) glochidia per female into the water column (Figure 1-1). The released larvae attach to the hosts mainly as free glochidia (Bauer, 1991). In addition, the shells of the adult mussels provide microhabitats which might be preferred by juvenile host fishes and could cause a spatial proximity between broadcasted glochidia and hosts.

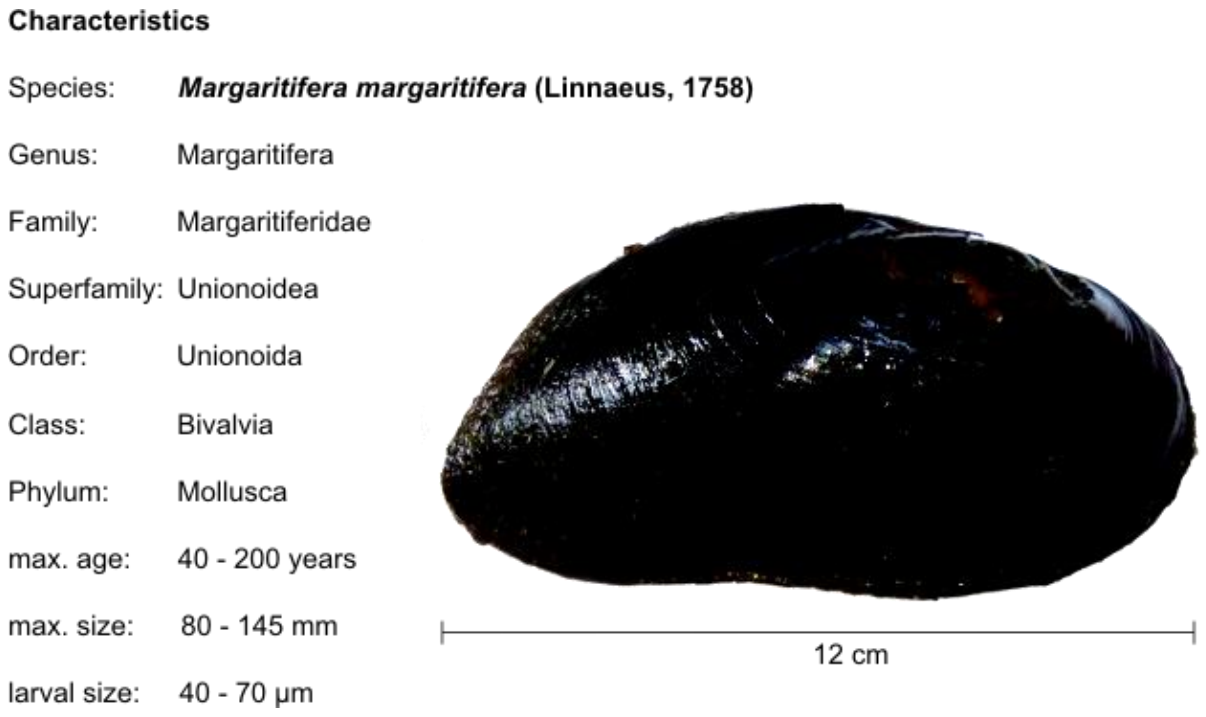

Figure 1-4. Species characteristics of *Margaritifera margaritifera.*

Although glochidia of *M. margaritifera* are able to attach to the gills of other fish species, they can only develop on suitable host species (Young and Williams, 1984a; Bauer, 1987a). Compared with other unionoid mussels, *M. margaritifera* has a narrow host range with only two confirmed hosts in Europe. According to Young and Williams (1984a), the only host fishes of freshwater pearl mussel in Europe are Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* forma *fario* L.), with brown trout being the exclusive host in many central European populations (Bauer, 1987a; Geist et al., 2006). For the Danube drainage, it was suggested that Danube salmon (*Hucho hucho* L.) is also suitable for hosting glochidia of *M. margaritifera* (Bauer, 1997; Taeubert et al., 2010) while in Northern Europe, Arctic charr (*Salvelinus alpinus*) might be a suitable host (Bauer, 1997). In addition, brook trout (*Salvelinus fontinalis*) might be suitable for hosting the eastern North American populations of *M. margaritifera* (Smith, 1976). After attachment, the larvae are encysted by migration of host epithelial cells which form a cyst around the attached larvae (Rogers-Lowery and Dimock, 2006). On suitable hosts, the metamorphosis of encysted larvae takes up to 11 months (Young and Williams 1984b; Ziuganov et al., 1994; Taeubert et al., 2013a). Hruška (1992) suggested that the temperature during the encystment phase is the main driver governing the development on the host. The latter author also found that *M. margaritifera* requires a mean water temperature ≥ 15 °C for at least 14 days at the end of the parasitic phase to excyst successfully. During encystment, glochidia grow approximately 6 - 10 times in size to about 0.4 and 0.5 mm and metamorphose into juvenile mussels (Ziuganov et al., 1994). After excystment, the juvenile mussels bury themselves into the river bed substratum, where they live for about five years and develop into surface living adult mussels (Young and Williams, 1984b; Bauer, 1997; Hastie et al., 2000). These first few years were identified to be the most sensitive phase in the development of *M. margaritifera* (Hastie et al., 2000; Geist and Auerswald, 2007; Österling et al., 2008; 2010).

1. 5. Objectives

Based on the high imperilment of these two freshwater mussel species, the development of effective conservation measures is necessary. The most important prerequisite for developing effective conservation and management strategies is the knowledge and the integration of aut- and synecological information of the endangered species (Geist, 2011).
The reproductive success of freshwater mussels is affected by interacting biotic and abiotic factors. This includes data on the habitat requirements as well as detailed knowledge on the interactions with other species. While there are several abiotic factors affecting the recruitment of the mussels, the most important biotic factor is the availability of suitable hosts. The host-dependent phase is considered to be the first critical step in the life-history of a large freshwater mussel and might limit their reproduction and distribution. Therefore, it is important to determine the host fish requirements of endangered mussel species like *U. crassus* and *M. margaritifera*, and to incorporate this knowledge in the conservation efforts.
The following specific objectives were tested in this study:

1) In a first step, different fish species were tested for hosting *U. crassus* larvae (Chapter 2 and 3). Since data on the host-specificity of *U. crassus* were rare in the available peer-reviewed literature, these investigations were a prerequisite for studies into mussel-fish relationships of the species. Especially fishes originating from the Danube drainage, which is considered one major distribution area of *U. crassus*, were included. In addition to standardized laboratory investigations, the fish community structure and the natural infestation rates of self-recruiting *U. crassus* populations were analysed to reflect the situation under natural conditions (Chapter 3).
2) In a second step, various populations (strains) within the same fish species were tested for hosting larvae of *U. crassus* and *M. margaritifera* (Chapter 3 and 4) to investigate the hypothesis that host-specificity varies among different populations of the same host species. This information is crucial to supportive breeding programmes which have been initiated in many European countries for *M. margaritifera* (Thomas et al., 2010; Gum et al., 2011). A possible ecotype-specific host parasite interaction is also relevant for an effective conservation management of wild fish stocks in streams of endangered mussel species.
3) In a third step, the effect of environmental conditions on the host-dependent phase of *M. margaritifera* was analysed. In particular, it was tested if the limitation of the mussel distribution to siliceous streams is related to the host-dependent phase (Chapter 4 and 5). In a second experiment, it was investigated if the completion of *M. margaritifera* development is possible under constant water temperatures between 11 and 12 °C or if the former postulated threshold of a mean water temperature ≥ 15 °C

for at least 14 days at the end of the parasitic phase hampers successful excystment (Chapter 5).

4) In the last step, it was tested if the infestation of the *M. margaritifera* had physiological effects on its hosts by considering lethal and sublethal endpoints (Chapter 6). This information helps to clarify if the relationship between this mussel species and its hosts can be considered as parasitism, mutualism or commensalism. In addition, knowledge on the physiological responses of the hosts to larvae infestation might be helpful to deduce optimal infestation rates for conservation purposes (Chapter 6).

2. Host-specificity of the endangered thick-shelled river mussel (*Unio crassus*, Philipsson 1788) and implications for conservation

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2. 1. Abstract

The complex life cycle of the endangered thick-shelled river mussel (*Unio crassus,* Philipsson 1788) includes an obligatory parasitic phase on a host fish. Consequently, knowledge on the interaction of *U. crassus* with its host species is crucial for the development of effective conservation strategies.
The objective of this study was to systematically assess the host-suitability of eight different fish species, including six native species which naturally co-occur with *U. crassus*, as well as two non-native species. All tested fish species were successfully infected with glochidia of *U. crassus*, which were present on their gills two days post exposure. *Phoxinus phoxinus* and *Squalius cephalus* were the most suitable hosts as indicated by both the highest total glochidial load and the highest fish-weight-normalized glochidial load after 16 days and 48 days. *Salmo trutta*, *Alburnoides bipunctatus* and *Cottus gobio* were less suitable, losing ~ 90 % of glochidia within 16 days. *Alburnus alburnus,* invasive *Neogobius melanostomus* and introduced *Oncorhynchus mykiss* lost more than 98 % of glochidia within 16 days, indicating they are unsuitable hosts. *U. crassus* larvae did not grow significantly (< 15 %) during their metamorphosis on suitable hosts, suggesting that the most obvious advantage of the host-dependent phase in the *U. crassus* life cycle is the dispersal by fish vectors. The observed differences in the development speed and the timing of excystment on different suitable host species are likely to increase the chances of successful dispersal and survival in adverse environmental conditions.
The sustainable conservation management of *U. crassus* populations is closely linked to the effective management of their host fish populations. In particular, the currently underestimated ecological functions of low-valued fish species such as *S. cephalus* and *P. phoxinus* clearly deserve better consideration in the conservation management of *U. crassus* habitats and stream ecosystems.

2. 2. Introduction

Freshwater bivalves are considered the most sensitive element of freshwater fauna (Geist, 2011), which is particularly true for stream-dwelling species such as the freshwater pearl mussel (*Margaritifera margaritifera* L.) and the thick-shelled river mussel (*Unio crassus*, Philipsson 1788). Stream-dwelling mussel species suffer severely from anthropogenic activities, including the creation of dams, flow modification, habitat destruction, water pollution and the increased fine sediment load caused by land-use effluents from agricultural and urban areas (Bogan, 1993, 2008; Lydeard et al., 2004). These factors can either directly or indirectly (e.g. via the decline in host fishes) result in the decline of mussel populations. Up to the first half of the 20th century, *U. crassus* was the most abundant unionid species in Europe (Israel, 1913; Zwanziger, 1920; Bednarczuk, 1986; Hochwald and Bauer, 1990). Under favourable conditions, densities could reach several hundred individuals per metre of stream bed (Tudorancea and Gruia, 1968; Zettler and Jueg, 2007). However, today *U. crassus* is considered highly endangered in several European countries and is listed in annexes II and IV of the European fauna and flora habitat´s (FFH) directive (Council of the European Communities, 1992). For example, in Germany the species is considered critically endangered and only a few intact populations remain (Engel and Wächtler, 1989; Zettler and Jueg, 2007).

As with all unionid species, *U. crassus* exhibit a complex life cycle including an obligate parasitic stage on a suitable host fish. *U. crassus* females brood the fertilized eggs in their marsupial demibranches and after the embryonic phase, they release glochidia larvae (Hochwald and Bauer, 1990). The glochidia are passively distributed by the water current and need to attach to a host fish for further development (Bednarczuk, 1986; Hochwald and Bauer, 1990; Bauer et al., 1991). Gills are considered by far the most important tissue for glochidia development in *U. crassus* (Hochwald, 1997). Vicentini (2005) described a unique spurting behaviour of glochidia release in gravid *U. crassus* females, presumably to attract potential hosts and to facilitate glochidia intake. Gill-encysted glochidia metamorphose into juvenile mussels before they drop off their hosts and bury into river bed substratum (Bednarczuk, 1986; Hochwald and Bauer, 1990). After 2 or 3 years, the mussels emerge on the stream bed surface, where they filter-feed on particles in the stream open water.

The natural distribution of *U. crassus* extends from the Pyrenees throughout central Europe to the Ural Mountains (Jäckel, 1962), while the species is not found in the British Isles and in several southern European regions (e.g. Italy, Balearic islands). The species occurs in small headwater streams and tributaries, as well as in large river systems such as the lower Danube in Hungary (Israel, 1910). The former distribution in a wide range of habitats all over Europe suggests that *U. crassus* has a broad and unspecific host range. In contrast to *M. margaritifera*, where the host-parasite interaction and the suitability of different host fish

species is well-known (Young and Williams, 1984b; Bauer, 1987a; Geist et al., 2006; Taeubert et al., 2010; Thomas et al., 2010), the host-specificity of *U. crassus* is less clear. To date, there is a lack of systematic studies on the suitability of different host fish species for *U. crassus* in the the peer-reviewed literature. It is likely that environmental changes affecting the host fish populations have a substantial impact on the drastic decline of *U. crassus* and other unionid species in some localities (Engel and Wächtler, 1989). In addition, it remains unclear if introduced fish species are suitable hosts for *U. crassus*. For these reasons, detailed knowledge about the host-specificity is crucial for development of effective conservation and management strategies of endangered mollusc species in general, and of *U. crassus* in particular. In addition, information about host suitability is a prerequisite for a comprehensive understanding of the colonization processes and the geographic distribution of *U. crassus.*

The main objective of this study was to assess the suitability of different fish species for infestation with glochidia of the thick-shelled river mussel. After isolation of viable *U. crassus* glochidia, infestation tests were carried out on a suite of fish species which naturally co-occur with *U. crassus*. In addition, two non-native fish species currently co-occurring with *U. crassus* in several regions were included. Glochidia numbers and sizes were analysed at different points in time to assess the host-parasite relationship of *U. crassus* with its fish hosts, and to deduce strategies for sustainable conservation management.

2. 3. Methods

2. 3. 1. Collection of glochidia

Adult *U. crassus* from the Ischler Achen, a tributary of the river Alz (Danube drainage; north of lake Chiemsee in Bavaria, Germany) were visually inspected for the presence and development status of glochidia in their marsupia by gently opening the shells with special tongs. Glochidia were fully developed on June 15 2010 at a water temperature of 17.5 °C. On the same day, nine gravid females were collected, transferred to the laboratory of the Aquatic Systems Biology Unit and maintained in aerated tanks (2.5 L) at 17.0 °C. Water exchanges and inspection for released glochidia were carried out daily. After 18 h, glochidia release started. The time-span of glochidia release ranged between 2 and 4 days for different mussels. Overall, glochidia from four different females were collected using a vacuum tube and stored in a 1 L container at 4.0 °C. The viability of the glochidia obtained was tested by examination of the clapping mechanism after adding NaCl to a small aliquot of suspended glochidia under a binocular microscope (SZX10, Olympus, Hamburg, Germany). On June 19 one amalgamated pool of glochidia was mixed to avoid the introduction of bias to the infestation experiment through possible differences in viability of glochidia originating

from different parent individuals, and from different times of release. The pooled glochidia were suspended in a 3 L container and acclimatized to groundwater (11.5 °C) over a period of 2 h. Three identical infestation baths (15 L each) were generated with a final concentration of ~ 8,500 glochidia L^{-1}.

2. 3. 2. Infestation procedure

Based on the natural co-occurrence of *U. crassus* with European minnow (*Phoxinus phoxinus* L.), chub (*Squalius cephalus* L.), brown trout (*Salmo trutta* L.), spirlin (*Alburnoides bipunctatus* Bloch 1782), bleak (*Alburnus alburnus* L.) and river bullhead (*Cottus gobio* L.), these fish species were selected as primary target species for the infestation experiments. In addition, introduced rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) and the invasive round goby (*Neogobius melanostomus*, Pallas 1814) were tested. *O. mykiss* is the dominant fish species in aquaculture facilities in the area and has established self-sustaining populations in the wild. *N. melanostomus* have colonized the upper Danube River and currently represent more than 50 % of the biomass in several sections (Joerg Brandner, pers. comm.). The origin of the eight fish species tested is given in Table 2-1.

Table 2-1: Origin of the species studied from the Danube drainage.

species	origin	subdrainage	latitude / longitude*
U. crassus	native	Inn	47.94897 / 12.41396
A. bipunctatus	native	Amper	48.03446 / 11.01351
A. alburnus	native	Isar	48.29228 / 11.64520
C. gobio	native	Amper	48.00840 / 11.02093
N. melanostomus	invasive	Danube	48.65077 / 13.16651
O. mykiss	introduced	Isar	48.33391 / 11.64180
P. phoxinus	native	Naab	49.82259 / 12.41610
S. trutta	native	Naab	49.82101 / 12.45822
S. cephalus	native	Amper	48.00840 / 11.02093

* Decimal degree latitude and decimal degree longitude (WGS 84).

Each replicate infestation bath was used for infestation of 20 individuals of *P. phoxinus, S. cephalus*, *S. trutta*, *A. bipunctatus*, *N. melanostomus*, *A. alburnus*, *O. mykiss* and 14 individuals of *C. gobio* for 45 min under constant agitation. All fish were simultaneously exposed to the baths to ensure identical conditions for glochidia uptake. In order to exclude

the potential influence of immune-response-related bias caused by previous contact with glochidia, only fish from waters without unionid populations were used in this study. To exclude the introduction of a bias due to tank effects, fish species were maintained under identical conditions in 1,400 L flow-through tanks which were sub-divided by grids. Tanks were supplied by groundwater (0.5 L s^{-1}). Fish were not fed during the experiment. Temperature loggers (Lascar Electronics Limited, Salisbury, UK) recorded water temperature every 30 minutes. Mean water temperature was 12.1 °C (SD = 1.3 °C).

2. 3. 3. Host fish suitability assessment

For quantifying both the initial infestation rate and the ongoing infestation levels, the glochidia number per fish was determined 2 days and 16 days post-infestation (pi). 21 fish of each species (7 out of each infestation bath replicate) were anaesthetized and sacrificed. Individual fish were measured (+/- 1 mm), weighed (+/- 0.01 g) and all eight gill arches were dissected. To account for differences in gill surface area and ventilation rates of different fish species, the total number of glochidia per fish was determined at 6.3 x magnification using a binocular microscope. Due to differences in size of tested fish species the weight-normalized infestation rates were calculated as described previously (Taeubert et al., 2010).

Daily inspection of strongly infested *S. cephalus* and *P. phoxinus* revealed glochidia presence on the gills up to 48 days post-infestation. Simultaneously, the first juvenile mussels were detected at the bottom of the *P. phoxinus* tanks 48 days pi. Completed metamorphosis and viability of excysted (released by the host) juveniles were evaluated under a binocular microscope by observing active movement. The infestation rate 48 days pi was determined by sacrificing the remaining test fish (*P. phoxinus* n = 10; *S. cephalus* n = 15; *S. trutta* n = 12; *A. bipunctatus* n = 16; *N. melanostomus* n = 11; *A. alburnus* n = 16) and counting glochidia. Because of the low number of *C. gobio* specimens (only 42 individuals of this species were available), the glochidial load of *C. gobio* could not be determined 48 days pi. The sizes of glochidia and of juvenile mussels were determined by measuring the maximum total shell length (+/- 2 µm) using a binocular microscope coupled to the cell D software programme, following the procedure described for *M. margaritifera* in Taeubert et al. (2010). Since *P. phoxinus*, *S. cephalus*, *S. trutta, A. bipunctatus* and *C. gobio* revealed glochidia presence 2 days pi and 16 days pi, glochidia from these time points were used for size determination.

2. 3. 4. Statistical analyses

Statistical analyses and the calculation of the equation and coefficient of determination (R^2) between fish weight and total glochidial load were performed in R Version 2.12.0 (© 2010, R

Foundation for Statistical Computing). Differences in mean glochidia infestation rates between the eight different species were tested by non-parametric Kruskal-Wallis sum of ranks test and post hoc pairwise Wilcoxon rank sum test since ANOVA assumptions were not fulfiled. Bonferroni correction was applied to correct for multiple tests. For the species with the highest variance in total weight - *S. cephalus* - the best-fit regression model was established for the link between fish size and total number of glochidia per specimen. Tested models included linear regression, logarithmic regression, exponential regression, power regression and polynomial regression.

2. 4. Results

Under laboratory conditions, four *U. crassus* females (10-15 years old, determined by annual growth increments on the shells) released a total number of ~ 380,000 glochidia within multiple spawning events. Glochidia were released either individually or in bulk. When stored as conglutinates at 4 °C, glochidia of *U. crassus* were viable for at least 3 days after release.
The procedure of a 45 min exposure of fish to an approximate concentration of 8,500 glochidia L^{-1} resulted in high initial infestation rates of all eight tested fish species (462 individuals) and successful encystment of mussel larvae was apparent on suitable hosts 16 days pi. Host fish mortality during the whole experiment was 5 % (5 *P. phoxinus*, 6 *S. trutta,* 2 *A. bipunctatus*, 7 *N. melanostomus*, 2 *A. alburnus*). Within the first 5 days after infestation only one *A. alburnus* died. Total number of glochidia per fish as well as the fish-weight-normalized number of glochidia 2 days pi, 16 days pi and 48 days pi strongly varied between the tested fish species (Figure 2-1, Table 2-2).

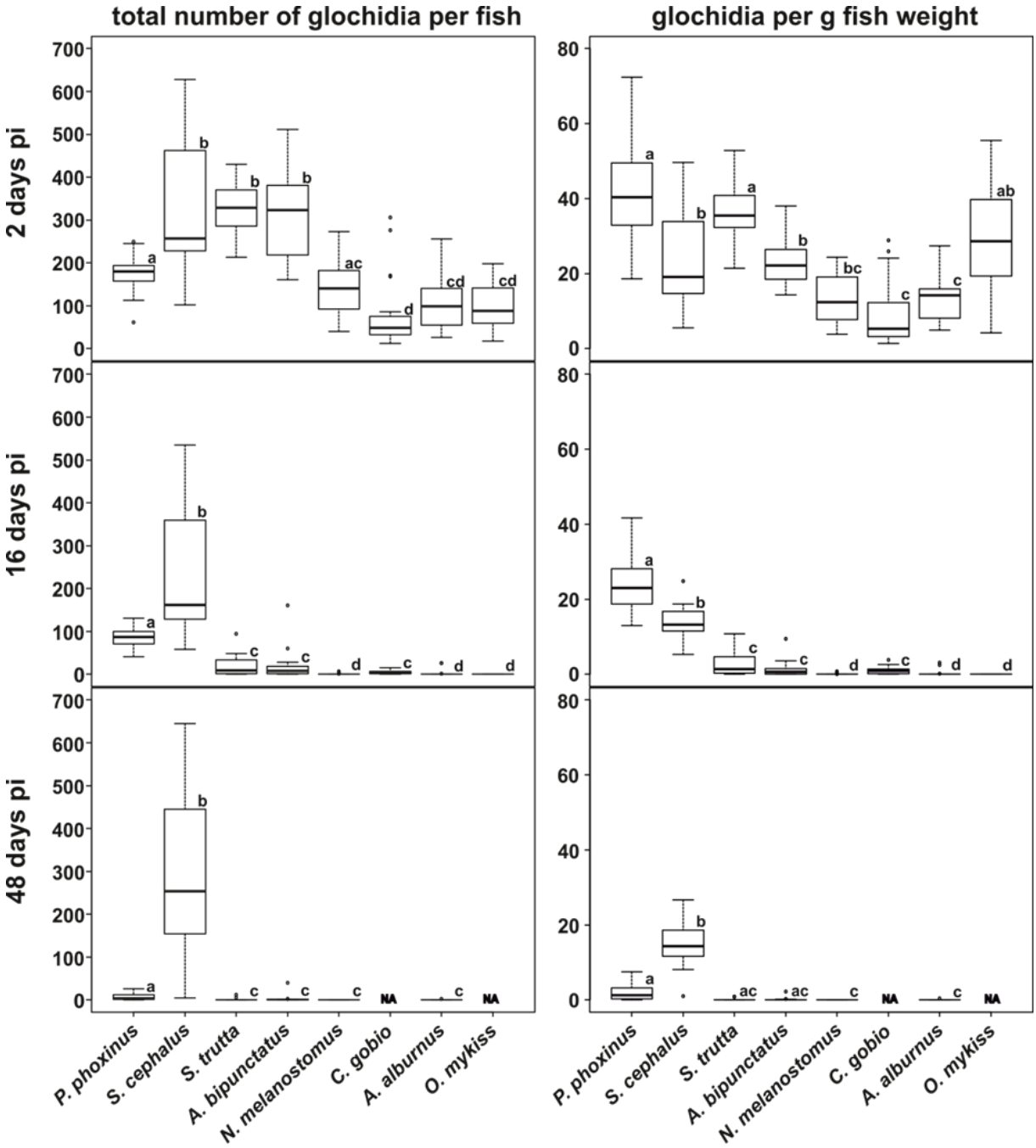

Figure 2-1: Total counts and fish-weight-normalized glochidial load on different fish species: *P. phoxinus*, *S. cephalus*, *S. trutta*, *A. bipunctatus*, *N. melanostomus*, *C. gobio*, *A. alburnus*, and *O. mykiss* 2 days pi, 16 days pi and 48 days pi. Note that 21 specimens of all potential host species were sampled 2 days pi and 16 days pi. Ten *P. phoxinus*, 15 *S. cephalus*, 12 *S. trutta*, 16 *A. bipunctatus*, 11 *N. melanostomus*, and 16 *A. alburnus* were sampled 48 days pi. Significant differences are indicated by different letters a, b, c, d ($p < 0.05$). Boxes are 0.75 and 0.25 percentiles and medians. Whiskers are the 1.5-fold interquartile range. Circles indicate data points outside the 1.5-fold interquartile range.

Table 2-2: Total counts and fish-weight-normalized glochidial load on different fish species:

P. phoxinus, *S. cephalus*, *S. trutta*, *A. bipunctatus*, *N. melanostomus*, *C. gobio*, *A. alburnus O. mykiss* 2 days pi, 16 days pi, and 48 days pi.

		2 days pi				16 days pi				48 days			
	species	**mean**	**sd**	**median**	**n**	**mean**	**sd**	**median**	**n**	**mean**	**sd**	**median**	**n**
Total number of glochidia per fish	*P. phoxinus*	175.0	43.9	180.0	21	87.2	23.3	87.0	21	7.8	9.3	4.0	10
	S. cephalus	332.5	164.5	257.0	21	245.7	154.0	162.0	21	284.3	192.9	254.0	15
	S. trutta	327.3	59.6	329.0	21	20.1	23.5	9.0	21	1.5	3.7	0.0	12
	A. bipunctatus	309.7	100.7	323.0	21	18.0	35.7	8.0	21	3.0	9.9	0.0	16
	N. melanostomus	148.0	68.7	140.0	21	0.8	1.9	0.0	21	0.0	0.0	0.0	11
	C. gobio	76.5	83.4	49.0	21	5.3	4.4	5.0	21	NA	NA	NA	NA
	A. alburnus	101.0	60.7	99.0	21	2.5	7.8	0.0	21	0.2	0.8	0.0	16
	O. mykiss	99.8	54.3	88.0	21	0.0	0.0	0.0	21	NA	NA	NA	NA
Glochidia per g fish weight	*P. phoxinus*	41.5	11.4	40.4	21	24.5	7.4	23.0	21	2.3	2.7	1.3	10
	S. cephalus	23.4	12.3	19.1	21	13.9	4.2	13.2	21	15.0	6.3	14.4	15
	S. trutta	36.2	7.1	35.4	21	2.7	2.9	1.3	21	0.1	0.3	0.0	12
	A. bipunctatus	22.6	5.8	22.1	21	1.2	2.1	0.7	21	0.2	0.6	0.0	16
	N. melanostomus	13.0	6.3	12.4	21	0.1	0.2	0.0	21	0.0	0.0	0.0	11
	C. gobio	9.2	8.1	5.3	21	1.0	1.0	0.9	21	NA	NA	NA	NA
	A. alburnus	12.9	5.7	14.2	21	0.3	0.8	0.0	21	0.0	0.1	0.0	16
	O. mykiss	29.4	13.8	28.7	21	0.0	0.0	0.0	21	NA	NA	NA	NA

Mean glochidial load per fish ranged between 76.5 glochidia per fish (*C. gobio*) and 332.5 glochidia per fish (*S. cephalus*) 2 days pi (Table 2-2). To account for different sizes of analysed fish species the fish-weight-normalized infestation rates were calculated. The fish-weight-normalized infestation rate of *P. phoxinus* (41.5 glochidia g^{-1}) was significantly higher ($p < 0.001$) than the infestation rates of *S. cephalus*, *A. bipunctatus*, *N. melanostomus*, *C. gobio* and *A. alburnus* 2 days pi. In contrast, *S. trutta* and *O. mykiss* revealed no significant difference in fish-weight-normalized infestation rates compared with *P. phoxinus* 2 days pi.

After 16 days, mean glochidial loads on all fish species were reduced compared with the initial infestation rates 2 days pi (Figure 2-1, Table 2-2), ranging between 0 in *O. mykiss* and 245.7 in *S. cephalus*. Along with the highest mean biomass of all tested fish species, *S. cephalus* had significantly higher total glochidial loads 16 days pi than all other tested fish species. In line with the results 2 days pi, *P. phoxinus* revealed the highest fish-weight-normalized glochidial load 16 days pi compared with all other species tested ($p < 0.001$). The mean fish-weight-normalized glochidia loss after 16 days was 41 % for *P. phoxinus* and *S. cephalus,* 89 % for *C. gobio*, 93 % for *S. trutta,* 95 % for *A. bipunctatus,* 98 % for *A. alburnus*, 99 % for *N. melanostomus*, and 100 % for *O. mykiss*. At 48 days pi, the mean infestation rate of *S. cephalus* remained significantly higher than the infestation rates of *P. phoxinus* ($p < 0.01$) and of the other fish species ($p < 0.001$ each) which carried no or very low numbers of glochidia at this time. This was also true for host fish-weight-normalized infestation rates which showed significant differences in glochidial load per g between *S. cephalus* and *P. phoxinus* ($p < 0.01$) as well as with all other species ($p < 0.001$ each). The glochidial load of *O. mykiss* was not assessed 48 days pi, since no glochidia were present on the gills of *O. mykiss* at the previous assessment 16 days pi (see NA, Figure 2-1). Gill tissue impairments found in *P. phoxinus* 48 days pi indicated a completed development and the recent excystment of transformed mussels. This matched the observation that after 48 days pi, the first completely metamorphosed and viable juvenile mussels were detected in the tanks of *P. phoxinus*.

The difference in glochidial load of *S. cephalus* between 16 and 48 days pi was not significant (Figure 2-1; $p = 0.470$) and resulted from higher mean body weight of *S. cephalus* sampled 48 days pi and the subsequent sampling of different individuals.

S. cephalus was the species with the highest individual variance in weight, with a similar logarithmic relationship between fish weight and total glochidial load 2 days pi, 16 days pi and 48 days pi (Figure 2-2). No obvious relationship between fish weight and total glochidial load was detected for *P. phoxinus, S. trutta, A. bipunctatus, N. melanostomus*, *C. gobio*, *A. alburnus* and *O. mykiss* owing to the low infestation rate and/or the low individual variance in fish weight.

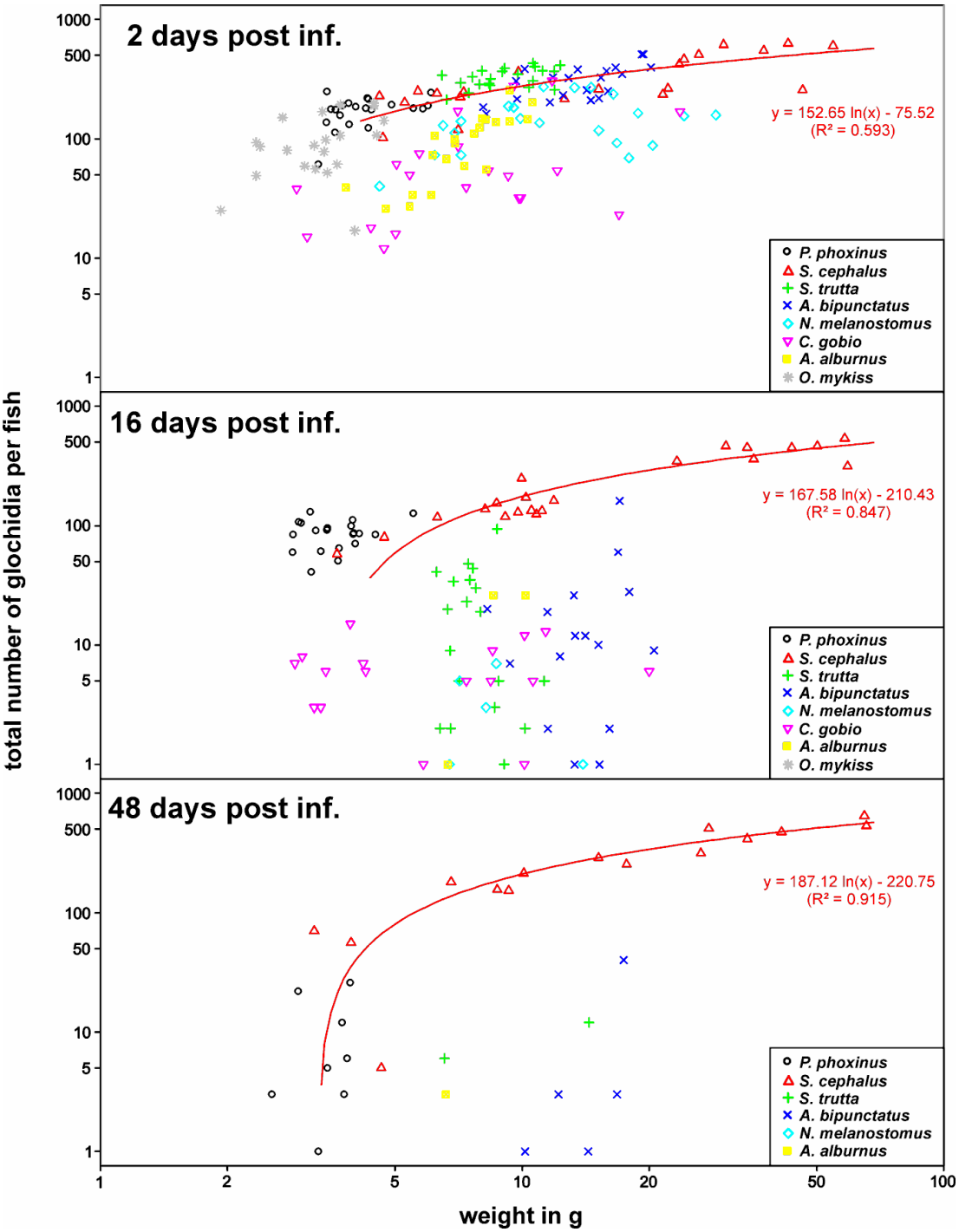

Figure 2-2: Relationship between fish weight and glochidial load in the species tested: *P. phoxinus*, *S. cephalus*, *S. trutta*, *A. bipunctatus*, *N. melanostomus*, *C. gobio*, *A. alburnus*, and *O. mykiss* and the total number of glochidia per fish 2 days pi, 16 days pi, and 48 days pi. The red line represents the logarithmic relationship between fish weight and glochidial load of *S. cephalus*, the species with the highest variance in weight. Note that 21 specimens of all potential host species were sampled 2 days pi and 16 days pi. Ten *P. phoxinus*, 15 *S. cephalus*, 12 *S. trutta*, 16 *A. bipunctatus*, 11 *N. melanostomus*, and 16 *A. alburnus* were sampled 48 days pi.

In the present study, no significant differences (p = 0.231) in median size of glochidia encysted on *P. phoxinus* (212.8 µm), *S. cephalus* (219.4 µm), *S. trutta* (217.7 µm), *A. bipunctatus* (219.9 µm) and *C. gobio* (219.2 µm) were observed 2 days pi (Figure 2-3). Pooling all species, median glochidia size between 2 days pi (217.7 µm) and 16 days pi (215.6 µm) did not differ significantly. This was also true for species specific comparisons between 2 days pi and 16 days pi for *S. cephalus*, *S. trutta*, *A. bipunctatus* and *C. gobio.* In contrast, a small (3.2 %) but significant difference (p = 0.015) in size of glochidia hosted by *P. phoxinus* between 2 days pi (212.8 µm) and 16 days pi (219.6 µm) was found (Figure 2-3). Shell length of juvenile mussels which excysted from *P. phoxinus* 48 days pi ranged between 244.0 µm and 255.3 µm, indicating a marginal (< 15 %) increase in size during metamorphosis.

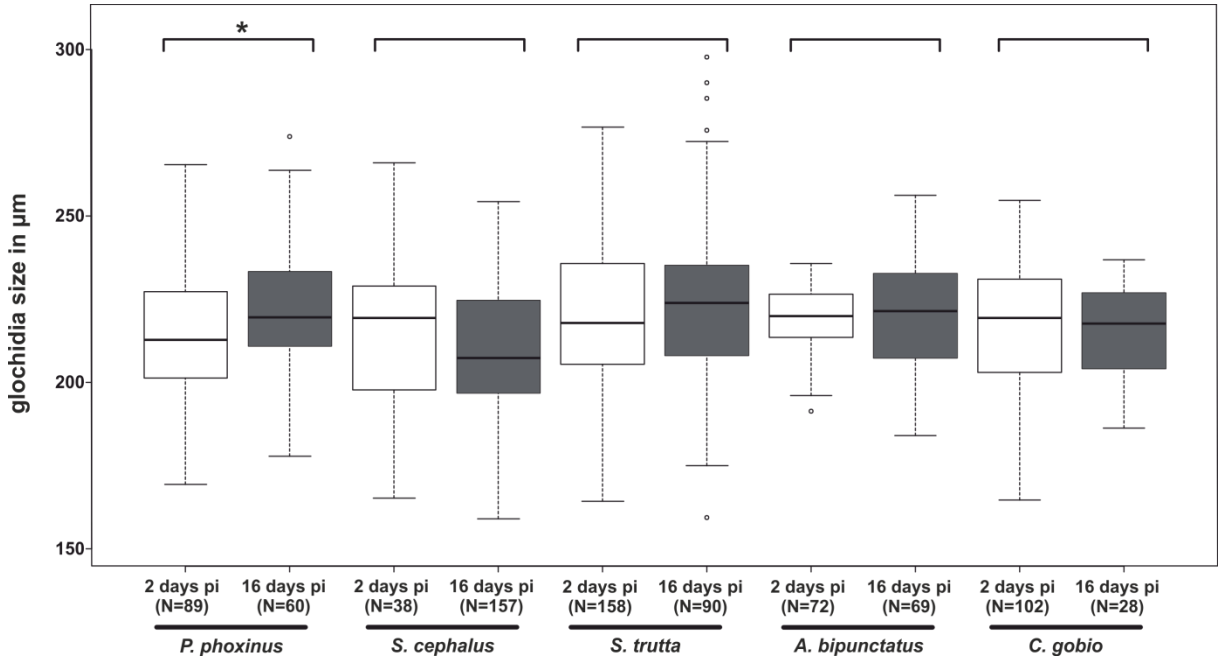

Figure 2-3: Size comparison of glochidia on different host fish species:
P. phoxinus, *S. cephalus*, *S. trutta*, *A. bipunctatus*, and *C. gobio* 2 days pi and 16 days pi. Significant differences are indicated by *****. Boxes are 0.75 and 0.25 percentiles and medians. Whiskers are the 1.5-fold interquartile range. Circles indicate data points outside the 1.5-fold interquartile range.

2. 5. Discussion

Despite the fact that *U. crassus* was the most abundant European river mussel a few decades ago (Israel, 1913; Zwanziger, 1920; Hochwald and Bauer, 1990), relatively little information about the species´ ecological requirements is available. The complex life cycle of unionoid mussels including an obligate parasitic phase is probably a major reason for their decline. Reproduction is susceptible to disruption by any factor that reduces the abundance, distribution, or mobility of host fish. Therefore, knowledge about basic ecological requirements such as host fish suitability is crucial when developing effective conservation strategies for this endangered freshwater mussel species (Barnhart et al., 2008; Geist, 2010). From unpublished literature, there is some indication that *P. phoxinus* and *S. cephalus* can be suitable hosts for *U. crassus* of the Elbe and Rhine drainages (Bednarczuk, 1986; Maaß, 1987; Hochwald, 1997). In contrast, there is no information available about the suitability of fish species for hosting *U. crassus* glochidia from the Danube drainage, one of the most important river systems for the remaining European *U. crassus* populations. Data concerning the suitability of various sympatric fish species such as *A. bipunctatus* and *A. alburnus* are completely lacking, while records of the suitability of species such as *C. gobio* and *S. trutta* have been contradictory (Bednarczuk, 1986; Maaß, 1987; Engel, 1990; Hochwald, 1997). While Bednarczuk (1986) stated that *C. gobio* was not a suitable host, Hochwald (1997) described metamorphosis success of *U. crassus* glochidia hosted by *C. gobio*. In addition *S. trutta* was considered as suitable for hosting *U. crassus* (Engel, 1990), whereas Maaß (1987) considered *S. trutta* unsuitable since all glochidia excysted undeveloped after 16 days. The suitability of invasive species in the upper Danube such as *N. melanostomus*, as hosts for *U. crassus* remained speculative, since Ondračková et al. (2005) found *N. melanostomus* hosting glochidia of duck mussel (*Anodonta anatina* (L.)) but no glochidia of *U. crassus* were detected on this species to date.

After successful isolation of viable glochidia from a self-sustaining Danubian *U. crassus* population, ~ 95,000 glochidia per female (380,000 glochidia / 4 females) were obtained. This is in line with the results of Hochwald and Bauer (1990) who collected 70,600 (SD = 7,000) glochidia per female originating from the Main drainage. However, Bednarczuk (1986) isolated total glochidia numbers between 105,200 and 237,600 per female originating from the Elbe drainage in northern Germany. These findings indicate at least a 10-fold lower glochidia number of *U. crassus* compared with *M. margaritifera* (2 - 4 million glochidia per female, Young and Williams, 1984b; Hastie and Young, 2003a). The high initial infestation rates and the encystment of glochidia on suitable hosts clearly demonstrate the applicability of the infestation procedure of different fish species used with glochidia of *U. crassus.*

The most suitable host fish for *U. crassus* in the present study was *P. phoxinus* in terms of the highest fish-weight-normalized glochidial loads at 2 and 16 days pi and the presence of

completely metamorphosed, viable mussels after 48 days. The lower infestation rate of *P. phoxinus* after 48 days compared with *S. cephalus* is probably due to an earlier completion of metamorphosis and the release of juvenile mussels. *S. cephalus* was also found to be an excellent host for *U. crassus,* showing both the second highest weight-normalized infestation rate 16 days pi and the highest weight-normalized infestation rate 48 days pi. The suitability of *P. phoxinus* and *S. cephalus* match the results of Hochwald and Bauer (1990), which indicated *P. phoxinus* and *S. cephalus* as being excellent hosts for the thick-shelled river mussel originating from the Rhine drainage. Even on suitable hosts the number of encysted *U. crassus* glochidia declines during the parasitic phase as indicated by the > 40 % lower glochidial loads on *P. phoxinus* and *S. cephalus* 16 days pi compared with 2 days pi. This loss of glochidia during the parasitic phase was also recorded for *M. margaritifera* in the case of brown trout (Meyers and Millemann, 1977; Young and Williams, 1984b; Bauer and Vogel, 1987). *S. cephalus* revealed similar infestation rates 48 days pi and 16 days pi indicating that the metamorphosis was still in progress, while *P. phoxinus* infestation rate decreased and several full developed juvenile mussels were found 48 days pi. In addition, gill tissue impairments found in *P. phoxinus* gills indicate a former occupation by glochidia and a variable progression of glochidia development on different host species. In line with the results of Hochwald and Bauer (1990), we found a late release of the first juvenile mussels and a similar delay in development of glochidia hosted by *S. cephalus* compared with *P. phoxinus*. For *U. crassus* originating from the Rhine system, Hochwald and Bauer (1990) reported completion of metamorphosis and release of the first living juvenile mussel after 26 days for *P. phoxinus* and 33 days pi for *S. cephalus* indicating a ~ 25 % extended development period of *U. crassus* glochidia on *S. cephalus*. In the present study the development period for glochidia was more than 15 days longer for all tested host fish compared with the findings of Hochwald and Bauer (1990). Theoretically, temperature is likely to be the most important factor for development progression, but similar water temperatures (~ 12 °C) were reported in both experiments. However, the results of the present study indicate that *U. crassus* requires between 550 and 650 degree-days for development on *P. phoxinus*. The different development progress of glochidia encysted on different host species show that host suitability itself has substantial influence on glochidia development. In streams with multiple host species, the variable progression of glochidia development in addition to various spawning events of adult mussels extends the drop-off period of juvenile mussels during one season. On a population level, an asynchronous excystment may be beneficial for surviving temporarily unfavourable environmental conditions such as high sheer-stress during periodical floods. At the same time, an extended drop-off period may increase the distribution range of juvenile mussels related to fish migrations. In contrast to the *Margaritiferidae, U. crassus* have a short life-span of usually 10

- 20 years and produce relatively low glochidia numbers per female. Consequently, a continuous recruitment is necessary for sustainable *U. crassus* populations. Another important factor is the divergent habitat use of different host species, which could enhance spatial distribution of juvenile mussels. *P. phoxinus* and juvenile *S. cephalus* show swarming behaviour in shallow river sections and can thus play a dominant role in local recruitment, whereas adult *S. cephalus* might be responsible for the wider distribution of *U. crassus* owing to their broader home ranges and more extensive migration behaviour.

Compared with *P. phoxinus* and *S. cephalus*, the low infestation rates 16 days pi indicate a highly diminished suitability (~ 90 % loss 16 days pi) of *S. trutta, A. bipunctatus* and *C. gobio* for hosting *U. crassus* glochidia. For *S. trutta,* several studies describe an excystment of undeveloped glochidia on *U. crassus* within 2-4 weeks pi, although successful encystment was apparent (Maaß, 1987; Hochwald and Bauer, 1990). In contrast, Engel (1990) reported that *S. trutta* were suitable for hosting *U. crassus* glochidia. The low suitability of *S. trutta* as host for *U. crassus* observed in our study is in contrast to the high suitability for glochidia from *M. margaritifera*, for which the species seems to be the most important or exclusive host in some areas (Geist et al., 2006; Taeubert et al., 2010).

In the case of *C. gobio*, the present results are contrary to those obtained by Hochwald and Bauer (1990) who observed good suitability in eight *C. gobio* specimens for glochidia development within the Rhine drainage. However, Bednarczuk (1986) and Maaß (1987) reported no susceptibility of *C. gobio* for infestation with *U. crassus* glochidia, testing only one specimen. Possible explanations for the different susceptibility of a species to glochidia infestation are i) host responses (e.g. immunity), ii) mussel-related traits (e.g. different genetic constitution or phenotypes), or iii) local co-evolution of host-parasite relationships. Taeubert et al. (2010) reported different suitabilities of various *S. trutta* strains for hosting glochidia of the freshwater pearl mussel (*M. margaritifera*) after weight-normalization. Various *C. gobio* lineages originating from different drainage systems may also respond differently to infestation with *U. crassus* glochidia. The hypothesis of mussel-strain dependency on the host-parasite interaction is supported by the different suitability of dace (*Leuciscus leuciscus* L.) for glochidia of different *U. crassus* subspecies / strains found in northern Germany (Engel and Wächtler, 1989). The latter authors found that glochidia of *U. crassus crassus* forma *maximus* metamorphosed successfully on *L. leuciscus*, while larvae of *U. crassus crassus* did not develop on this fish species.

The present results strongly suggest that *A. alburnus*, invasive *N. melanostomus* and introduced *O. mykiss* are unsuitable hosts for *U. crassus*. These fish species shed nearly all glochidia within the first 16 days pi. However, 2 days pi *N. melanostomus*, *A. alburnus* and *O. mykiss* had average infestation rates indicating a minimal time period of two days for the innate immune response of non-suitable hosts. In addition, differences in initial infestation

rates (2 days pi) were primarily dependent on species-specific variation of ventilation rate and gill surface area. The minimal time period of two days for innate immune response is comparable to the observation by Fustish and Millemann (1978) who described a well-developed hyperplastic reaction in coho salmon (*Oncorhynchus kisutch*, Walbaum 1792) 4.5 days after infestation with *M. margaritifera* glochidia. The high initial infestation rates of unsuitable host fish such as *N. melanostomus* and *O. mykiss* might decrease the recruitment success of *U. crassus* since glochidia attached to unsuitable hosts are then lost to suitable hosts. This is partly compensated by the high number of glochidia produced per specimen, but may become relevant at higher ratios of unsuitable to suitable hosts in a river. Despite the fact that evolution of host-specificity is associated with host availability in *U. crassus* habitats, there is little information (Bauer et al., 1991) about host infestation rates under natural conditions and the importance of certain fish species for recruitment of *U. crassus*. In addition to host suitability, the behaviour and abundances of fish species are assumed to substantially influence the reproductive success of *U. crassus*. On the one hand the size of *U. crassus* glochidia of more than 200 µm facilitates a fast deposition in the river open water column enhancing contact with benthic feeding hosts (Barnhart et al., 2008) such as *C. gobio.* On the other hand partially surface feeding fish such as *P. phoxinus* and *S. cephalus* can easily take up glochidia following the spurting behaviour of female *U. crassus* (Vicentini, 2005). This behaviour is unique for *U. crassus* and has been observed in 2009 and 2010 in the studied stream as well. Since spurting behaviour presents the glochidia individually (Vicentini, pers. comm.), the use of a glochidia suspension for artificial infestation of different fish species is similar to the natural conditions.

However, the results of this lab-based study only provide insights into the relative suitability of different fish hosts under standardized conditions. They do not necessarily reflect the situation in the wild since the likelihood of host infestation also depends on the host behaviour, their local distribution, and the likelihood of encounter. Further investigation concerning abundance and distribution of sympatric fish species are needed to clarify host suitability in the wild.

In summary, the suitability of the tested fish species as host for *U. crassus* from the Danube drainage can be classified by infestation rates as follows: i) good hosts (*P. phoxinus, S. cephalus*) ii) poor hosts (*S. trutta, A. bipunctatus* and *C. gobio*) iii) unsuitable hosts (*A. alburnus, N. melanostomus*, *O. mykiss*). The different host fish, which are suitable for *U. crassus* glochidia are not phylogenetically closely related, suggesting the absence of adaptation to certain evolutionary fish lineages. The artificial infestation approach used in this study cannot clarify the role of poor hosts for natural reproduction of *U. crassus*. Therefore further investigations concerning natural infestation rates of these fish species are necessary.

The glochidial load of suitable hosts rises with fish size but the logarithmic relationship between fish weight and total glochidial load of *S. cephalus* indicates a declining relative susceptibility with increasing host size. Possible physiological explanations are higher ventilation rates of smaller fish and the reduction in relative gill area (= gill surface area / body weight) with increasing fish size (Pauly, 1981; Rombough and Moroz, 1997). An involvement of a more intense immune response resulting in declining susceptibility of larger hosts is unlikely, as evident from the similarity of the mathematical relationship between fish weight and glochidial loads of *S. cephalus* at different times. Under natural conditions host fish size is often governed by habitat conditions. With regard to artificial infestations for conservation purposes the host fish size has to be considered in order to improve captive breeding success. Based on total fish biomass and stocking density more mussels can be bred on smaller fish (*P. phoxinus*, juvenile *S. cephalus*) whereas larger fish (adult *S. cephalus*) are more robust with regard to handling and diseases.

The observed marginal increase in size of *U. crassus* glochidia encysted on *P. phoxinus* during the parasitic phase and the larger size of the excysted juvenile mussels is in contrast to observations by Maaß (1987), who found no size difference between glochidia and juvenile mussels. Possible explanations for the size variation found in this study are i) marginal growth during the parasitic phase, ii) decreased likelihood of a successful metamorphosis of the smallest and weakest glochidia iii) natural error in the data due to a high variation in size of glochidia 2 days pi (Figure 2-3). However, the different suitability of various host species in addition to the different development speed of glochidia on different hosts indicate a parasitic character of the interaction between host fish and *U. crassus*, but the most obvious advantage from this host-parasite interaction is the dispersal – especially in upstream direction – of sedentary *U. crassus*.

2. 6. Conclusions and implications for conservation

Detailed knowledge about the basic ecological requirements such as suitable host fish species is essential for the development of effective and sustainable conservation strategies for endangered *U. crassus* populations. In addition, this information is necessary for a general understanding of the evolution of host-parasite interactions. The present study detected substantial differences in the suitability of tested fish species for hosting glochidia of *U. crassus* from the Danubian drainage. Host suitability varies in both the infestation rates and the progression of metamorphosis during the parasitic phase. Due to decreasing susceptibility with increasing host size it is crucial to consider fish size in different conservation measures, e.g. for development of captive breeding programmes. The two species identified as the most suitable host fishes for *U. crassus* - *S. cephalus* and *P.*

phoxinus - are not among the highly valued species in fisheries management and have occasionally even been actively removed from streams by electrofishing in order to reduce competition with higher valued species, e.g. *S. trutta*. The results of this study clearly show that both species should be actively supported in *U. crassus* catchments and that the conservation management of freshwater mussel species requires the inclusion of suitable fisheries management in order to be successful.

3. The relationship between endangered thick-shelled river mussel (*Unio crassus*) and its host fishes

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3. 1. Abstract

Successful conservation strategies require consideration of species interactions. In their life cycle, freshwater mussels of the family Unionidae depend on a suitable host fish on which their larvae metamorphose into juveniles. This study investigated the host-parasite interaction of different fish species with the endangered European thick-shelled river mussel (*Unio crassus*) under artificial and natural conditions. *Chondrostoma nasus*, *Cottus gobio*, *Leuciscus idus*, *Phoxinus phoxinus*, *Squalius cephalus*, *Scardinius erythrophthalmus* and three different strains of *Gasterosteus aculeatus* were identified as suitable hosts. In contrast, *U. crassus* was not able to metamorphose on *Acipenser ruthenus* and *Rutilus rutilus*. In natural *U. crassus* streams, sixteen different fish species were found, with pronounced differences in the occurrence and abundance of suitable hosts. Data from the laboratory infections and from the field investigations strongly suggest that *S. cephalus* is the most important host for *U. crassus* in the Danube drainage, despite the fact that some populations exclusively depend on other hosts. The results of this study indicate that (i) an evaluation of host fish importance in unionid mussels should not exclusively rely on standardized infestation experiments without considering natural fish communities, (ii) host suitability can vary within different strains of the same host species, (iii) development time of *U. crassus* on different hosts varies considerably, and (iv) natural recruitment in functional *U. crassus* populations can be secured by highly different fish community structures and densities. The wide host range of *U. crassus* is likely to increase the chances of successful restoration of priority populations for conservation.

3. 2. Introduction

Human alterations to aquatic ecosystems have been shown to lead to significant changes in community compositions and decreases in species richness (Galbraith et al., 2010). In

particular, freshwater mussels have become priority species in aquatic conservation, since many populations severely declined during the last decades (Lydeard et al., 2004; Bogan, 2008; Geist, 2010). Development of effective conservation strategies in general, and for freshwater mussels in particular, depends on the integration of aut- and synecological information (Geist, 2011). This includes data on the habitat requirements as well as detailed knowledge on the interactions with other species. This interaction is particularly important for unionid freshwater mussels which all have an obligate parasitic phase on a suitable host fish.

Among the European unionid species, the thick-shelled river mussel (*Unio crassus*, Philipsson 1788) has become a major target species for conservation (Zettler and Jueg, 2007; Geist, 2010, 2011). *Unio crassus* colonizes a wide range of habitats and was considered the most abundant unionid species in central and northern Europe in the 20th century (Israel, 1913; Zwanziger, 1920; Jäckel, 1962; Bednarczuk, 1986; Hochwald and Bauer, 1990). Today, *U. crassus* is listed as endangered in the IUCN Red List of threatened species (van Damme, 2011) and in the annexes II and IV of the European fauna and flora habitat´s (FFH) directive (Council of the European Communities, 1992; Bouchet et al., 1999).

During spring and summer, *U. crassus* females release ~ 100,000 glochidia larvae per spawning event to the free-flowing water (Bednarczuk, 1986; Hochwald, 1997). The glochidia reach their hosts passively by the water current and preferentially attach to the gills, where the mussel larvae become encysted by host epithelial cells and develop into juvenile mussels (Hochwald and Bauer, 1990). Depending on the water temperature and the host species, the metamorphosis lasts 20 to 50 days (Bednarczuk, 1986; Hochwald and Bauer, 1990; Hochwald, 1997; Taeubert et al., 2012a). After metamorphosis, the juvenile mussels excyst and bury themselves into the stream bed substratum for a period of 1 - 3 years. After this period, the juveniles emerge as filter feeders at the substratum surface (Bednarczuk, 1986; Hochwald and Bauer, 1990; Hochwald, 1997). Although some studies investigated the suitability of different fish species for hosting *U. crassus* glochidia from the Rhine and Elbe drainages (Bednarczuk, 1986; Maaß, 1987; Engel, 1990; Hochwald and Bauer, 1990; Hochwald, 1997), the suitability of many co-occuring fishes as hosts remains unknown. Especially for species originating from the Danube drainage – one major distribution area of *U. crassus* – only little information about the host-parasite interaction is available (Taeubert et al., 2012a). For instance, the host-suitability of several sympatric fish species like *Chondrostoma nasus, Leuciscus idus* and *Acipenser ruthenus* are completely lacking, while other species such as *Scardinius erythrophthalmus*, *Gasterosteus aculeatus* and *Rutilus rutilus* were not previously assessed in the Danube drainage.

Moreover, most of the available data exclusively refer to artificial infestations in the laboratory which provide insights into the relative suitability of different fish hosts under standardized conditions (Bednarczuk, 1986; Maaß, 1987; Hochwald, 1997; Taeubert et al., 2012a).

However, such approaches do not necessarily reflect the situation under natural conditions and are thus not reliable when the contribution of different fish species to the recruitment of *U. crassus* needs to be evaluated. To our knowledge, no study has yet tested the metamorphosis success of *U. crassus* from wild fishes.

The core objective of this study was to assess the host-parasite interaction between different fish species and *U. crassus* under artificial and natural conditions. On the one hand, suitability of different fish species was tested by quantifying the infestation rate at different time points, as well as the metamorphosis success over the whole period of excystment. This experiment included fish species which have to our knowledge not been tested before such as *Acipenser ruthenus*, *Chondrostoma nasus* and *Leuciscus idus*, as well as three different strains of stickleback from the Danube and Elbe drainages. On the other hand, the fish species composition in functional *U. crassus* streams (i.e. with recent recruitment) and metamorphosis success of *U. crassus* from infested wild fishes were analysed.

3. 3. Methods

3. 3. 1. Collection of glochidia for artificial infestation

During the spawning period of *U. crassus* in 2011, glochidia were obtained from the self-recruiting population Ischler Ache (IA; Danube drainage; Germany) following the procedure described in Taeubert et al. (2012a). Gravid females were identified by observing their swollen marsupia after gently opening the shells < 5 mm, following recommendations by Moorkens and Costello (2004). Due to different times of glochidia release and the short life-span of released glochidia, artificial infections were carried out at three different time points. On May 25th and July 3rd, one pool of glochidia originating from 2 females and on July 9th one pool of glochidia originating from 3 females were used to produce a homogenous suspension for the artificial infestation experiments. The final glochidia concentration in infestation baths was ~ 7,800 glochidia L^{-1} (total 15 L) on May 25th, ~ 6,800 glochidia L^{-1} (total 15 L) on July 3rd and ~ 3,700 glochidia L^{-1} (total 45 L) on July 9th.

3. 3. 2. Artificial infestation procedure and maintenance of fishes

In order to test if glochidia from the three different infestations were viable and able to metamorphose into juvenile mussels, European minnow (*Phoxinus phoxinus* L.) – previously identified as a suitable host of *U. crassus* (Taeubert et al., 2012a) – was used as a positive control in each experiment. For artificial infestation, nine fish species were used, including chub (*Squalius cephalus* L.), European minnow, ide (*Leuciscus idus* L.), nase (*Chondrostoma nasus* L.), river bullhead (*Cottus gobio* L.), roach (*Rutilus rutilus* L.), rudd

(*Scardinius erythrophthalmus* L.), sterlet (*Acipenser ruthenus* L.), and the three-spined stickleback (*Gasterosteus aculeatus* L.). To test for intra-species variation, three *G. aculeatus* strains originating from the river "Stör" (Elbe drainage), "Moosach" (Danube drainage) and "Glonn" (Danube drainage) were additionally included. The origin of the 11 artificially tested fish species and strains is given in Table 3-1.

Table 3-1: Origin of the studied species from the upper Danube drainage and location of the investigated *U. crassus* streams.

species	**subdrainage**	**drainage**	**latitude / longitude**[a]
Unio crassus	Inn	Danube	47.949 / 12.414
Acipenser ruthenus	Naab	Danube	49.760 / 12.186
Chondrostoma nasus	Naab	Danube	49.823 / 12.416
Cottus gobio	Isar	Danube	48.161 / 11.609
Gasterosteus aculeatus (Elbe)	Stör	Elbe	53.913 / 09.526
Gasterosteus aculeatus (Glonn)	Amper	Danube	48.363 / 11.383
Gasterosteus aculeatus (Moosach)	Isar	Danube	48.393 / 11.727
Leuciscus idus	Amper	Danube	48.363 / 11.383
Phoxinus phoxinus	Naab	Danube	49.823 / 12.416
Rutilus rutilus	Isar	Danube	48.292 / 11.645
Scardinius erythrophthalmus	Inn	Danube	47.984 / 12.436
Squalius cephalus	Naab	Danube	49.823 / 12.416
investigated *U. crassus* stream	**subdrainage**	**drainage**	**latitude / longitude**[a]
Emersacker Weiherbach (EW)	Zusam	Danube	48.483 / 10.685
Mooshamer Weiherbach (MW)	Isar	Danube	47.900 / 11.526
Ischler Ache (IA)	Inn	Danube	47.949 / 12.414
Staffelsee Ach (SA)	Ammer	Danube	47.742 / 11.120

[a] decimal degree latitude and decimal degree longitude (WGS 84)

The tested fishes were simultaneously exposed to the infestation baths to ensure identical conditions for glochidia uptake. Each infestation was carried out for 30 min under constant agitation. In the first infestation experiment (1st inf on May 25th), 50 specimens of *P. phoxinus* and 50 individuals of each *G. aculeatus* strain were used. A number of 50 *C. gobio*, 50 *S. erythrophthalmus*, 41 *C. nasus* and 21 *P. phoxinus* were infested on July 3rd in the second infestation experiment (2nd inf). During the third infestation (3rd inf) on July 9th, 50 *S. cephalus*, 50 *A. ruthenus,* 50 *R. rutilus* and 21 *P. phoxinus* were used. Due to limited availability of *L. idus*, this species was tested in a subsequent experiment using glochidia

from the 1st infestation. Three *L. idus* were exposed for 15 min to the glochidia suspension used before for the artificial infestation of *G. aculeatus* and *P. phoxinus*.

To avoid immune-related unsuitability of tested fishes caused by previous infestations, only fishes which had no previous contact to glochidia of unionid species were used for the laboratory study. After each infestation, fishes were maintained under identical conditions in 120 L circular flow- through tanks that were supplied by groundwater (0.5 L s^{-1}) and fed with a mixture of frozen Tubifex and commercial trout chow (0.7 mm Aqua Pro, Skretting) until 22 days post-infection (pi). For the observation of the excystment process, fishes were separated by species and maintained in 20 L aquaria 22 days pi. The aquaria were supplied by the same groundwater (0.05 L s^{-1}). Water temperature in each aquarium was recorded by temperature loggers (Lascar Electronics Limited, Salisbury, UK) every 30 minutes (Table 3-2). In order to separate the fishes from the mussels after excystment and to prevent possible predation by the hosts, a grid was installed 3 cm above the bottom of the aquaria. The number of specimens used for the quantification of excystment is given in Table 3-3.

3. 3. 3. Assessment of infestation rates and metamorphosed mussels

Infestation rates 3 days post-infestation (pi) and 22 days pi as well as weight-normalized infestation rates were determined as described in Taeubert et al. (2010). For two fish species (*C. nasus*, *S. erythrophthalmus*), high mortality reduced the number of available specimens for analysing the infestation rates (8 specimens three days pi, no specimen 22 days pi). Since no glochidia were found on *R. rutilus* and *A. ruthenus* three days pi, these fish species were not additionally assessed 22 days pi. Tanks and aquaria were inspected every 24 h for the presence of viable juvenile mussels. Mussels were collected using a vacuum tube and their viability was checked under a binocular microscope by observing active pedal movement. The mean number of excysted mussels per fish (mussels per g fish weight) was calculated for each day and summed up at the end of excystment.

In order to analyse the growth of glochidia during metamorphosis, the size of encysted glochidia three days pi and 22 days pi was determined by measuring the maximum total shell length (± 2 µm) of *U. crassus* on the two fish species with the highest numbers of glochidia 22 days pi (*P. phoxinus* and *S. cephalus*), following the procedure described for *Margaritifera margaritifera* L. in Taeubert et al. (2010).

3. 3. 4. Natural infestation and fish species composition in the wild

The fish communities of four *U. crassus* streams, the Emersacker Weiherbach (EW), the Mooshammer Weiherbach (MW), the Ischler Ache (IA) and the Staffelseeach (SA) were assessed by electrofishing directly downstream of the largest mussel populations (Table 3-

1). All four streams are among the most important *U. crassus* streams in Bavaria and contain self-recruiting populations with a number of ≥ 7,000 adult *U. crassus* (Ansteeg, 2006; Hochwald, 2008; Hochwald and Ansteeg, 2009; Schneider, 2012). Electrofishing was carried out in two 300 m long sections per stream, wading from downstream to upstream direction with a single anode following the German standard (VDFF, 2000). The EW (~ 1.2 m width) and the MW (~ 1.7 m width) were analysed using a 3 kW portable electrofishing backpack unit (ELT 62 II-D, Grassl, Schoenau, Germany) on June 20th 2011 and June 21st 2011. Since the analysed sections of the Ischler Ache (IA) and the Staffelsee Ach (SA) were between 6 and 10 m wide, both streams were assessed using an 11 kW electrofishing unit (EL 65 II, Grassl, Schoenau, Germany) on July 26th and August 3rd.

Stunned fishes were collected with a dip net and maintained in plastic tanks with permanent oxygen supply. The total length of all specimens was measured (± 0.5 cm) and fishes > 10 cm were weighted (± 0.5 g) individually. A representative number (N > 10) of smaller specimen of the same length were pooled and weighted. To minimize negative effects on the fish populations, the infestation rate was checked non-invasively by gently opening the operculum and inspecting the gill arches. For practicability reasons, three categories of infestation were distinguished, depending on the total number of glochidia on the gill arches: No, weak (< 10 glochidia per fish) and strong (≥ 10 glochidia per fish). Caught fishes were directly released at the sampling sites, except for a small number of infested fishes that were maintained in aerated containers and brought to the laboratory for analysing the excystment. Since it was not possible to accurately determine the infestation rates of *A. alburnus*, *A. anguilla*, *C. gobio* and *R. sericeus* without harming these species, the infestation of these species was not assessed in the field. However, 10 individuals of *C. gobio* and *R. sericeus* as well as a subset (for details see Table 3-4) of infested *S. cephalus* (MW, IA), *R. rutilus* (EW), *S. trutta* (EW), *S. erythrophthalmus* (EW), *P. fluviatilis* (MW, IA) and *A. bipunctatus* (IA) were transported to the laboratory of the Aquatic Systems Biology Unit and maintained in 20 L aquaria to analyse the excystment process as described above.

3. 3. 5. Statistical analyses

Differences in total glochidia infestation rates and weight-normalized glochidia infestation rates, as well as differences in glochidia sizes between species were tested using non-parametric Kruskal-Wallis sum of ranks test and post hoc pairwise Wilcoxon rank sum test since ANOVA assumptions were not fulfiled. Bonferroni correction was applied to correct for multiple tests. All statistical analyses were performed in R version 2.12.0 (© 2010, R Foundation for Statistical Computing).

3. 4. Results

3. 4. 1. Artificial infestations - infestation and excystment rates

The artificial infestation resulted in encystment of *U. crassus* glochidia from all tested fish species except for the unsuitable species *A. ruthenus* and *R. rutilus* 3 days pi. From all infestation experiments viable juvenile mussels were found. Highest numbers of metamorphosed juveniles were collected from *P. phoxinus*, which was used as a positive control in each infestation experiment (Table 3-2). However, the viability of glochidia varied strongly between the three infestation experiments indicated by the different initial infestation rates and the variability in metamorphosis success of glochidia encysted on *P. phoxinus*. The weight-normalized glochidial load of *P. phoxinus* in the 2nd inf was ~ 4-fold lower compared with the weight-normalized glochidial load of *P. phoxinus* from the 1st inf, although the glochidia concentration of the infestation baths was similar for both experiments (Table 3-2, Figure 3-1). Consequently, the mean number of successfully metamorphosed juveniles on *P. phoxinus* ranged from 1.5 glochidia fish^{-1} (2nd inf) up to 29.4 glochidia fish^{-1} (1st inf). In line with the twofold lower concentrated glochidia suspension used in the 3rd inf, the weight-normalized glochidial load of *P. phoxinus* was 4.4-fold lower than the weight-normalized glochidial load of *P. phoxinus* from the 1st inf 3 days pi in this experiment. However, except for *G. aculeatus* “Moosach” 22 days pi, *P. phoxinus* revealed significantly ($p < 0.05$) higher fish-weight-normalized glochidial loads 3 and 22 days pi compared with all other fish species used in this study.

Table 3-2: Results of artificial host fish infestations with mean numbers of glochidia 3 days pi, 22 days pi and juvenile mussels after excystment.

	3 days pi			22 days pi				excystment					
fish species	glochidia per fish	Glochidia per g fish weight	n fish	glochidia per fish	glochidia per g fish weight	n fish	weight-normalized gloch. loss [%]	mussels per fish	mussels per g fish weight	n fish	weight-normalized gloch. loss [%]	start - end of excystment [days pi]	temperature $\bar{X}$ ± SD [°C]
P. phoxinus	218.9	53.0	15	97.5	25.4	15	52	29.4	5.9	10	89	28 - 59	14.1 ± 1.2
G. aculeatus (Elbe)	11.5	14.9	15	2.7	4.1	15	72	1.5	0.9	8	94	33 - 52	14.2 ± 1.2
G. aculeatus (Moosach)	48.2	33.6	15	22.2	16.3	15	51	0.9	0.8	10	98	30 - 34	14.2 ± 1.3
G. aculeatus (Glonn)	18.0	14.0	15	5.9	4.9	15	65	0.5	0.5	10	96	29 - 43	14.6 ± 1.1
P. phoxinus	63.5	13.7	8	22.5	4.8	2	65	1.5	0.2	2	99	29 - 38	14.3 ± 0.4
C. gobio	29.0	3.7	15	8.5	1.4	15	62	1.0	0.2	7	95	25 - 38	14.1 ± 0.5
S. erythrophthalmus	78.1	4.7	15	NA	NA			1.0	0.1	5	98	38 - 38	13.3 ± 1.3
C. nasus	33.1	5.1	8	NA	NA			1.4	0.2	6	96	42 - 53	12.2 ± 1.9
P. phoxinus	49.0	10.3	8	29.0	6.3	8	39	11.0	2.0	5	81	32 - 55	13.2 ± 0.5
S. cephalus	190.2	6.2	15	121.7	4.7	15	24	84.8	2.9	10	53	39 - 69	13.4 ± 0.6
A. ruthenus	0	0	15	NA[a]	NA[a]								
R. rutilus	0	0	15	NA[a]	NA[a]								

NA not assessed due to high mortality of host fishes.

NA[a] not assessed because no infestation was found 3 days pi.

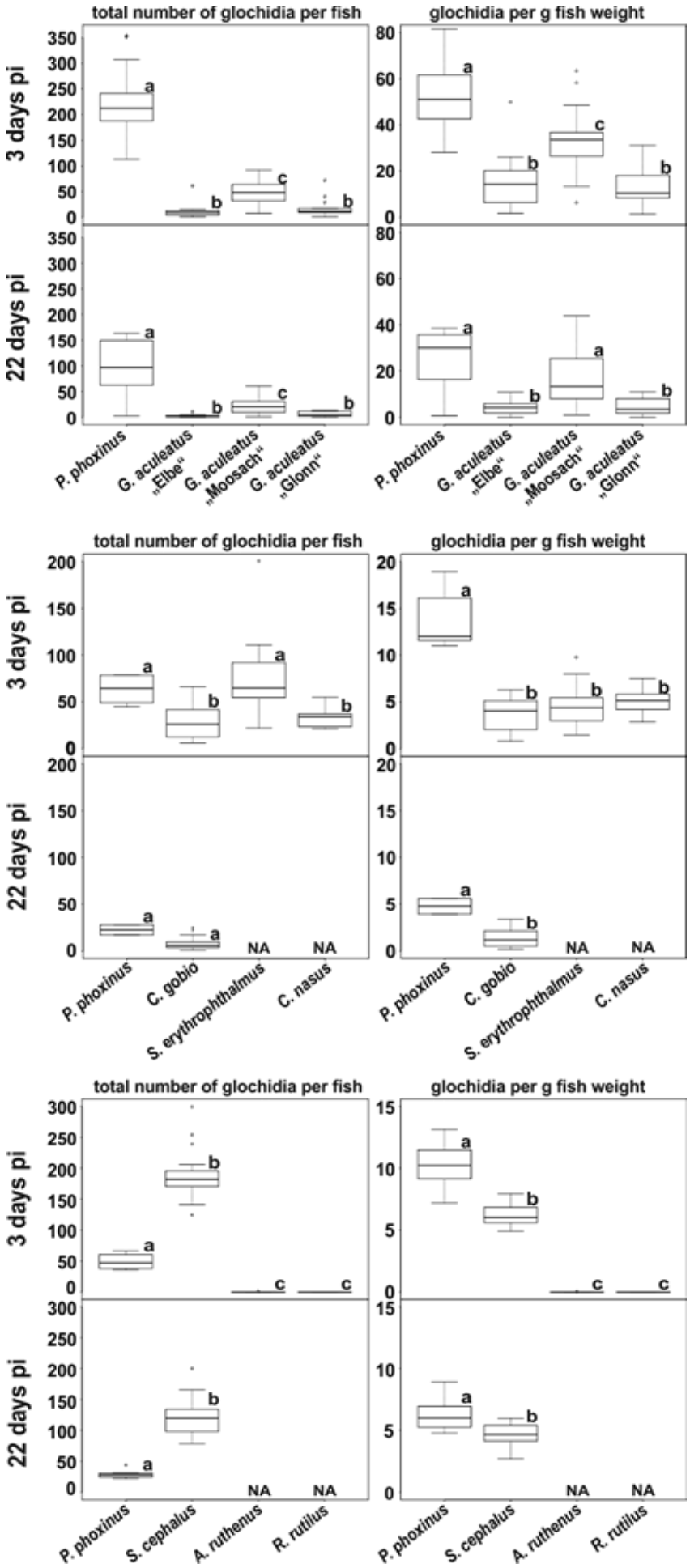

Figure 3-1: Total counts and fish-weight-normalized glochidial load on different fish species: *P. phoxinus*, *G. aculeatus* "Elbe", *G. aculeatus* "Moosach", *G. aculeatus* "Glonn", *C. gobio*, *S. erythrophthalmus*, *C. nasus*, *S. cephalus*, *A. ruthenus* and *R. rutilus* 3 days pi and 22 days pi. Significant differences are indicated by different letters a, b, c ($p < 0.05$). Boxes are 0.75 and 0.25 percentiles and medians. Whiskers are the 1.5-fold interquartile range. Circles indicate data points outside the 1.5-fold interquartile range. NA indicates fish species which were not assessed 22 days pi due to high fish mortality (*S. erythrophthalmus*, *C. nasus*) or no glochidia presence 3 days pi (*A. ruthenus*, *R. rutilus*).

G. aculeatus originating from the different drainage systems revealed pronounced differences in infestation rates, as evident from the significantly ($p < 0.05$) and by > 60 % higher total infestation rates of *G. aculeatus* “Moosach” compared with the *G. aculeatus* strains originated from “Glonn” and “Elbe” (Table 3-2; Figure 3-1), 3 and 22 days pi. Similarly, the fish-weight-normalized infestation rates of *G. aculeatus* “Moosach” were 50 – 75 % higher than fish-weight-normalized infestation rates of *G. aculeatus* strains from “Glonn” and “Elbe”. The weight-normalized glochidia loss 22 days pi ranged between 51 % for *G. aculeatus* “Moosach” and 72 % for *G. aculeatus* “Elbe”. The excystment rates for *G. aculeatus* – especially of *G. aculeatus* “Moosach” – were probably underestimated in this study, due to the relatively high fish mortality during the excystment process following an uncompleted metamorphosis of most of the encysted glochidia on this strain. Therefore, calculated glochidia loss of *G. aculeatus* “Moosach” was as high as glochidia mortality rates of *G. aculeatus* “Elbe” and *G. aculeatus* “Glonn”. However, the three *G. aculeatus* strains yielded 6 to 10-fold lower numbers of metamorphosed mussels than *P. phoxinus* after weight-normalization (Table 3-2). *Cottus gobio*, *C. nasus* and *S. erythrophthalmus* did not reveal significant differences in the weight-normalized glochidial loads 3 days pi. Along with the unavoidable differences in mean size of tested fish species, the total glochidial load of *C. gobio* and *C. nasus* were significantly ($p < 0.05$) lower than the glochidial loads of *S. erythrophthalmus*. Twenty-two days pi, *C. gobio* revealed significantly lower ($p < 0.05$) weight-normalized glochidial loads than *P. phoxinus* while the total glochidial loads did not differ significantly ($p = 0.061$). The number of glochidia on *C. nasus* and *S. erythrophthalmus* could not be assessed 22 days pi, due to high mortality rates of these species. Weight-normalized mussel excystment rates in *C. gobio* and *C. nasus* were almost equal to the excystment rates in *P. phoxinus* (all 0.2 mussels per g fish weight), while 0.1 mussels per g fish weight excysted from *S. erythrophthalmus*.

The total glochidial load of *S. cephalus* was significantly higher ($p < 0.001$) than the glochidial load of *P. phoxinus* 3 days pi and 22 days pi, while after weight-normalization, *P. phoxinus* revealed significantly higher ($p < 0.01$) glochidial loads both 3 days pi and 22 days pi. *Squalius cephalus* had the highest total excystment rate (84.8 mussels $fish^{-1}$) and the second highest weight-normalized excystment rate (2.9 mussels g fish $weight^{-1}$) of all artificial infestation experiments in this study. In addition, *S. cephalus* revealed the lowest weight-normalized glochidia loss 22 days pi (24 %) and after excystment (53 %), compared with all other tested fish species.

The infestation rates of *A. ruthenus* and *R. rutilus* differed significantly from the infestation rates of *S. cephalus* and *P. phoxinus*, with no glochidia present on their gills 3 days pi. Therefore, infestation rates of *A. ruthenus* and *R. rutilus* were not additionally assessed 22 days pi (see NA, Figure 3-1). In addition to the three standardized artificial infestations

experiments, 8 living juvenile mussels were detected in the aquaria of *L. idus*, indicating a successful development of *U. crassus* also in this species.

3. 4. 2. Timing of excystment

The timing of *U. crassus* excystment varied between different host species. Applying the concept of day degrees (dd), i.e. the sum of daily water temperatures, the first mussel was released after 350 dd (25 days pi) by *C. gobio* (Table 3-2). Along with the lowest mean temperature during infestation and excystment, *C. nasus* released the first living juvenile mussel 42 days pi (510 dd). *Unio crassus* hosted by *S. cephalus* excysted between 520 and 930 dd, while mussels hosted by *P. phoxinus* excysted between 420 and 730 dd pi under similar temperature conditions (Table 3-2).

3. 4. 3. Glochidia sizes

Pronounced differences in glochidia sizes between 3 days pi and 22 days pi were observed in *P. phoxinus* and *S. cephalus* (Figure 3-2). The sizes of glochidia hosted by *P. phoxinus* (228.8 µm ± 7.7, n = 50) and *S. cephalus* (233.2 µm ± 6.3, n = 50) 22 days pi were significantly larger ($p < 0.001$) than those of glochidia 3 days pi in *P. phoxinus* (222.8 µm ± 3.7; n = 50) and *S. cephalus* (224.4 µm ± 7.5; n = 50). However, size of glochidia encysted on *P. phoxinus* and *S. cephalus* three days pi did not differ significantly ($p = 1$).

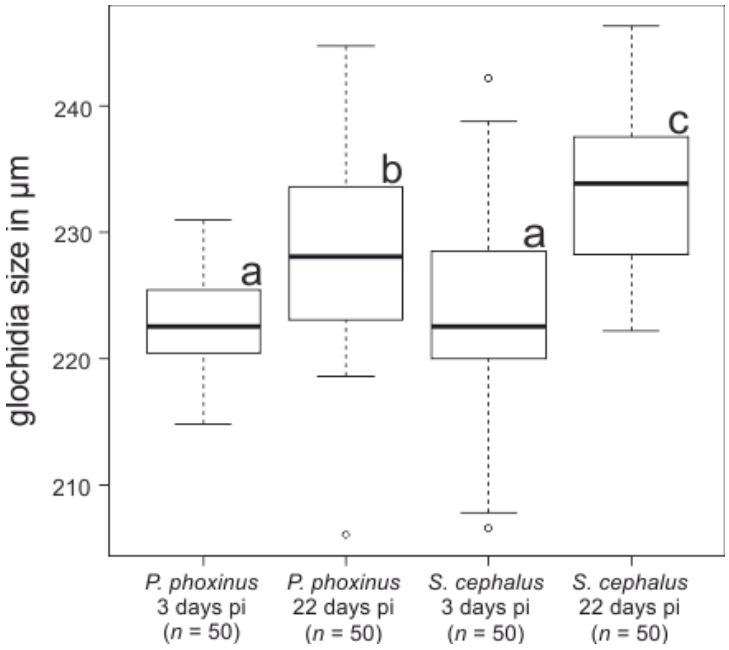

Figure 3-2: Size distribution of glochidia on *P. phoxinus* and *S. cephalus* 3 days pi and 22 days pi. Significant differences are indicated by different letters a, b, c ($p < 0.05$). Boxes are 0.75 and 0.25 percentiles and medians. Whiskers are the 1.5-fold interquartile range. Circles indicate data points outside the 1.5-fold interquartile range.

3. 4. 4. Fish species composition in *U. crassus* streams

Sixteen different fish species including freshwater bream (*Abramis brama* L.), spirlin (*Alburnoides bipunctatus* (Bloch 1782)), bleak (*Alburnus alburnus* L.), European eel (*Anguilla anguilla* L.), barbel (*Barbus barbus* L.), nase (*C. nasus*), bullhead (*C. gobio*), northern pike (*Esox lucius* L.), dace (*Leuciscus leuciscus*), perch *Perca fluviatilis*, bitterling (*Rhodeus sericeus* (Pallas 1776)), roach (*R. Rutilus*), brown trout (*Salmo trutta fario* L.), rudd (*S. erythrophthalmus*), European catfish (*Silurus glanis* L.) and chub (*S. cephalus*) were found to occur sympatric with *U. crassus* (Table 3-3). The fish species composition per stream was highly variable and ranged between five (EW, MW) and ten species (IA). Host fish density, including *C. nasus*, *C. gobio, S. erythrophthalmus* and *S. cephalus* was 458 specimen ha^{-1} for the EW, 1165 specimen ha^{-1} for the MW, 164 specimen ha^{-1} for the IA and 72 specimen ha^{-1} for the SA. The proportion of suitable hosts ranged between 11 % and 81 % of caught specimens in the investigated streams (Table 3-3). The only fish species which was caught in all four streams was *R. rutilus*. *Squalius cephalus* was found in three streams and had the highest abundances in this study (190 individuals).

Table 3-3: Fish species composition in four self-recruiting *U. crassus* streams.

fish species	Emersacker Weiherbach			Mooshamer Weiherbach			Ischler Ache			Staffelsee Ach		
	total	specimen per ha	%	total	specimen per ha	%	total	specimen per ha	%	total	specimen per ha	%
Abramis brama							3	8	1.0	1	2	0.3
Alburnoides bipunctatus										162	342	55.3
Alburnus alburnus							70	198	24.1			
Anguilla anguilla							4	11	1.4	17	36	5.8
Barbus barbus							4	11	1.4	70	148	23.9
Chondrostoma nasus										**1**	**2**	**0.3**
Cottus gobio	**32**	**444**	**11.1**									
Esox lucius				3	35	2.5	1	3	0.3			
Leuciscus leuciscus							3	8	1.0	5	11	1.7
Perca fluviatilis				12	141	9.8	50	141	17.2			
Rhodeus sericeus	189	2625	65.6									
Rutilus rutilus	46	639	16.0	5	59	4.1	97	274	33.3	1	2	0.3
Salmo trutta	20	278	6.9							3	6	1.0
Scardinius erythrophthalmus	**1**	**14**	**0.3**									
Silurus glanis				3	35	2.5	1	3	0.3			
Squalius cephalus				**99**	**1165**	**81.1**	**58**	**164**	**19.9**	**33**	**70**	**11.3**
host fish[a]	33	458	11.4	99	1165	81.1	58	164	19.9	34	72	11.6
host fish (inc. potential hosts)[b]	53	736	18.3	111	1306	90.9	111	313	38.1	204	431	69.6

[a] *C. nasus*; *C. gobio*; *S. erythrophthalmus*; *S. cephalus*.

[b] *A. bipunctatus*; *C. nasus*; *C. gobio*; *L. leuciscus*; *P. fluviatilis*; *S. trutta*; *S. erythrophthalmus*; *S. cephalus*.

3. 4. 5. Natural infestations - infestation and excystment rates

Behaviour and local distribution patterns of host fishes under natural conditions are important factors for host suitability assessment, since encounter of glochidia and fishes is a prerequisite for successful infestation in the wild. The quantification of excysted mussels from natural infested fishes is probably the most comprehensive approach of assessing host suitability of different fish species.

Strong natural infestation (≥ 10 glochidia fish^{-1}) was found in *S. erythrophthalmus* (only one specimen caught), *S. squalius* (7 % of caught specimens) and *P. fluviatilis* (2 % of caught specimens). In contrast to the strong infestation, an infestation with less than 10 glochidia fish^{-1} (weak infestation) was found in *S. trutta*, *S. glanis*, *R. rutilus*, *A. bipunctatus* (Figure 3-3). In total, 29 % of 190 caught *S. cephalus* and 11 % of 62 caught *P. fluviatilis* were infested (Figure 3-3).

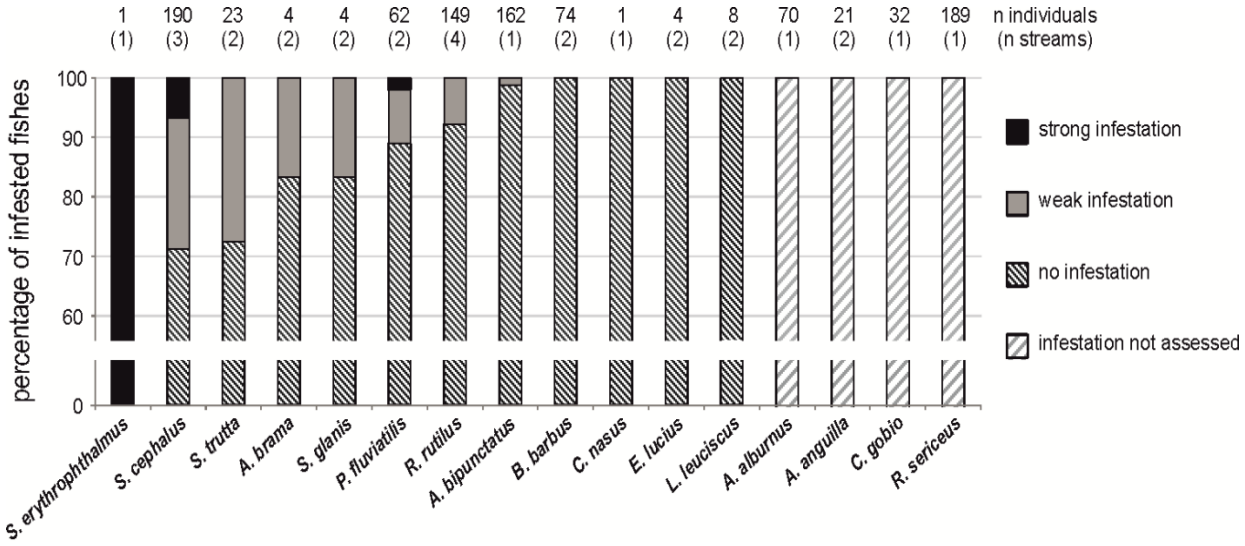

Figure 3-3: Percentage of infested fishes and infestation ratio under natural conditions in four *U. crassus* streams with recent recruitment.

Squalius cephalus originating from MW and IA as well as *S. erythrophthalmus* from EW revealed the highest number of excysted viable mussels per fish as well as high weight-normalized excystment rates (Table 3-4). Interestingly, the total and weight-normalized excystment rates of *S. cephalus* from the MW were 20 % and 28 % higher than the excystment rates of *S. cephalus* analysed in the artificial infestation experiment. In addition, excystment of living juvenile mussels was detected in *C. gobio* from EW and *P. fluviatilis* from IA. One living *U. crassus* was detected in the aquaria of two weakly infected *S. trutta*, while only dead glochidia could be recovered from *R. rutilus* and *R. sericeus*. *Alburnoides bipunctatus* from the SA did not excyst juvenile mussels or dead glochidia after transfer to

the laboratory although two weakly infested specimens were used for analysing the excystment.

Table 3-4: Excystment rates of natural infested fish from the four studied *U. crassus* streams after transfer to the laboratory.

stream	species	excystment			
		mussels per fish	mussels per g fish weight	dead glo. per g fish weight	n fish
Emersacker Weiherbach	***Cottus gobio***	7.5	0.5	0.21	10
	Rhodeus sericeus	0.0	0.0	1.80	10
	Rutilus rutilus	0.0	0.0	3.20	10
	Salmo trutta	0.5	0.0	0.01	2
	Scardinius erythrophthalmus	136.0	1.5	0.60	1
Mooshamer Weiherbach	*Perca fluviatilis*	0.0	0.0	0.15	5
	Squalius cephalus	106.0	4.0	0.10	8
Ischler Ache	*Leuciscus leuciscus*	0.0	0.0	0.00	3
	Perca fluviatilis	3.7	1.4	0.03	10
	Squalius cephalus	36.3	0.9	0.02	5
Staffelseeach	*Alburnoides bipunctatus*	0.0	0.0	0.00	3

3. 5. Discussion

Environmental factors such as water pollution, high fine sediment loads and the decline of host fish populations are considered the main factors contributing to the decline of freshwater mussel populations (Lydeard et al., 2004; Geist, 2010). In *Unio crassus*, low densities or absence of host fishes were suspected to be the primary factor for decline in certain regions (Engel and Wächtler, 1989). Herein, we assessed the suitability of different fish hosts and compared these results with the situation in functional *U. crassus* streams. The results of this study indicate that (i) an evaluation of host fish importance in unionid mussels should not exclusively rely on standardized infestation experiments without considering natural fish communities, (ii) host suitability can vary within different strains of the same host species, (iii) development time of *U. crassus* on different hosts varies considerably, and (iv) natural

recruitment in functional *U. crassus* populations can be secured by highly different fish community structures and densities.

3. 5. 1. Differences in assessing host suitability in the laboratory and in the wild

The suitability of different fish species for hosting *U. crassus* can be assessed in different ways. On the one hand, there is the artificial infestation approach using a glochidia suspension for parallel infestation of different fish species. This approach identified *P. phoxinus* and *S. cephalus* as the best hosts for *U. crassus*. However, not a single specimen of *P. phoxinus* was detected in any of the functional streams investigated here, suggesting the need to also consider the natural fish community structure for conservation recommendations. In addition, a homogenous glochidia suspension used in artificial infestations does not reflect the typical situation in the wild, since host fish abundance, behaviour and local distribution patterns in their natural environment are also important. On the other hand, assessments of host fish suitability in the wild can be limited by small sample numbers (as evident for *S. erythrophthalmus*). Also, the quantification of the suitability of different fishes is not possible based on such data since the infestation rate of caught fish species can result from multiple subsequent infestations within one season. In addition, a previous contact of wild fishes and glochidia following decreased susceptibility of older host fish due to an induced immune response (Bauer and Vogel, 1987) cannot be excluded while assessing host suitability in the field. Quantitative information on host suitability, however, along with information on the time required for metamorphosis, is particularly important for captive breeding. As evident from the results of this study, the most comprehensive method for analysing the host-specificity of a mussel species is a combination of the artificial infestation approach and the natural infestation approach.

3. 5. 2. Artificial infestations - infestation and excystment rates

While most of the previous studies (e.g. Bednarczuk, 1986; Hochwald, 1997; Taeubert et al., 2012a) only focused on comparisons of infestation rates without quantifying complete excystment, the results presented herein quantified the metamorphosis success over the whole period of excystment. The generally observed high rates of glochidia loss in the order of 53 – 99 % suggest that the mere consideration of infestation rates provides a proxy, but does not allow reliable assessments of host suitability. A substantial proportion of glochidia loss already occurs during metamorphosis, as evident from the ~ 40 – 50 % decline of glochidial load 22 days pi on *P. phoxinus* in the 1st and 3rd inf. It is likely, that the higher weight-normalized glochidia loss (65 %) of *P. phoxinus* in the 2nd infestation was caused by lower glochidia condition. In *S. cephalus*, only a ~ 25 % weight-normalized glochidia loss was

observed during metamorphosis which is much lower than previously observed in this species (Taeubert et al., 2012a). One explanation of these differences could be the use of two different strains with different susceptibility in both studies. This is further supported by the observed different infestation rates of stickleback populations from different regions in this study. *Gasterosteus aculeatus* can be classified - depending on the strain - as a good or poor host of *U. crassus*. Similar observations were previously evident from different infestations rates of *S. trutta* strains after standardized exposure to glochidia of the freshwater pearl mussel *M. margaritifera* (Taeubert et al., 2010).

The low infestation rates of *C. gobio, S. erythrophthalmus* and *C. nasus* were probably caused by a poor condition of glochidia used in this infestation experiment as seen from the low infestation rates in the reference hosts. However, the fish-weight-normalized excystment rates as well as the weight-normalized glochidia loss of *C. nasus*, *S. erythrophthalmus* and *C. gobio* were similar (excystment rate) or even lower (glochidia loss) compared with *P. phoxinus*. These results indicate the suitability of *C. nasus*, *S. erythrophthalmus* and *C. gobio* for hosting *U. crassus* after artificial infestation. The detected good suitability of *S. erythrophthalmus* and *C. gobio* are in line with Hochwald (1997). In contrast, Taeubert et al. (2012a) defined a different strain of *C. gobio* as a poor host for *U. crassus* from the IA. The combined results of this study and the study of Taeubert et al. (2012a) support the hypothesis of a different suitability of various strains within one fish species for hosting *U. crassus*, since both studies used mussels from the IA for artificial infestation of different *C. gobio* strains.

To our knowledge this is the first study which describes the suitability of *C. nasus* for hosting *U. crassus*. Since *C. nasus* was formerly highly abundant in medium-sized rivers of the upper Danube region a few decades ago, a considerable contribution of this fish species to the recruitment of *U. crassus* appears likely. Today, *C. nasus* is listed as endangered in the upper Danube area (Bohl et al., 2003), which might be one reason for the substantial decline of *U. crassus* especially in the medium-sized rivers of this region.

A. ruthenus and *R. rutilus* did not reveal any glochidia presence on the gills 3 days pi, which might be the result of immune-related rejection until 3 days pi and / or reduced attachment rate of glochidia on both species. This is in line with Fustish and Millemann (1978) who found that unsuitable coho salmon (*Oncorhynchus kisutch*, Walbaum 1792) sloughed all *M. margaritifera* glochidia until 4.5 days pi.

The remarkably low number of released juvenile mussels from excellent hosts like *P. phoxinus* and *S. cephalus* compared with the number of gill-encysted glochidia 22 days pi indicate a marginal or non-existent contribution of glochidia encysted on other parts of the fish (e.g. fins) to the recruitment success of *U. crassus*. In conclusion, *U. crassus* can be classified as a preferred gill parasite. This is in line with Hochwald (1997) who found an

increased mortality of glochidia encysted on the fins of *P. phoxinus* compared with glochidia encysted on the gills.

3. 5. 3. Timing of excystment

The variable progression of glochidia development on different host species and the ~ 25 % extended development period on *S. cephalus* compared with *P. phoxinus* under identical temperature conditions in the 3rd inf was also found by Hochwald (1997) and Taeubert et al. (2012a). In addition to the variable progression of glochidia development on different hosts, our results suggest a different progression of *U. crassus* development on the same host species. For example on *P. phoxinus* (1st inf), the first mussels where found after 400 degree-days, while the last mussels metamorphosed after 730 dd, indicating pronounced variation even within the same host strain. The different duration of glochidia development together with the shifted development progress on different host species strongly extends the overall excystment period of juvenile mussels during one season, which might be beneficial for a wide distribution of excysted mussels. In turn, a wide distribution reduces the competition for nutrients and enhances the survival of locally or temporarily unfavourable conditions.

3. 5. 4. Glochidia sizes

The detected increase (2.5 – 4.0 %) of glochidia size between 3 days pi and 22 days pi is most likely related to growth during metamorphosis and not to higher survival rates of larger glochidia as evident from the presence of larger specimens at 22 days pi compared with the smaller original sizes at 3 days pi. This is additionally supported by the lower loss rates coinciding with a higher growth rate of glochidia on *S. cephalus* compared with *P. phoxinus*. However, the growth is marginal and the parasitic relationship can be considered as phoretic (Barnhart et al., 2008). The most obvious advantage of this host parasite-relation is the dispersal of juvenile mussels, especially in upstream direction.

3. 5. 5. Fish species composition of *U. crassus* streams

The detection of 16 sympatric fish species in the four studied *U. crassus* streams indicates the high number of potential host species co-occurring with the mussel. In contrast, oligotrophic streams with occurrence of the freshwater pearl mussel *M. margaritifera*, only had an average number of five fish species (Geist et al., 2006). The use of different host fish species under natural conditions for recruitment of *U. crassus* is most likely, whereas *M. margaritifera* is exclusively limited to only two host species, *S. trutta* and *Salmo salar* L. The narrow host range of *M. margaritifera* hampers successful restoration of threatened

populations and reintroductions. In contrast to *M. margaritifera*, the wide host range of *U. crassus* increases the chance of restoring host fish populations in the context of *U. crassus* conservation.

Despite this wide host range, *S. cephalus* probably is the single most important host for *U. crassus* in the Danube drainage due to its high susceptibility to glochidia, its wide distribution and its high abundance. Interestingly, *P. phoxinus*, another excellent host in the artificial infestation experiments, was not detected in the four investigated streams with self-recruiting *U. crassus* populations. This finding indicates the necessity of evaluating the fish species composition of *U. crassus* streams specifically to estimate the contribution of different fish species to the recruitment of this endangered mussel species under natural conditions. It also shows the limitations of transferring laboratory-derived data on the host suitability to the situation in the wild. However, *P. phoxinus* may play an important role for *U. crassus* recruitment in other types of streams which were not investigated in this study. For one stream, the EW, *C. gobio* is important for successful recruitment of *U. crassus*. Since *S. erythrophthalmus* is a stagnophilic fish species, it is likely that the single specimen caught in the EW was drifted downstream from a connected pond. However, *S. erythrophthalmus* might be an important host for lake populations of *U. crassus* and a possible target species for semi-artificial rearing of this endangered mussel.

The host fish density of four self-recruiting *U. crassus* streams varied between 72 specimen ha^{-1} and 1 165 specimen ha^{-1} and 11 – 81 % of all fish specimens. This high variation in host fish abundances indicates a complex relationship between *U. crassus* and its hosts. It is not possible to provide universal minimum values for the required host fish density, since *U. crassus* recruitment depends on multiple variables. For example, adverse substratum conditions may limit *U. crassus* recruitment during post-parasitic phase as shown for *M. margaritifera* (Hastie and Young, 2000; Geist and Auerswald, 2007; Geist, 2010) and *M. durrovensis* (Costello et al., 1998). High mortality rates during the post-parasitic phase would in turn need to be compensated by higher excystment rates from host fishes. However, although a successful recruitment of *U. crassus* is not assured by high host fish densities alone, it is likely that an increase in host fishes facilitates *U. crassus* recruitment, especially in rivers where only few sites provide suitable substratum conditions for the development of juvenile mussels. In the freshwater pearl mussel (*M. margaritifera*), juvenile mussel density was positively related to the number of glochidia infections per stream area in streams with ongoing recruitment (Österling et al., 2008). Species occurring sporadically or in low densities in *U. crassus* streams are likely of minor importance for overall recruitment success, even if some of these species were found to be suitable hosts in this study. This is likely true for *A. brama*, *A. anguilla*, *B. barbus*, *E. lucius* and *S. glanis*. It needs to be noted, however, that no definite conclusion can be made whether the fish communities found in the

different streams are entirely natural or anthropogenically influenced. In any case, the present fish community structure seems to be sufficient for securing sustainable recruitment of *U. crassus*.

3. 5. 6. Natural infestation - infestation and excystment rates

Since excystment of living juvenile mussels after natural infestation is the best evidence of suitability for hosting *U. crassus* glochidia, *S. cephalus* can be considered one of the most important hosts for *U. crassus* in the upper Danube drainage. *Cottus gobio* is also a suitable host for *U. crassus* as evident from the excystment of juvenile mussels from natural infested fish and local populations of *U. crassus;* e.g. the population in the EW highly depends on this host fish. *Rutilus rutilus* and *R. sericeus* shed only dead glochidia and can be classified as unsuitable for hosting *U. crassus*. In addition, these results demonstrate the necessity of evaluating the metamorphosis success for suitability assessment of different fish species because unsuitable fishes are infested in the same way as suitable fish species.

Since the infestation rate under natural conditions could be the result of multiple infestations, quantification of infestation and / or excystment rates of different fish species results in inaccuracies and is not suitable for comparison of different fish species. In addition, the absence of glochidia on the gills of one fish species does not necessarily justify the interpretation that this species is an unsuitable host. Since *L. leuciscus* from IA and SA were not infested at the sampling time point, it is not known if these strains were suitable hosts for *U. crassus*. However, Engel and Wächtler (1989) reported that *L. leuciscus* was suitable for hosting a subspecies of *U. crassus.*

3. 6. Conclusions

The results of this study improve the general understanding of host-suitability of different fish species by quantifying infection rates and metamorphosis success of *U. crassus* under artificial and wild conditions. Table 3-5 provides a summary of the suitability of different fish species for hosting *U. crassus* from the upper Danube drainage. The detected mismatch between the excellent suitability of *P. phoxinus* under artificial conditions and the absence of this fish species in the four self-recruiting *U. crassus* streams show the necessity of also analysing the fish community structure for appropriate host suitability assessment. Before the start of captive breeding activities, the suitability of used host fish populations should be evaluated, because the degree of host-specificity and glochidia development varies greatly between fish species as well as between populations within species.

Table 3-5: Suitability of different fish species from the upper Danube drainage for hosting *U. crassus* as found in this study and the study of Taeubert et al. (2012a).

species	good hosts	good – poor hosts	poor hosts	no hosts
Alburnoides bipunctatus			X	
Alburnus alburnus				X
Acipenser ruthenus				X
Chondrostoma nasus	X			
Cottus gobio		X[a]		
Gasterosteus aculeatus		X[a]		
Leuciscus idus		X[b]		
Neogobios melanostomus				X
Onchorynchus mykiss				X
Perca fluviatilis		X[b]		
Phoxinus phoxinus	X			
Rhodeus sericeus				X
Rutilus rutilus				X
Salmo trutta			X	
Scardinius erythrophthalmus	X			
Squalius cephalus	X			

[a] Depending on the strain.

[b] Further investigations are needed.

Host fish density and fish species composition was very different among four self-recruiting *U. crassus* streams which indicates the use of different host fishes under natural conditions. The size of the host range of *U. crassus* explain its wide distribution and the ability of colonizing different habitats. However, *S. cephalus* can be considered the most suitable host of *U. crassus* in the Danube drainage and should be actively supported in *U. crassus* streams and tributaries. In streams with a high density of host fishes, but a lack of *U. crassus* recruitment, other factors, especially the substrate composition, should be given priority for conservation and management measures. Despite the fact that *U. crassus* can use different host fish species, successful conservation of this endangered mussel requires an integrative approach including fisheries management.

4. Suitability of different salmonid strains as hosts for the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.)

The content of this chapter was published:
Taeubert JE, Denic M, Gum B, Lange M, Geist J. 2010. Suitability of different salmonid strains as hosts for the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 728-734.

4. 1. Abstract

The complex life cycle of endangered European freshwater pearl mussels *Margaritifera margaritifera* L. involves an obligatory parasitic phase on a host fish. Knowledge on the host-parasite interaction and on the suitability of different host fish species and strains is required both for the management of wild fish and mussel populations as well as for improving the efficiency of captive breeding methods. In this study, the suitability of different salmonid strains for hosting glochidia was tested, including Danube salmon (*Hucho hucho* L.) and three brown trout (*Salmo trutta* L.) strains from inside and outside the freshwater pearl mussel distribution range. All brown trout strains as well as Danube salmon were successfully infected with freshwater pearl mussel glochidia and encystment of mussel larvae was detected. One brown trout strain originating from the natural *M. margaritifera* distribution range was identified as the most suitable host revealing the highest fish-weight-normalized infection rates and highest glochidia growth rates, whereas endemic Danube salmon was least suitable. Under natural conditions, the role of Danube salmon may be attributed to the long-distance dispersal of glochidia in the Danube system, whereas sedentary brown trout appear to be the most important hosts at local scale. Successful infection of suitable hosts and the maintenance of these host-parasite systems in calcareous water were demonstrated in this study. These results indicate that neither the infection process nor the encystment phase of freshwater pearl mussels is dependent on low lime concentrations. The results of this study suggest that careful selection and management of appropriate host fish strains is mandatory for sustainable conservation management of freshwater pearl mussel populations.

4. 2. Introduction

The freshwater pearl mussel *Margaritifera margaritifera* L. is considered one of the most endangered species in Europe (Young, 1991). The majority of central European *M. margaritifera* populations consist exclusively of individuals older than 50 years and successful juvenile recruitment has not occurred for decades.

Freshwater pearl mussels have a complex life cycle linked to stage-specific ecological requirements. In summer, larvae of *M. margaritifera* (glochidia) with a size of about 0.07 mm are released by gravid females in a highly synchronized spawning event within one or two days (Young and Williams, 1984b; Hastie and Young, 2003a). The glochidia are obligate ectoparasites on the gills of salmonid fishes and reach their host passively with the water current. After their inhalation by the host fish, glochidia encyst on the gills and become encapsuled by epithelial cells of the host (Young and Williams, 1984a; Bauer, 1987b; Ziuganov et al., 1994). Under natural conditions, young-of-the-year fish (YOY) have been reported to be the most important hosts since contact with glochidia induces an immune response and results in reduced susceptibility in older fish (Bauer, 1987c; Bauer and Vogel, 1987). After a period of up to 11 months and a growth to 0.4 - 0.5 mm, the juvenile mussels are released and bury into the river substratum, where they live for about 5 years before they appear at the substratum surface and develop into adult mussels (Young and Williams, 1984b; Bauer, 1991, 1997; Hastie and Young, 2000). The post-parasitic phase was identified as the most sensitive phase in the life cycle of *M. margaritifera* (Hastie and Young, 2000; Geist and Auerswald, 2007; Geist, 2010).

According to Young and Williams (1984b), the only host fish of freshwater pearl mussel in Europe are Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta fario* L.) with brown trout being the exclusive host in many central European populations (Bauer, 1987a; Geist et al., 2006). In addition, Bauer (1997) suggested that Danube salmon (*Hucho hucho* L.) is moderately susceptible for glochidia of *M. margaritifera*. To our knowledge this observation has neither been tested nor quantified in other studies. Glochidia are able to attach to the gills of many fish, but development is only successful on suitable host species (Young and Williams, 1984a). Unsuitable host fish such as rainbow trout (*Oncorhynchus mykiss* (Walbaum 1792)) and minnow (*Phoxinus phoxinus* L.) typically shed glochidia within the first 48 hours after infection (Young and Williams, 1984a, 1984b). Even on suitable hosts, the number of encysted glochidia gradually declines and many do not metamorphose successfully (Meyers and Millemann, 1977; Young and Williams, 1984b; Bauer and Vogel, 1987). The obligate parasitic phase of *M. margaritifera* implies a host-dependent distribution area (Geist and Kuehn, 2008). The geographic range of *M. margaritifera* is confined to smaller areas than the distribution of suitable hosts, which can be explained by more specific

habitat requirements of *M. margaritifera* compared with salmonids (Bauer, 1997). Bauer (1992) suggested the *M. margaritifera* distribution being limited to streams which are low in lime and nutrients, whereas Atlantic salmon, brown trout and Danube salmon tolerate a much wider range of carbon and nutrient content.

An increasing number of supportive conservation measures and breeding programmes for *M. margaritifera* have been initiated in many European countries. Information on the suitability of different host fish strains is crucial both for supportive breeding and for the management of wild fish stocks in pearl mussel streams. Geist et al. (2006) expected different susceptibilities and immunity reactions of different brown trout strains and there is anecdotal evidence from European *M. margaritifera* conservation programmes regarding variable success in use of different host strains. The ecotype-specific parasite-host-interaction becomes particularly relevant because of the increasing fragmentation of *M. margaritifera* subpopulations and the need for effective conservation of freshwater pearl mussels.

The core objective of this study was to analyse the suitability of three different brown trout strains for infection with *M. margaritifera* glochidia, including strains from inside and outside the pearl mussel distribution range along with the lacustrine form of brown trout (*Salmo trutta lacustris* L.) and the Danube salmon. In addition, the effects of fish size and condition factors on infection rates and glochidia growth were tested. The infection of hosts with *M. margaritifera* glochidia under carbonate-rich water conditions was investigated to clarify wether the restriction of *M. margaritifera* distribution to siliceous streams results from autecological limitations of glochidia to low-carbonate water during the infection and parasitic phase.

4. 3. Methods

Freshwater pearl mussel glochidia were obtained from five adult females (aged 60-80 years) from the Wolfsbach, a tributary of Saechsische Saale, Elbe drainage, Germany. Gravid mussels were collected under licence number “UNB Vogtlandkreis licence-fn. 364.622/hbg from 06/02/09”, transferred into aerated buckets and maintained at 16 °C. Water exchanges and inspection for released glochidia were carried out daily. After 5 days, glochidia were released on August 25 and 26, collected and suspended in a 5 litre container, and stored for a maximum time of 36 h at 8 °C (according to Lòpez et al., 2007) before host fish infection. All infections were carried out in the laboratory of the Unit of Functional Aquatic Ecology and Fish Biology. Glochidia were slowly adapted to ground water and re-suspended for infection. In order to account for possible differences in viability of glochidia originating from different parent individuals, and from different timepoints of release, one single, well-mixed glochidia

suspension was generated. Simultaneous host fish infections at a concentration of 25,000 glochidia L^{-1} were performed for 45 min under permanent agitation.

Three different strains of brown trout (*Salmo trutta*) and Danube salmon (*Hucho hucho*) were used. The three strains of trout included fish from a carbonate-poor environment within the pearl mussel distribution range ("Baernau", Fischzuchtbetrieb Roesch, Germany; 69 specimens), a strain of brown trout from a carbonate-rich environment outside the pearl mussel distribution range ("Moosach", Unit of Functional Aquatic Ecology and Fish Biology; 69 specimens), and a strain of the lake-dwelling ecophenotype of brown trout ("lacustrine brown trout", Landesfischzuchtanstalt Mauka, Germany; broodstock from Walchensee; 18 specimens). Danube salmon originated from a previously genetically characterized lineage of the species (Geist et al., 2009) from "Fischereilicher Lehr- und Beispielbetrieb Lindbergmuehle", Germany (69 specimens). Phenotypes and origins of host fish strains and pearl mussel glochidia are illustrated in Figure 4-1.

Young-of-the-year (YOY) fish were used throughout the experiment for standardization and in order to exclude the introduction of any bias related to immune responses resulting from possible previous contact with glochidia.

After infection, the different strains and species were maintained in regular plastic upflow incubation trays and fed approximately 1 % of total body weight with commercial trout chow twice each week.

Water temperature was recorded in 5 min intervals by temperature loggers (Lascar Electronics Limited, Salisbury, UK) and ranged between 11.5 °C and 12 °C throughout the experiment. The groundwater used for infection and exposure contained 118 mg L^{-1} calcium and 41.7 mg L^{-1} magnesium. The specific conductance (SC) was 1035 µs cm^{-1}.

To quantify the infection rate, glochidia numbers per fish were determined 8 weeks after infestation (October 21, 2009) in Moosach and Baernau brown trout as well as in Danube salmon, and after 10 weeks in lacustrine brown trout (November 4, 2009). After this period of time, any bias due to incomplete encystment is highly unlikely (Bauer 1987c, 1991). Fifteen fish of each strain were anaesthetized and sacrificed. Individual fish were measured (+/- 1 mm) and weighed (+/- 0.01 g). As an indicator of fish constitution, the condition factor was calculated using the formula (cf = w * 100 / l ^ 3) with w = weight (in grams) and l = total length (in cm). After dissecting all 8 gill arches, the number of glochidia was determined at 10 x magnification using a binocular microscope (Wild, Heerbrugg, Switzerland). In order to test for links between infection rates and glochidia growth, the diameter (+/- 2 µm) of the encapsulated larvae was measured on November 4, 2009. Altogether 5,760 glochidia of brown trout Moosach (n = 3 fish), brown trout Baernau (n = 3 fish), Danube salmon (n = 3 fish) and lacustrine brown trout (n = 6 fish) were included.

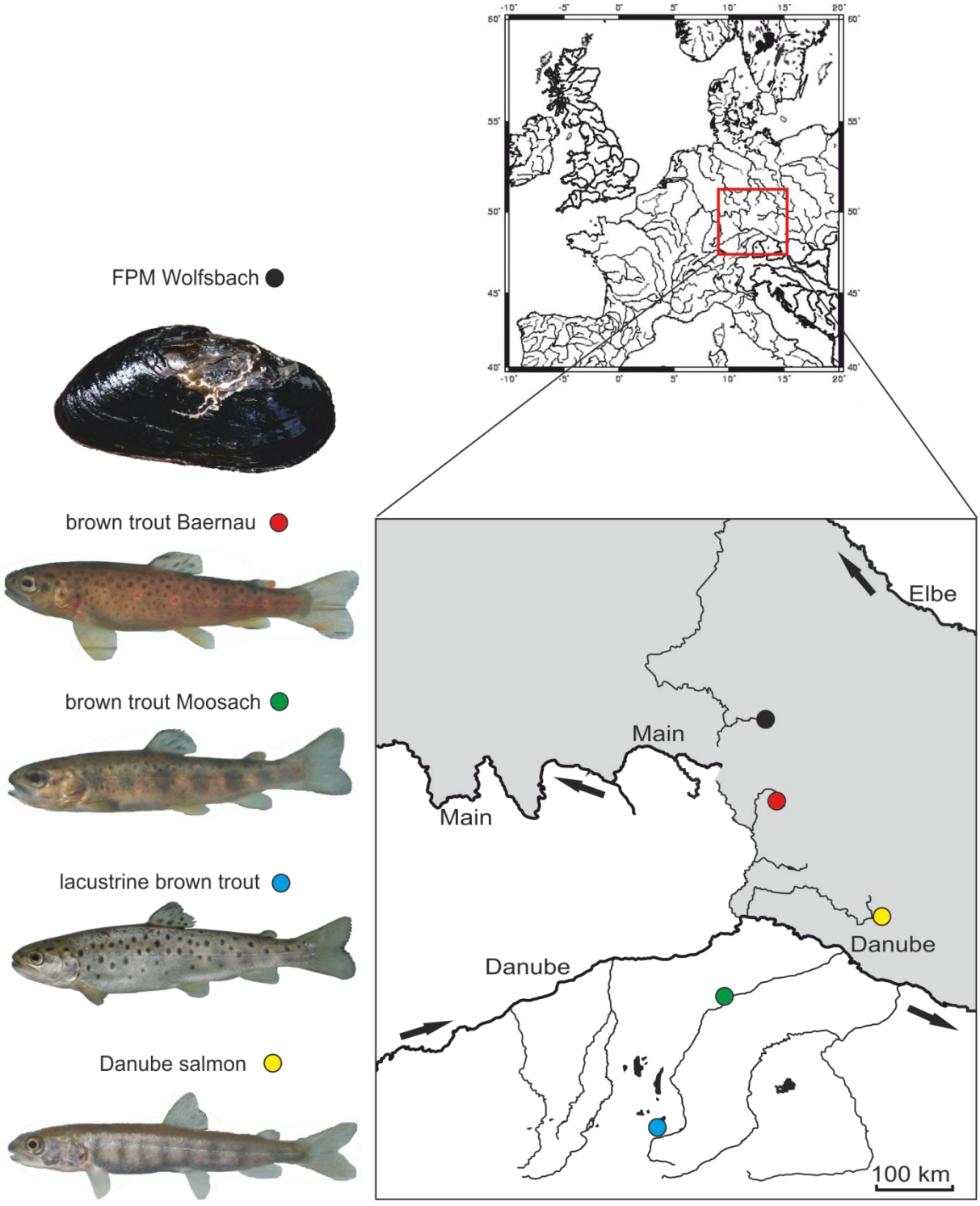

Figure 4-1: Distribution of species used in this study:
Locations (coloured circles) and phenotypes of salmonid strains. Freshwater pearl mussel distribution area is highlighted in grey. Flow directions of streams are indicated by arrows.

For each species and strain, a best-fit linear model for the link between fish size and total number of glochidia per specimen was established. Statistical analyses and the calculation of the equation, coefficient of determination (R^2) and Spearman rank correlation coefficient

were performed in R Version 2.6.1 (© 2008, The R Foundation for Statistical Computing). Differences in mean glochidia infection rates, glochidia size, and fish condition factor between the four different treatments were tested using non-parametric Kruskal-Wallis sum of ranks tests and Mann-Whitney U post hoc tests because data were not normally distributed. Bonferroni correction was applied to correct for multiple tests.

4. 4. Results

4. 4. 1. Infection procedure in calcareous water

As evident from the relatively high infection rates after 56 days, glochidia remained viable and infective for at least 36 hours post-release when stored at 8 °C. Despite the high degree of specialisation of pearl mussels to silicious streams with low carbonate concentrations, glochidia infection was demonstrated to be successful even at high carbonate concentrations (118 mg L^{-1} Ca^{2+} and 41.7 mg L^{-1} Mg^{2+}). No impairment of host fishes was evident after 45 min exposure to 25,000 glochidia L^{-1}. During the first three days after infection, no host fish mortality was observed. Within 28 days after infection, only 8 fish (3 Baernau brown trout and 5 Moosach brown trout) out of 248 specimens (3.2 %) died. In all species and strains, encystment of glochidia was observed in each of the four gill arches and no significant differences between infection rates at the right and the left gills were found ($p = 0.60$).

4. 4. 2. Susceptibility of different host fish species and strains

Margaritifera margaritifera larvae were found to infect all tested brown trout strains as well as Danube salmon. However, comparison of brown trout Baernau, brown trout Moosach, Danube salmon and lacustrine brown trout showed different susceptibilities for *M. margaritifera* glochidia (Figure 4-2 (A)). Median infection rates were 879 glochidia per fish for brown trout Moosach, 745 glochidia per fish for lacustrine brown trout, 699 glochidia per fish for brown trout Baernau and 43 glochidia per fish for Danube salmon. Danube salmon infection rate was significantly lower than the infection rates of the three brown trout strains ($p < 0.001$). The mean infection rates of the three brown trout strains did not significantly differ both for total infection numbers (Figure 4-2 (A)) as well as for host size-normalized infection rates (Figure 4-2 (B)). In contrast, host fish-weight normalized infection rates revealed highly significant differences in glochidial load between Moosach and Baernau trout ($p < 0.001$, Figure 4-2 (C)). Lacustrine brown trout had an intermediate glochidial load (Moosach - lacustrine $p = 0.111$; Baernau - lacustrine $p = 0.445$).

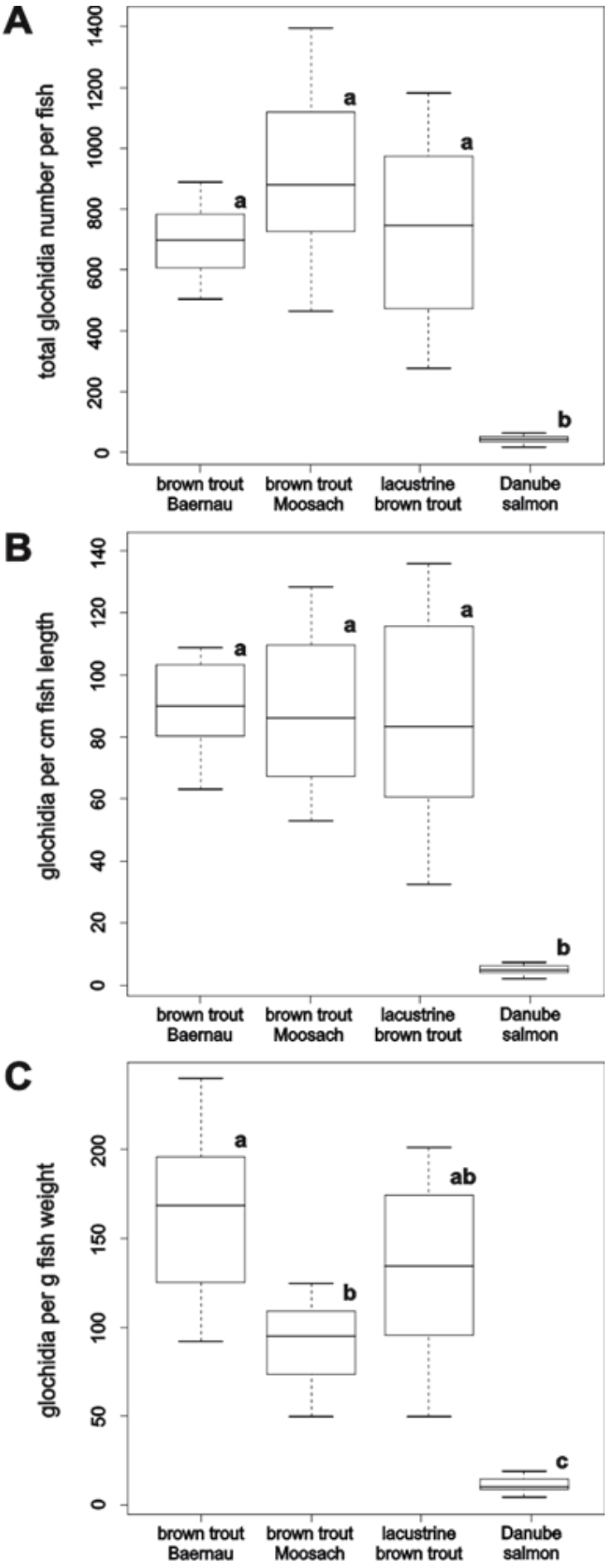

Figure 4-2: Glochidial load on different species and strains:
Box-Whisker-Plot of brown trout Baernau (n=15 fish), brown trout Moosach (n=15 fish), Danube salmon (n=15 fish) and lacustrine brown trout (n=15 fish) and the load with glochidia. (**A**) Total sum of glochidia per fish; (**B**) total sum of glochidia normalized per cm total fish length; (**C**) total sum of glochidia normalized per g total fish weight. Lacustrine brown trout were sampled 2 weeks after brown trout Baernau, brown trout Moosach and Danube salmon. Significant differences are indicated by different letters a, b, c ($p < 0.05$). Boxes are 0.75 and 0.25 percentiles and medians.

Brown trout Moosach and lacustrine brown trout revealed a weak positive linear relationship between fish weight and glochidial load, whereas this trend was not observed in the smaller Baernau brown trout and Danube salmon (Figure 4-3).

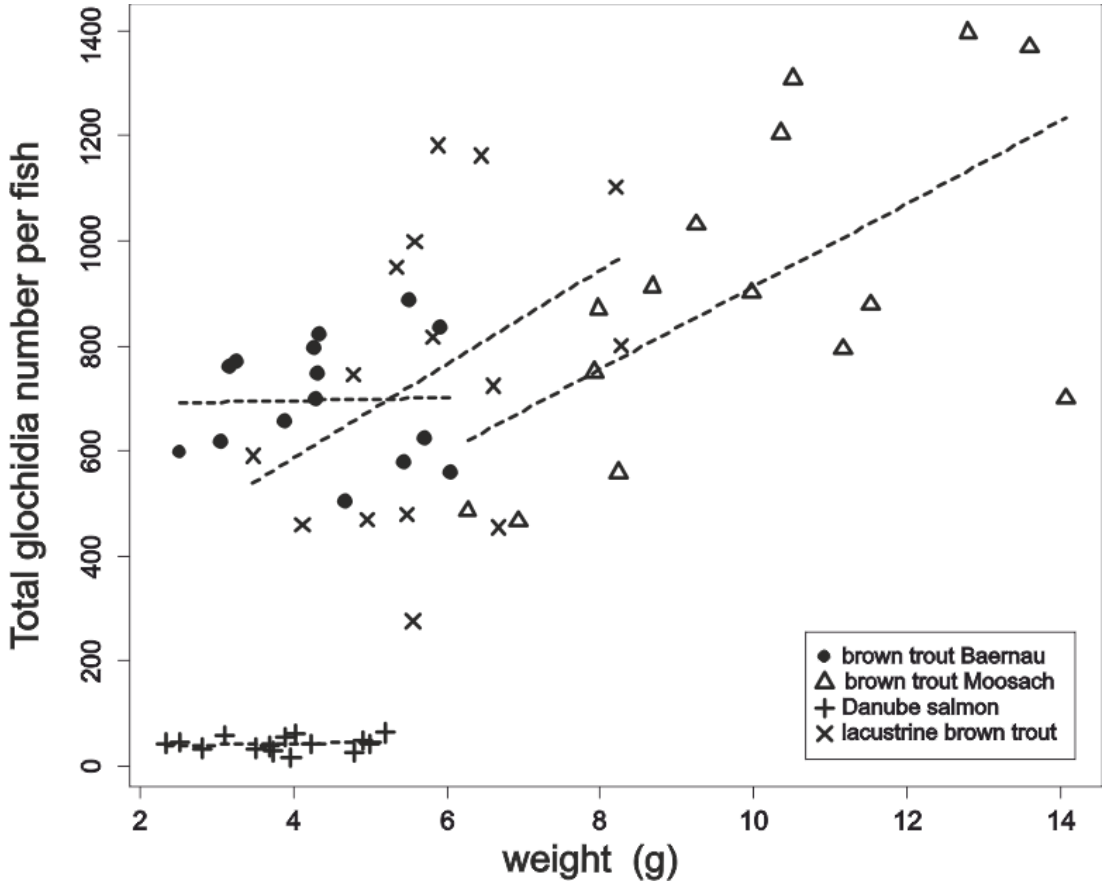

Figure 4-3: Weight of species / strains and glochidial load:
Scatterplot of total fish weight of brown trout Baernau, brown trout Moosach, Danube salmon and lacustrine brown trout and the load with glochidia. Note that lacustrine brown trout were sampled 2 weeks after the other brown trout strains and Danube salmon.

The best-fit equations and spearman rank correlation coefficients of these species-specific relations are shown in Table 4-1. The condition factor (cf) did not show any significant differences between the three brown trout strains (brown trout Baernau: cf = 0.91, brown trout Moosach: cf = 0.93 lacustrine brown trout: cf = 0.91, Figure 4-4) but was significantly lower in Danube salmon (cf = 0.62, $p < 0.001$).

Table 4-1: Relation between fish weight / length and number of glochidia.

Equation, coefficient of determination (R^2) and Spearman rank correlation coefficient of the relation between total fish weight and length of brown trout Baernau, brown trout Moosach, Danube salmon and lacustrine brown trout and the number of glochidia. Note that lacustrine brown trout were sampled 2 weeks after the other brown trout strains and Danube salmon.

	weight					**length**				
species / strain	**equation**	**R^2**	**p**	**spearman**	**p**	**equation**	**R^2**	**p**	**spearman**	**p**
Danube salmon	y = 1.74x + 36.04	0.013	0.683	0.132	0.638	y = 2.73x +19.64	0.012	0.699	0.091	0.746
brown trout Baernau	y = 2.83x + 684.49	< 0.001	0.922	0.025	0.929	y = 4.17x + 664.5	< 0.001	0.941	-0.005	0.985
brown trout Moosach	y = 78.42x + 128.03	0.374	**0.015**	0.579	**0.026**	y = 182.80x - 959.30	0.328	**0.026**	0.571	**0.026**
lacustrine brown trout	y = 89.13x + 229.30	0.164	0.134	0.361	0.185	y = 154.39x - 570	0.164	0.134	0.359	0.189

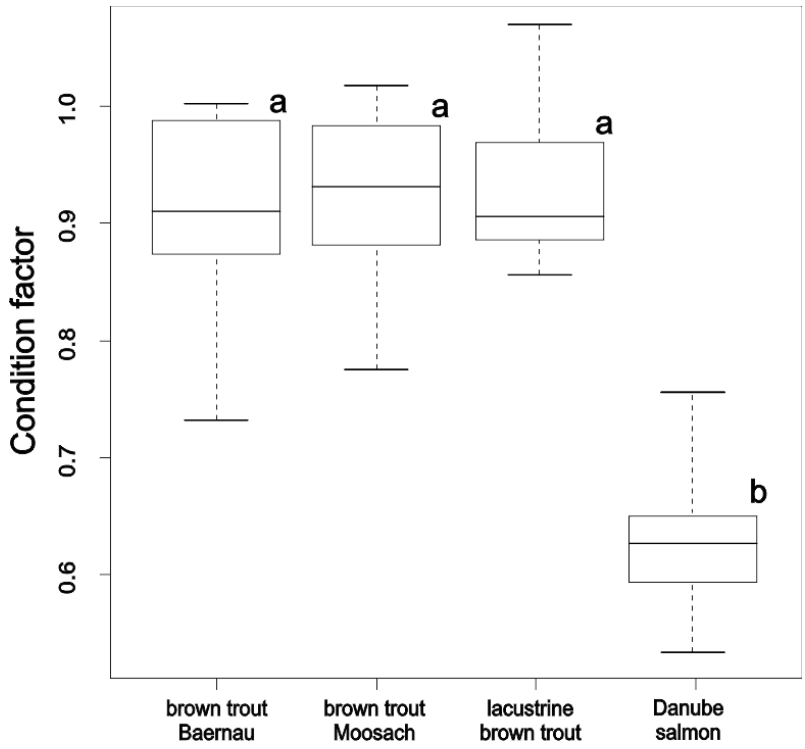

Figure 4-4: Condition factors of species and strains:
Condition factor of brown trout Baernau (n = 15 fish), brown trout Moosach (n = 15 fish), Danube salmon (n = 15 fish) and lacustrine brown trout (n = 15 fish). Note that lacustrine brown trout were sampled 2 weeks after the other brown trout strains and Danube salmon. Significant differences are indicated by different letters a, b ($p < 0.05$). Boxes are 0.75 and 0.25 percentiles and medians.

4. 4. 3. Glochidia size on different host species and strains

Mean glochidia sizes after 70 days were 218.7 µm (SD = 37.6) on brown trout Baernau, 218.3 µm (SD = 55.7) on lacustrine brown trout, 200.4 µm (SD = 46.9) on brown trout Moosach, and only 168.2 µm (SD = 54.6) on Danube salmon (Figure 4-5). These differences were highly significant for the comparison of mean glochidia sizes on Danube salmon and the three brown trout strains as well as for the Moosach brown trout and Baernau brown trout ($p < 0.001$). Lacustrine brown trout glochidia had a similar median size to glochidia from brown trout Baernau. Glochidia size was highly variable among different specimens of the same species / strain. In lacustrine brown trout, individual-specific mean glochidia sizes between 173.0 µm (SD = 45.0) and 263.6 µm (SD = 38.2) and a weak positive linear relation ($R^2 = 0.27$; $p < 0.01$) of glochidia size and total number of glochidia on the corresponding gill arches was found. Baernau fish showed the lowest variance in mean glochidia size (213.9 µm, SD = 37.7, and 222.9 µm, SD = 37.2), while brown trout Moosach (142.4 µm, SD = 33.8, and 222.3, SD = 37.0) and Danube salmon (133.2 µm, SD = 47.3, and 197.3, SD = 63.2) were intermediate.

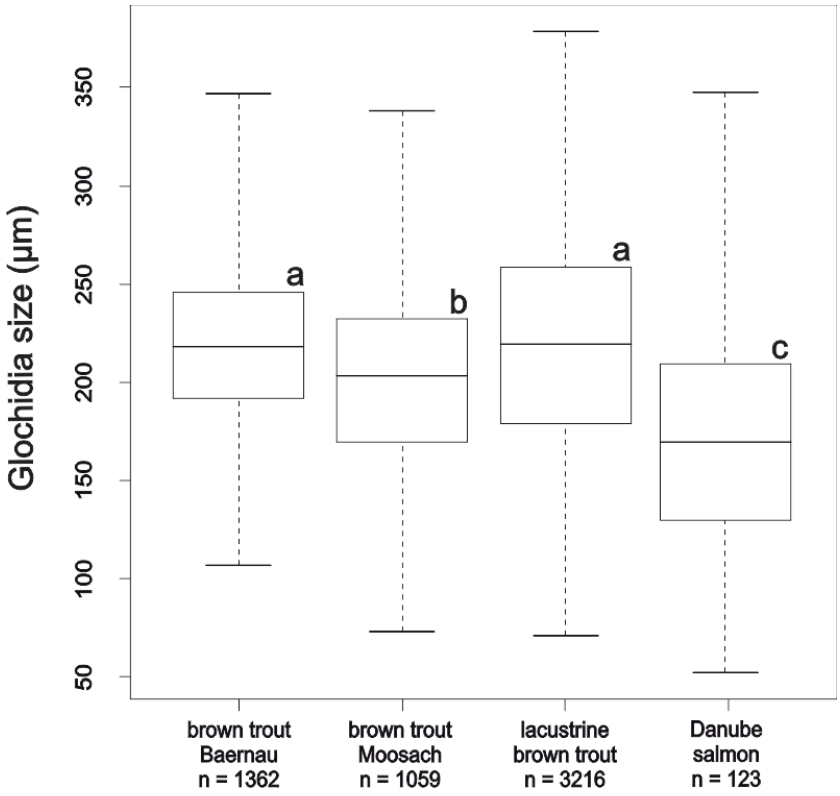

Figure 4-5: Glochidia size on different species and strains:
Glochidia size on brown trout Baernau, brown trout Moosach, Danube salmon and lacustrine brown trout. Significant differences are indicated by different letters a, b, c ($p < 0.05$). Boxes are 0.75 and 0.25 percentiles and medians.

4. 5. Discussion

Knowledge on host-parasite interactions between fishes and mussels is crucial both for mussel conservation and for fisheries management. The results of this study show that freshwater pearl mussel glochidia infection was successful on Danube salmon as well as on three ecophenotypes of brown trout, including strains from outside the pearl mussel distribution range. The different infection rates and glochidia growth among host fish species and ecophenotypes indicate that a careful selection of hosts can increase the efficiency of conservation measures, e.g. in pearl mussel culturing and propagation.

4. 5. 1. Infection procedure in calcareous water

Based on the results of this study, the limitation of freshwater pearl mussel distribution to waters low in carbonate is not caused by a narrow ecological valence in the larval stage. Concentrations of calcium and magnesium in the infection bath and for host maintenance exceeded those of the stream (Ca^{2+} = 12.68 mg L^{-1}, Mg^{2+} = 4.63 mg L^{-1}) from which glochidia originated by factors of 10. Similarly, specific conductance ranged between 130 and 180 µS

cm^{-1} in the pearl mussel stream compared with > 1,000 µS cm^{-1} in the infection bath and during host maintenance. The finding that the short-time exposure to calcareous water after glochidia release and before encystment did not inhibit glochidia development can be explained by the adaptation of glochidia to high osmolarity of fish blood. Following encystment, glochidia are covered by host epithelial cells and are protected from direct contact with ambient water. The most limiting factor responsible for the specific distribution pattern of *M. margaritifera* is likely to interact with the post-parasitic phase when juvenile mussels are particularly sensitive to adverse substratum conditions (Hastie and Young, 2000; Geist and Auerswald, 2007; Geist and Kuehn, 2008; Geist, 2010). A long time exposure (45 min) of YOY fish to relatively low concentrations of glochidia suspension (25,000 L^{-1}) resulted in high infection rates of suitable hosts, suggesting that even small remnant populations of *M. margaritifera* can be used as source of glochidia for successful supportive breeding and conservation programmes. The glochidia viability after 36 hours at 8 °C matches the observations by Ziuganov et al. (1994) who reported glochidia activity even after 6 days.

4. 5. 2. Susceptibility of different host fish species and strains

The most suitable host for *M. margaritifera* in our study was brown trout originating from Baernau, the only tested strain from within the natural *M. margaritifera* distribution range. The significantly different fish weight-normalized infection rates of Moosach and Baernau trout illustrate the importance of standardizing the assessment of infection rates for comparing the suitability of different hosts. Considering fish weight-normalized infection rates, the Moosach trout showed lowest values 8 weeks post-infection although the condition factors did not show any significant differences between the trout strains studied. It is thus likely that variations in genetic imprinting have an impact on host suitabilities.

Possible explanations for the significantly lower infection rate of Danube salmon compared with the three different brown trout strains include: i) the significantly lower condition factor of Danube salmon compared with the brown trout strains, which may be linked with the physiological status and immunological competence of these hosts, ii) the possible specific adaptation of *M. margaritifera* larvae to the genus *Salmo* with its two European forms, Atlantic salmon and brown trout, owing to the endemism and restriction of *Hucho hucho* to a small portion of the *M. margaritifera* distribution range, and iii) the possible mismatch between pearl mussel glochidia from the Elbe drainage and *Hucho hucho* host fish from the Danube basin. In addition, differences in host fish infestation rates may also result from species-, strain- and size-specific variation of ventilation rates and gill surface areas (Young and Williams, 1984b). Although Geist and Kuehn (2005) showed that the present *M. margaritifera* population differentiation does not always match with present drainage

systems, the gravid *M. margaritifera* from the brook Wolfsbach used in this study differ from Danube drainage populations. Therefore, an infection of Danube salmon with glochidia originating from its native distribution area would clarify its role in *M. margaritifera* reproduction. However, the fact that 8 weeks post-infection some glochidia were encysted on the gills of YOY Danube salmon shows that the immunological rejection reaction is not as effective as observed for unsuitable hosts (Young and Williams, 1984a, 1984b). This confirms the results of Bauer (1997), who described a diminished suitability of Danube salmon. Fish which are unsusceptible for *M. margaritifera* glochidia like coho salmon (*Oncorhynchus kisutch* (Walbaum 1792)) sloughed glochidia from their gills 4.5 days post-infection by a well-developed hyperplastic reaction (Fustish and Millemann, 1978). According to Young and Williams (1984b) and Bauer (1987a), even on suitable hosts the number of encysted glochidia gradually declines and many do not metamorphose successfully.

The low density of *H. hucho* compared with *S. trutta* in most streams in addition to the low survival rate of juvenile mussels recorded in Young and Williams (1984b) indicates a low importance of Danube salmon (mean rate: 43 glochidia per fish) in *M. margaritifera* reproduction. However, Beasley (1996) also found comparatively low natural encystment rates with mean values of 10 glochidia per salmon and 41 glochidia per trout. Most of the remaining central European pearl mussel populations are entirely trout-dependent (Geist et al., 2006) and are located in small brooks where neither Danube salmon nor Atlantic salmon occur. However, *Hucho hucho* might significantly contribute to the long-distance dispersal of *M. margaritifera* in the larger streams of the Danube basin where this species is endemic. The higher infection rates and the higher growth rates of *M. margaritifera* glochidia on brown trout compared with Danube salmon indicate that brown trout plays the dominant role for the local recruitment of most *M. margaritifera* populations.

In addition to the relative comparisons of host infections and glochidia development in this study, a pairwise comparison of these variables from both the Danube and the Elbe system would be a suitable approach to test for susceptibilities of specific ecophenotypes from the same catchment. In 2009, only glochidia from the Elbe drainage and host fish from the Danube drainage were available for this experiment. However, the high median infection rates of all used brown trout strains 56 days after infection (699 glochidia per Baernau brown trout, 745 glochidia per lacustrine brown trout and 879 glochidia per Moosach brown trout) are similar to reported infection rates on autochthonous brown trout. For instance, Young and Williams (1984a) found 610 glochidia per host fish one day after infection. These findings indicate that infection rates on hosts from outside the *M. margaritifera* drainage of origin do not necessarily result in lower infection rates than on autochthonous host fish.

Lacustrine brown trout and brown trout Moosach exhibit a positive linear relation between weight / length and glochidial load whereas no correlation was observed in Baernau brown

trout and Danube salmon. This can be explained by the low variation in length and weight of these two strains. Consequently, the hypothesis that the initial glochidial load rises with fish size can only be confirmed for brown trout Moosach and lacustrine brown trout in this study. We did not find a negative correlation of fish length and glochidial load as described for some natural populations in northern Scotland (Hastie and Young, 2001). Under field conditions, variances of host fish size are often governed by stream size and environmental conditions. Many *M. margaritifera* reproduction programmes use hatchery reared YOY fish for infection with glochidia without considering these size effects. The results of this study suggest that knowledge of an ideal host fish size could improve captive breeding success.

4. 5. 3. Glochidia size on different host species and strains

The highest mean glochidia size in combination with the lowest host-specimen-specific variability in glochidia size indicates that Baernau brown trout facilitate the best glochidia development in this study. Growth variation of *M. margaritifera* glochidia on the hosts may be influenced by initial infection rates, fish size and fish activity as well as by physiological defense mechanisms of the host. All strains used in this study showed a variance in size of glochidia caused by different growth of glochidia. Since glochidia are not globular, the measurement in their native orientation at the gill arch could theoretically result in a reduced precision of length measurements. The large number of measurement replications (n = 5,760) used for relative comparison of fish species and strains along with the great similarity of measured glochidia sizes on the right compared with left gills suggest the absence of such a bias in the dataset presented here.

The hypothesis that a high number of glochidia on a gill arch results in decreased growth of these glochidia was tested on lacustrine brown trout, the fish with the highest individual variances in mean glochidia size. The weak positive relationship of glochidia size and total number of glochidia on the corresponding gill arches is in line with results by Bauer and Vogel (1987), who showed that glochidia growth is not density-dependent as evident from the negative correlation between growth and mortality of glochidia. Consequently, host suitability - even at an individual level - seems to have the highest impact on glochidia development.

5. Variable development and excystment of freshwater pearl mussel (*Margaritifera margaritifera* L.) at constant temperature

The content of this chapter was published:
Taeubert JE, Gum B, Geist J. 2013. Variable development and excystment of freshwater pearl mussel (*Margaritifera margaritifera* L.) at constant temperature. *Limnologica* 43(4): 319–322.

5. 1. Abstract:

The highly endangered freshwater pearl mussel (*Margaritifera margaritifera* L.) has strongly declined throughout Europe and is a priority species in aquatic conservation. The complex life cycle of *M. margaritifera* includes an obligate development phase of glochidia larvae on a suitable host fish. Knowledge on the progression of the parasitic phase and on the factors governing excystment of juvenile mussels are particularly crucial for artificial breeding and conservation measures. The core objective of this study was to study excystment of *M. margaritifera* after maintaining the infested hosts under constant water temperatures between 11 and 12 °C and to determine the sum of daily water temperatures (day degrees) required by *M. margaritifera* for completion of metamorphosis. In a standardized laboratory experiment, excystment of juvenile mussels from brown trout (*Salmo trutta*) was found between 1,700 and 3,400 day degrees post-infestation, indicating highly variable development times of individual glochidia and the absence of a previously postulated threshold temperature of ≥ 15 °C for successful excystment of living juveniles. Consequently, the parasitic phase does not seem to limit the current distribution range and reintroduction of the species into cool headwater areas, as well as the culturing under constant water temperature conditions in typical salmonid fish hatchery setups. The concept of day degrees of development may also be useful to test the ecological implications of observed genetic differences among different populations.

5. 2. Introduction

The freshwater pearl mussel (*Margaritifera margaritifera* L.) is highly endangered in central Europe and has become a priority species in aquatic conservation (Bauer, 1991; Young, 1991; Geist, 2010; 2011). The complex life cycle of *M. margaritifera* includes an obligate parasitic phase on suitable host fish species such as brown trout (*Salmo trutta* L.) or Atlantic

salmon (*Salmo salar* L.) (Young and Williams, 1984a; Hastie and Young, 2001). In summer, the freshwater pearl mussel larvae (glochidia) are released by gravid females and reach their fish hosts passively with the water current. After inhalation, the glochidia attach to the gills and become encysted by epithelial cells of the host (Young and Williams, 1984b; Hastie and Young, 2003b). During encystment, glochidia grow approximately 6 - 10 fold in size and metamorphose into juvenile mussels (Ziuganov et al., 1994) before they excyst and bury into the river substratum. After about 5 years, the juvenile mussels appear at the substratum surface and live as filter feeders (e.g. Young and Williams, 1984a; Bauer, 1991, 1997; Hastie and Young, 2000). Although several studies investigated the suitability of different host fish species as well as the post-parasitic phase (Young and Williams, 1984a, 1984b; Taeubert et al., 2010), there is only limited knowledge concerning the factors which govern excystment. Hruška (1992) found that *M. margaritifera* require a mean water temperature ≥ 15 °C for at least 14 days at the end of the parasitic phase for successful excystment which would restrict the geographic distribution of this endangered mussel species to streams with higher temperature conditions during summer. To our knowledge, this observation has never been empirically verified. Information of the influence of temperature on excystment is also crucial for the assessment of pearl mussel habitat quality and is essential for identifying suitable areas for reintroduction. Since artificial breeding efforts in *M. margaritifera* increased in recent years (Hastie and Young, 2003b; Preston et al., 2007; McIvor and Aldridge, 2008; Thomas et al., 2010; Gum et al., 2011), detailed knowledge of the timing of excystment can be helpful for effective temperature management during mussel propagation and culturing (Hastie and Young, 2003b).

The core objective of this study was to determine the sum of daily water temperatures (day degrees) which *M. margaritifera* require for completion of metamorphosis under constant water temperatures between 11 and 12 °C which are typical for the most groundwater-fed salmonid hatcheries. Obtained results were compared with available literature from different countries and can help deduce strategies for facilitating artificial breeding of this endangered mussel species.

5. 3. Methods

Glochidia collection from mussels of the Zinnbach population in Germany, and infection procedures were carried out on September 8th, 2010 following the protocol described in Taeubert et al. (2010). For standardization and in order to avoid any bias due to an acquired resistance to glochidia of unionoid mussels in the host fish (Dodd et al., 2005), only hatchery reared fish with no previous contact to glochidia were used in this experiment. Mean infestation rate was ~ 1,500 glochidia per fish. In the laboratory of the Aquatic Systems Biology Unit, 100 infested brown trout (*Salmo trutta*) were maintained in a 300 L flow-through

circular tank with ground water supply (0.5 L s^{-1}). Initially, fish were fed 2 % of body weight twice a week with commercial trout chow (1.8 mm Aqua Pro, Skretting). In order to reduce faeces and to facilitate the recovery of excysted juvenile mussels, fish were only sparsely fed once a week after the start of excystment (3rd February 2011). To collect the excysted juvenile mussels, the tank outflow was filtered through a bucket with an incorporated 200 µm screen and the recovered material was checked for the presence of juvenile mussels under a binocular (SZX10, Olympus, Hamburg, Germany) using 6.3 x magnification. Viability of juvenile mussels was assessed based on two criteria: Active contraction of the adductor muscle, and active movement (evident from movement of the foot). In contrast, dead juveniles could be easily identified by wide gaping of the valves and by the absence of any reaction. During the experimental period, water temperature was recorded every hour by temperature loggers (Lascar Electronics Limited, Salisbury, UK). No changes in mean water temperature before and during excystment were found. The mean temperature between infestation and the start of excystment (September – February) was 11.5 °C (SD = 0.2 °C). During the excystment period (February – July), a mean water temperature of 11.8 °C (SD = 0.2 °C) was recorded (Figure 5-1).

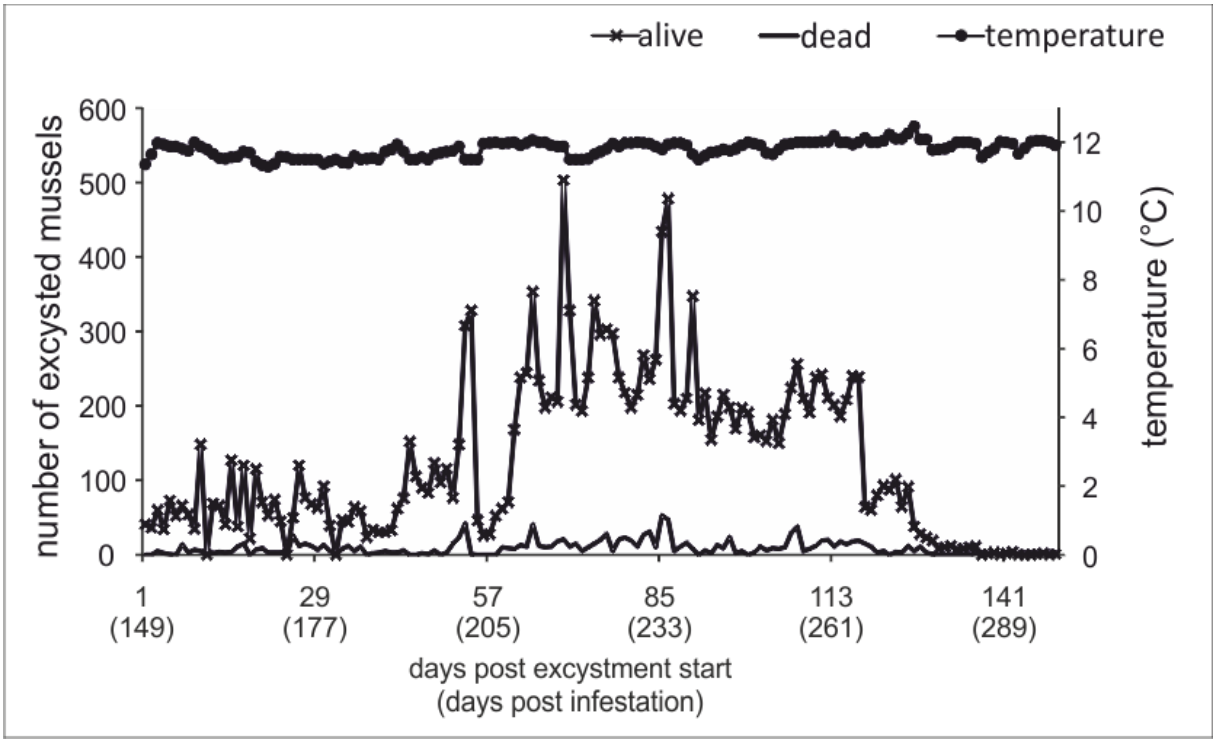

Figure 5-1: Excystment chronology of juvenile *M. margaritifera*, subdivided into counts of living and dead specimens.

5. 4. Results

The excystment of living juvenile *M. margaritifera* started on February 3rd and continued up to July 1st. Over this period of 149 days, a total of 19,891 juvenile mussels were collected, including 18,595 living juvenile mussels and 1,296 dead mussels (7 % of all collected mussels). A positive linear relationship between the excystment rates of living juvenile mussels and dead juveniles was found (Figure 5-2).

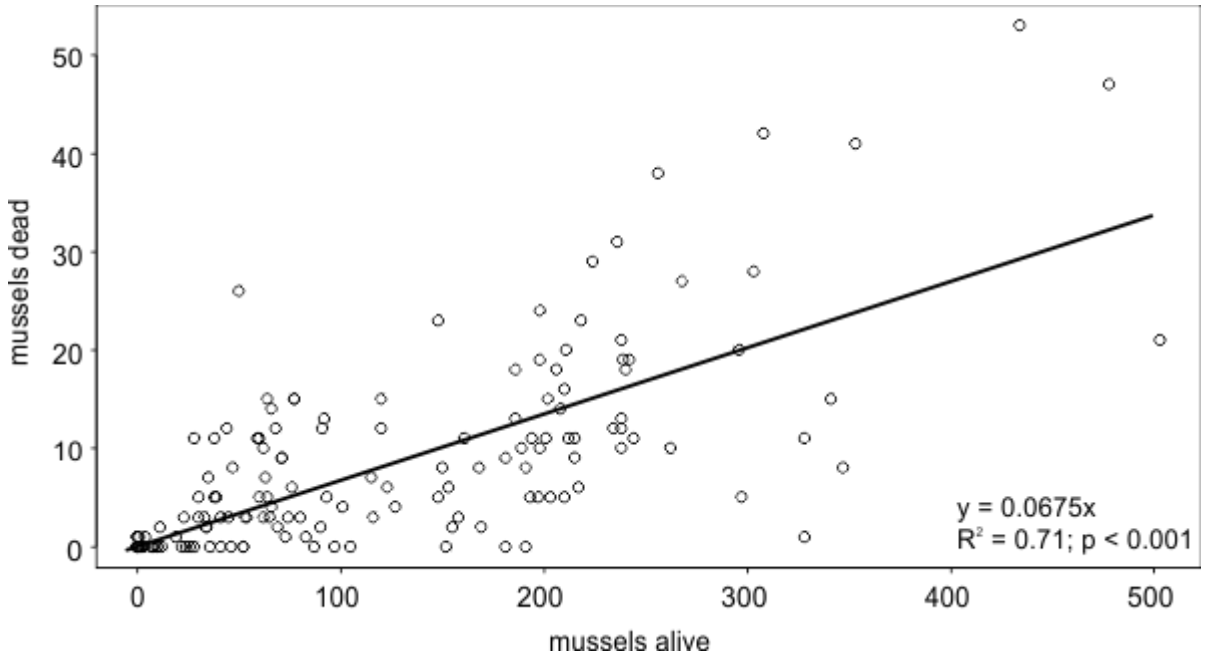

Figure 5-2: Relationship between the excystment of living juvenile mussels and obviously dead juveniles.

On average, ~ 185 viable mussels fish^{-1} were recovered, following initial infestation rates of 1,500 glochidia fish^{-1}. This indicates a ~ 88 % loss of glochidia during metamorphosis. It was not possible to observe active foot movement for every single mussel with closed valves due to high number of excysted juveniles. However, for every day of the excystment period, active foot movement of several juvenile mussels was recorded. The host fish mortality between the infestation and the start of excystment was 5 %, while 42 % of the host fish died until the end of excystment phase.

The sum of daily water temperature (day degrees) from infestation to completion of metamorphosis ranged between ~ 1,700 day degrees and ~ 3,440 day degrees (dd). However, at the beginning and the end of the excystment period only few mussels were found, while more than 80 % of all juvenile mussels excysted over a 73-day period between 2,220 and 3,080 dd. The peak daily excystment rate with 503 mussels (2.6 % of total excysted mussels) was found 2,530 dd (217 days) post-infestation (Figure 5-1).

5. 5. Discussion

In order to prevent extinction of priority *M. margaritifera* populations, several European countries have initiated conservation measures and breeding programmes. These programmes often use artificial infestation approaches and semi-natural cultivation of juvenile freshwater pearl mussels (Gum et al., 2011). After successful excystment from suitable hosts, juvenile mussels are collected and pre-cultured in small containers before they are transferred into cages and placed in natural rivers or semi-natural flow channels (Hruška, 1999; 2001; Thomas et al., 2010; Gum et al., 2011). One critical step during artificial propagation is the timing of the excystment process since the small size of excysted mussels impedes their collection. Host fish were often not fed before and during excystment to reduce the amount of fish faeces and facilitate mussel collection. Detailed knowledge of the timing of the excystment process is crucial to minimize holding of infested fish and time-consuming control for the presence of juvenile mussels. In addition, this knowledge helps to optimize and standardize artificial breeding of *M. margaritifera* and to avoid the loss of juvenile mussels due to delayed collection (Hastie and Young, 2003b). However, according to the available literature, the start of excystment of *M. margaritifera* appears highly variable. For example Ziuganov et al. (1994) described the start of excystment between 18 days post-infestation and 11 months post-infestation under natural temperature conditions (Table 5-1).

Table 5-1: Summary of available temperature and day degree data for the excystment of *M. margaritifera.*

country	host fish species	water temp. (°C)	start of excystment		end of excystment		additional information	reference
			day degree pi	days pi	day degree pi	days pi		
Germany	*S. trutta*	11.8 ± 0.2	1,702	149	3,448	297	constant temperatures	this study
Russia	*S. salar*	-	-	18	-	-	1987; adult *S. salar*	Ziuganov et al. (1994)
Germany	*S. trutta*	-		~ 21		~ 28	river IV	Bauer (1979)
Czech Republik	*S. trutta*	15.5 - 17.0	1,300	~ 84	1,430	-	constant temperatures	Hruška (1992)
Germany	*S. trutta*	-		~ 270		-	river I	Bauer (1979)
Great Britain	*S. trutta*	-	-	283	-	297	-	Young and Williams (1984b)
Czech Republik	*S. trutta*	-	1,760 (1,818)	~ 300	1,820 (1,860)	-	natural temperature regime	Hruška (1992)
Germany	*S. trutta*	2.0 - 5.0	1,500	~ 300	-	-	natural temperature regime	Schmidt and Vandré (2010)
Russia	*S. salar*	-	-	~ 330	-	-	1989; 2 years old *S. salar*	Ziuganov et al. (1994)
Great Britain	-	-	2,381 (2,229 - 2,619)	-	-	-	-	Thomas et al. (2010)

pi = post-infestation

Data from different years in brackets.

Hruška (1992) established the concept of day degrees for the duration of metamorphosis of *M. margaritifera* and recorded excystment start after 1,300 dd under constant water temperatures between 15.5 and 17.0 °C. In contrast, Thomas et al. (2010) found excystment following ~ 2,400 dd (Table 5-1). The high variation between different experiments might be caused by i) different temperature regimes, ii) the use of different host fish species and strains, and / or iii) the use of different strains of *M. margaritifera*. Since the freshwater pearl mussel population from the Zinnbach is genetically closely related to other populations from the Elbe and the northern Danube catchment (Geist and Kuehn, 2005), it is likely that the observed excystment patterns will be similar for these populations as well. From an evolutionary perspective, it would be highly interesting to compare the observed patterns of genetic differentiation among pearl mussel populations (e.g. Geist and Kuehn, 2008; Geist et al., 2010) with ecological factors such as temperature regimes and the timing of excystment. Interestingly, *M. margaritifera* from this study was found to excyst between ~ 1,700 and ~ 3,400 dd post-infestation. To our knowledge, such a long period of excystment has never previously been reported. The prolonged excystment period indicates a highly variable development speed of glochidia, which can result from a variable suitability of different specimen of a host population and / or a variable suitability of different encystment sites within one host. For example, glochidia encysted on the gill rakers might reveal a slower development than glochidia encysted on the gill filaments caused by different nutrition supply. Different suitabilities of various specimens within a host population were also found in other studies with unionoid mussel larvae (Roberts and Barnhart, 1999; Taeubert et al., 2012b). The resulting differences in time points of excystment may counterbalance the highly synchronized spawning event of *M. margaritifera*. Under natural conditions, an extended excystment period facilitates the distribution of juvenile mussels over larger areas due to host migration (Watter and O`Dee, 1999). It probably also increases the chances of survival due to higher exploitation of suitable habitats and a reduced competition for nutrients (Taeubert et al., 2012a). In natural stream ecosystems with occurrence of *M. margaritifera* typically only the headwaters reveal approximately constant temperatures throughout the year. Further downstream daily and seasonal fluctuations of temperature might compress the development and result in a shorter excystment period.

The low number of excysted living mussels per fish (12 %) compared with the high initial infestation is in line with Young and Williams (1984a) and Hastie and Young (2001) who found that only 5 - 10 % of the initially attached glochidia metamorphosed and excysted as juvenile mussels in *M. margaritifera*. In other species of Unionoidae, most unsuccessful glochidia are also lost from the host, typically within the first few days (e.g. Roberts and Barnhart 1999). The metamorphosis success and the long recovery time of juvenile mussels are even more surprising considering the high mortality of fish (42 %). Since gill tissue of

dead fish decomposes rapidly, resulting in a quick release of encysted glochidia, this effect might have depressed the apparent success of metamorphosis, and also shortened the time course of recovery. Although the ratio of dead to living juveniles was constant throughout the excystment period, it remains unclear if the condition of the living individuals is constant throughout the excystment period. For the investigation of differences in post-parasitic survival of juvenile mussels released at different time points during the excystment phase, it is crucial to provide ideal rearing conditions to the juvenile *M. margaritifera*. The increasing effort invested into culturing of *M. margaritifera* is likely to contribute to higher survival rates also in the sensitive early post-parastic phase (e.g. Geist, 2010; Gum et al., 2011).

As evident from the successful excystment under constant water temperature of ~ 12 °C, the hypothesis of Hruška (1992) that encysted glochidia require a mean water temperature of ≥ 15 °C for at least 2 weeks for successful excystment is not supported by this study. Our results indicate that *M. margaritifera* distribution is not limited by a threshold temperature of 15 °C and that colonization of cool headwaters is not inhibited by the excystment process. Consequently, such areas of high habitat quality can also be considered for reintroductions. However, there is anecdotal evidence that increased water temperatures may enhance recruitment of *M. margaritifera* populations (Hruška, 1992; Hastie et al., 2003) and it is likely that higher temperatures facilitate an earlier start as well as a shorter period of excystment. On the other hand, higher water temperatures have detrimental effects on brown trout, the most important host of *M. margaritifera* in central Europe (Geist et al., 2006).

The relationship between temperature and development speed should be considered for captive breeding and reintroduction purposes. Maintenance of infested host fish under constant water temperatures between 11 and 12 °C which is typical for most ground-water fed salmonid hatcheries in central Europe, ensures a continuous supply with juvenile mussels between March and May (excystment of 80 % of the mussels), which is an excellent time for starting cultivation of post–parasitic juveniles. For culturing, it is probably ideal to maintain infested fish at a temperature between 10 and 12 °C until metamorphosed glochidia reach their final size, and to then increase temperature to ensure high drop-off rates within short periods of time. The first months after the release from the host were considered as the most sensitive phase in the cultivation of *M. margaritifera* (Gum et al., 2011) and a long growth period during summer is beneficial for surviving the first winter. This is supported by Buddensiek (1995) who found that juvenile mussels > 900 µm had a 50 % chance to reach the growing period of the second year, while 100 % of juveniles < 700 µm died during their first winter. The use of in vitro culturing methods of freshwater mussels (Lima et al., 2012) may provide suitable alternatives which are more independent from seasonality.

In addition to Taeubert et al. (2010) who found a successful artificial infestation of *M. margaritifera* under carbonate-rich water conditions, this study verified a successful

development and excystment of *M. margaritifera* in calcareous water. Therefore the restriction of *M. margaritifera* distribution to siliceous streams cannot be explained by autecological limitations of glochidia to low-carbonate water during the infection and parasitic phase. The specific distribution of *M. margaritifera* is likely due to limitations of the post-parasitic phase when juvenile mussels are particularly sensitive to adverse substratum conditions (Hastie and Young, 2000; Geist and Auerswald, 2007; Geist and Kuehn, 2008; Geist, 2010).

In summary, *M. margaritifera* glochidia encysted on *S. trutta* were able to metamorphose successful under constant water temperatures between 11 and 12 °C and excystment of *M. margaritifera* does not depend on a threshold temperature of 15 °C. Excystment of juvenile mussels was found between 1,700 and 3,400 dd post-infestation indicating a highly variable development speed of glochidia within a host population.

6. Critical swimming speed of brown trout (*Salmo trutta*) infested with freshwater pearl mussel (*Margaritifera margaritifera*) glochidia and implications for artificial breeding of an endangered mussel species

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Taeubert JE, Geist J. 2013. Critical swimming speed of brown trout (*Salmo trutta*) infested with freshwater pearl mussel (*Margaritifera margaritifera*) glochidia and implications for artificial breeding of an endangered mussel species. *Parasitology Research* 112(4): 1607–1613.

6. 1. Abstract

Unionoid freshwater mussels need to attach to a host fish for completion of their life cycle. It remains unclear whether the relationship between these mussels and their host fishes can be considered parasitic, mutualistic or commensal. Herein, we studied the effects of *Margaritifera margaritifera* infestation on *Salmo trutta*, the most important host of this endangered mussel species in central Europe. Glochidial load of host fish increased with increasing glochidia concentration, but the highest ratios of encysted glochidia to exposed glochidia were found at low concentration (15,000 glochidia L^{-1}) during infestation. Host fish mortality occurred at infestation rates of ~ 350 glochidia per g fish weight and was highest (60 %) at the highest infestation rates (~ 900 glochidia per g fish weight). On a sublethal level, swimming performance of hosts was inversely related to infestation rates, with infestation of ~ 900 glochidia per g fish weight reducing critical swimming speed of *S. trutta* significantly by ~ 20 % compared to infestation with 6 glochidia per g fish weight. The high mortality and the impaired swimming capability of highly infested hosts indicate a parasitic interaction between *M. margaritifera* and its host. For conservation and reintroduction of *M. margaritifera* via glochidia-infested *S. trutta*, we recommend glochidial loads of 5 - 100 glochidia per g fish weight, while for artificial breeding of juvenile *M. margaritifera* under laboratory conditions, higher infestation rates of up to 300 glochidia per g fish weight are ideal to balance high yields of mussels and welfare of host fishes.

6. 2. Introduction

Inter-species relations are defined by the effect of the interaction between the involved species and can either be classified as i) beneficial to both species (mutualism), ii) beneficial to one species, while the other species is not affected (commensalism), iii) beneficial to one species and detrimental for the interacting partner (parasitism or predation), and iv) detrimental to both interacting species (competition). Clarification of the mode of interaction can be useful in understanding population-level dynamics between species, yet remains unresolved for some interspecies relationships.

Freshwater mussels from the superfamily Unionoidea have a complex life cycle including an obligate phase on a suitable host (Barnhart et al., 2008). For example, the larvae (glochidia) of the freshwater pearl mussel *Margaritifera margaritifera* L. - one of the most endangered species in Europe (Young, 1991; Geist, 2010, 2011) – are released by the females and reach their host passively via the water current. After being inhaled by the host fish, glochidia encyst on the gills and become encapsuled by epithelial cells of the host within 6 to 12 h (Young and Williams, 1984a; Rogers-Lowery and Dimock, 2006). During a period of up to 11 months they grow from ~ 0.07 mm to 0.4 - 0.5 mm and metamorphose into juvenile mussels (Bauer and Vogel, 1987). After the excystment from the host, the juveniles bury into the river substratum, where they live for about five years before they appear at the substratum surface and develop into adult mussels (Young and Williams, 1984b; Bauer, 1991, 1997; Ziuganov et al., 1994; Hastie and Young, 2000). The post-parasitic stage is the most sensitive phase in the life of *M. margaritifera* (Hastie and Young, 2000; Geist and Auerswald, 2007; Österling et al., 2008; 2010; Geist, 2010). According to Young and Williams (1984b), the only host fishes of freshwater pearl mussel in Europe are Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) with brown trout being the exclusive host in many central European populations (Bauer, 1987c; Geist et al., 2006). While some authors considered the interaction of *M. margaritifera* and its hosts as a parasitic relationship (Karna and Millemann, 1978; Bauer, 1987a, 1987c), other authors highlight the positive effects of the co-occurrence of salmonid fishes and freshwater pearl mussels (Ziuganov et al., 1994; Skinner et al., 2003). The hypothesis of a parasitic relationship is supported by the high growth rates of glochidia during metamorphosis. In contrast, the mutualistic relationship between freshwater pearl mussel and salmonids is supported by the fact that rivers with pearl mussels reveal a higher resistance to eutrophication and provide manifold microhabitats for young salmonids (Ziuganov et al., 1994). Ziuganov and Nezlin (1988) considered the mussel / fish interaction to be a variety of symbiosis-protocooporation rather than simple parasitism.

The core objectives of this study were to clarify the effect of *M. margaritifera* infestation on its host fish, *S. trutta*, and to deduce optimal infestation rates for conservation purposes. Therefore, *S. trutta* was infested with different concentrations of glochidia to determine the

links between glochidia concentrations and infestation success. To assess the effects of glochidia interaction on host fish, lethal and sublethal endpoints were considered. For assessment of sublethal effects of *M. margaritifera* infestation on its host, the swimming performance of infested fish was tested. Since most of the central European *M. margaritifera* populations consist exclusively of individuals older than 50 years and successful juvenile recruitment has not occurred for decades, artificial breeding of *M. margaritifera* has increased in recent years to save the species from extinction (Hastie and Young, 2003b; Preston et al., 2007; McIvor and Aldridge, 2008; Thomas et al., 2010; Gum et al., 2011). The use of different glochidia concentrations for infestation of *S. trutta* was thus also intended to find optimal infestation rates for both captive breeding of *M. margaritifera* under artificial conditions as well as reintroduction of *M. margaritifera* by stocking of infested host fish.

6. 3. Methods

6. 3. 1. Collection of glochidia and infestation procedure

Glochidia from 20 adult *M. margaritifera* were collected on 24 August 2011 and stored at 4 °C following the protocol described in Taeubert et al. (2010). The viability of obtained glochidia was checked by observing their response after addition of NaCl to a small aliquot of suspended glochidia under a binocular microscope (SZX10, Olympus, Hamburg, Germany). On 25 August, glochidia were slowly adapted to ground water (11 °C) and resuspended in preparation for host infestation. Four different infestation baths (each 10 L) were created, with 150,000 glochidia L^{-1} (treatment A), 75,000 glochidia L^{-1} (treatment B), 15,000 glochidia L^{-1} (treatment C) and 1,500 glochidia L^{-1} (treatment D). One additional treatment of the same volume, but without glochidia was used as a control (reference). In each treatment, ten *S. trutta* (originated from the hatchery of the “Landesfischzuchtanstalt Mauka”, Germany) were exposed to glochidia for 15 min under permanent agitation to ensure homogenous distribution of glochidia. After infestation, treated fish were held in one 1,400 L fish tank (sub-divided with screens) under identical conditions to exclude bias due to maintenance effects.

6. 3. 2. Mortality and critical swimming performance (U_{crit}) measurement

The mortality of infested fish was checked every 12 h post-infestation. Critical swimming performance of infested and control fish was analysed in a modified swimming flume, first described in Blažka et al. (1960) (Figure 6-1). An impeller pump (SRP.18.30.806.08 Grundfos Arnold AG, Switzerland) was linked by an 1,150 mm long spacer pipe, with incorporated flow straightening pipes, to a 1,000 mm long Plexiglas pipe with an inner diameter of 292 mm. The Plexiglas pipe included three identical water tunnels which were

used to test three fish simultaneously. Each water tunnel was 670 mm long with an inner diameter of 112 mm. The water tunnels were capped with wire screen at each end to prevent fish from leaving. A shocking grid was not used, according to animal care regulations. A semitransparent black foil, covering 300 mm of the tunnels centres, provided shelter that encouraged fish to stay in the middle of each section. Pump speed and, hence, water velocity in the three tunnels was controlled by a frequency controller (Movitrac LTE-B, SEW Eurodrive, Germany), calibrated using a HFA flow-measuring instrument (Höntzsch Instrumente, Waiblingen, Germany). Critical swimming speed (U_{crit}) values did not need correction for blocking effects by specimens, since the maximum cross-sectional area (< 8.7 cm^2) of each fish did not exceed 10 % of the cross-sectional area (98.5 cm^2) of the swimming chambers (Bell and Terhune, 1970).

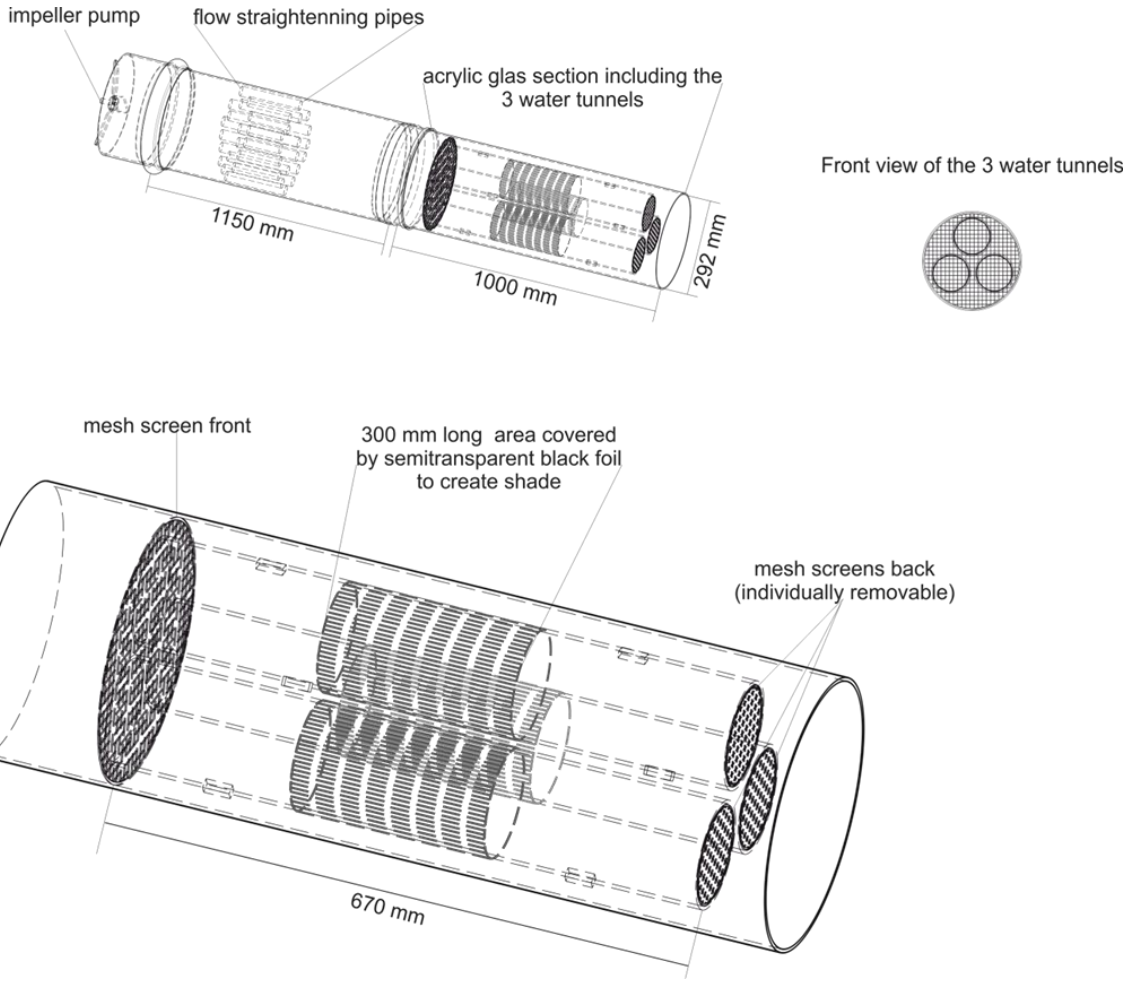

Figure 6-1: Schematic view of the modified swimming tunnel which is set up in a circular tank (8 m diameter).

Water level in the tank was at least 0.1 m over the whole construction. The impeller pump generates a water current (see arrow) through flow straightening pipes. The three water tunnels (test sections) are enclosed in a transparent Plexiglas pipe which allows the observation of test fish.

Analyses of U_{crit} started 24 hours post-infestation. Infested fish were randomly placed in the tunnels and allowed to acclimatize at a water velocity of 5 cm s^{-1} for 15 min. After the acclimatization period, water velocity was increased in increments of 5 cm s^{-1} at 5 min intervals until the fish fatigued (Nelson et al., 2002, 2003; Santos et al., 2007). Fatigue was defined as the point at which the fish spent more than 20 s at the screen even after being gently prodded with a plastic wire. U_{crit} was calculated according to Brett (1964) using the formula:

$U_{crit} = U_i + [(T_i / T_{ii}) * U_{ii}]$

where U_i represents the highest velocity maintained for the whole 5 min [cm s^{-1}], U_{ii} is the velocity increment (5 cm s^{-1}), T_i is the time to fatigue at the last current velocity [min], and T_{ii} is the interval time (5 min). U_{crit} was calculated both as absolute [cm s^{-1}] and relative [body length s^{-1}] swimming speed.

6. 3. 3. Determination of infestation rate

After U_{crit} determination, fish were anesthetized and sacrificed. Individual fish were measured (+/- 1 mm), weighed (+/- 0.01 g) and gill arches were dissected. Infestation rates were determined under binocular microscope (SZX10, Olympus, Hamburg, Germany) using 10 x magnification. For further details see Taeubert et al. (2010).

6. 3. 4. Statistical analyses

Statistical analyses, including the best-fit linear model for the link between glochidial load and critical swimming speed were performed in R Version 2.12.0 (© 2010, R Foundation for Statistical Computing). Differences in mean critical swimming speed between the five different infested groups of *S. trutta* were tested by ANOVA. Although unequal sample sizes were used, the assumptions of normality and homoscedasticity were met. TukeyHSD post hoc test was used to determine significant differences between means of different groups.

6. 4. Results

Mean infestation rates of *S. trutta* differed after exposure to different concentrations of glochidia (Table 6-1). The glochidial load of fish infested with the highest concentrated glochidia suspension (treatment A) ranged between 53,636 and 63,883 glochidia per fish, while glochidial loads of fish infested within treatment B varied between 12,857 and 27,342 glochidia per fish. The lowest glochidial load, ranging between 191 and 484 glochidia per fish, was found for *S. trutta* infested with the lowest glochidia concentration (treatment D).

Table 6-1: Infestation rates of *S. trutta* after exposure to different concentrations of glochidia.

Treatment	glochidia conc. used for infestation	infestation rate $\bar{X}$ ± SD		U_{crit} $\bar{X}$ ± SD		fish length $\bar{X}$ ± SD	no. of tested fish	fish mortality 12 h post-infestation [%]
	glochidia L^{-1}	glochidia per fish	glochidia per g fish weight	[cm s^{-1}]	[body length s^{-1}]	[cm]		
A	150,000	58,747 ± 4793	906 ± 156	46.75 ± 4.57 [a]	2.48 ± 0.21 [a]	18.93 ± 0.64	4	60
B	75,000	21,833 ± 5378	353 ± 91	50.19 ± 6.34 [ab]	2.71 ± 0.37 [ab]	18.54 ± 0.82	8	10
C	15,000	6,823 ± 1320	113 ± 20	51.56 ± 4.42 [ab]	2.75 ± 0.26 [ab]	18.70 ± 0.56	8	0
D	1,500	358 ± 106	6 ± 1	56.00 ± 4.62 [b]	3.01 ± 0.26 [b]	18.58 ± 0.36	8	0
Reference	0	0	0	54.81 ± 3.00 [ab]	2.96 ± 0.24 [ab]	18.45 ± 0.70	8	0

Significant differences are indicated by different letters - a and b.

6. 4. 1. Glochidia uptake

After comparison of the available glochidia in each infestation bath with the mean glochidial load of host fish infested within these baths, the highest glochidia uptake (45 %) was found for *S. trutta* infested in treatment C (15,000 glochidia L^{-1}). In contrast, fish infested in treatment D (1,500 glochidia L^{-1}) revealed only 24 % of the available glochidia on their gills. Intermediate glochidia uptakes of 39 % and 29 % were found for *S. trutta* infested within treatment A (150,000 glochidia L^{-1}) and treatment B (75,000 glochidia L^{-1}).

6. 4. 2. Host fish mortality

Host fish mortality (60 %) was highest for *S. trutta* with the highest total (58,747 ± 4793 glochidia per fish) and weight-normalized infestation rates (906 ± 156 glochidia per g fish weight). One host fish out of ten within treatment B, with the second highest weight-normalized infestation rate (353 ± 91 glochidia per g fish weight), died 24 h post-infestation. In contrast, none of the *S. trutta* with mean weight-normalized glochidial loads of 113 ± 20 glochidia per g fish weight (treatment C) and 6 ± 1 glochidia per g fish weight (treatment D) died 24 h post-infestation.

6. 4. 3. Swimming performance

The mean critical swimming speed (46.75 cm s^{-1}) of *S. trutta* in treatment A was significantly lower ($p < 0.05$) than that of *S. trutta* in treatment D (56.00 cm s^{-1}). This is also true for the body-length-normalized swimming speed ($p < 0.05$) for treatment A (2.48 body length s^{-1}) compared with treatment D (3.01 body length s^{-1}). However, both U_{crit} and the body-length-normalized swimming speed of *S. trutta* in treatment B (50.19 cm s^{-1}), treatment C (51.56 cm s^{-1}), as well as the uninfested reference group (54.81 cm s^{-1}), were intermediate and did not differ significantly from absolute and body-length-normalized U_{crit} of fish with the highest and lowest infestation rates (treatments A and D). Swimming performance of fish with high infestation levels (treatment A and B) was similar to swimming performance of the weakest fish with no or lower glochidial loads. Although U_{crit} of individual fish is highly variable within treatments, values below 2.4 body length s^{-1} were only found in fish infested with more than 10,000 glochidia (Figure 6-2). A negative linear relationship between the mean infestation rate and mean U_{crit} of each treatment was found. The best-fit equations and coefficient of determinations are given in Figure 6-2.

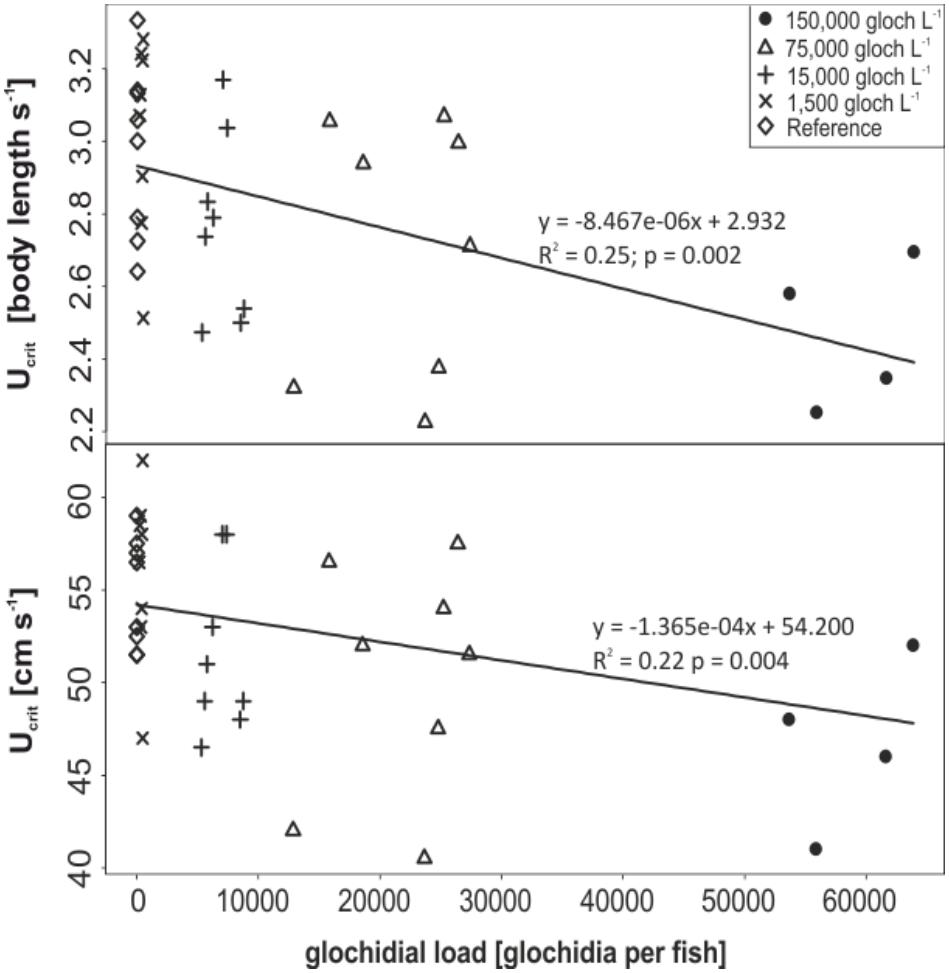

Figure 6-2: Linear relationships between glochidial load and absolute as well as body-length-normalized critical swimming speed (U_{crit}) of *S. trutta*.

6. 5. Discussion

Understanding the nature of the relationship between host fish and freshwater mussels is crucial for determining evolutionary benefits and disadvantages to both species. At the same time, the increasingly common artificial propagation of *M. margaritifera* necessitates development of optimized infestation procedures which provide high yields of juvenile mussels and consider the welfare of host fishes during the larval phase of this endangered freshwater mussel. We investigated host responses to glochidia infestation by assessing the mortality and the swimming performance of fish with glochidial loads between 350 and 60,000 glochidia per fish. In addition to the effects of glochidia infestation on the host, the proportion of encysted glochidia allowed the calculation of glochidia uptake, which is helpful for deducing strategies for artificial infestation procedures.

6. 5. 1. Glochidia uptake

For artificial propagation, a high proportion of glochidia used for infestation should encyst and develop into juvenile mussels, especially when restoring small remnant populations of *M. margaritifera* where only limited numbers of glochidia can be harvested. The most effective glochidia uptake was found for the 15,000 glochidia L^{-1} suspension (45 % of provided glochidia encysted on the gills of the hosts). At lower glochidia concentrations, the likelihood of encounter between glochidia and host fish decreases, while at higher glochidia concentrations the available gill area might be the limiting factor for higher glochidia uptakes (Taeubert et al., 2012a; 2012b). In addition to the glochidia concentration of the infestation baths, the duration of infestation may play an important role for the glochidia uptake. As recommended by Taeubert et al. (2010), a relatively low concentrated glochidia suspension (25,000 L^{-1}) can result in high infestation rates of suitable hosts after a long time exposure of 45 min. However, extending infestation times beyond the 15 min used in this study can result in oxygen depletion and may not be appropriate for generating different infestation rates on the host fish. However, the most important factor determining the uptake and, thus, the success of artificial propagation measures of *M. margaritifera* is the viability of glochidia.

6. 5. 2. Host fish mortality

The results of this study indicate that host fish mortality increases with glochidial loads, particularly at very high infestation rates. Glochidial loads of 900 glochidia per g fish weight, resulting from infestation within a 150,000 glochidia L^{-1} glochidia suspension, were considered particularly harmful. This is in line with Meyers and Millemann (1977), who found mortality rates of 90 % within 48 h post-infestation after exposing Atlantic salmon (*Salmo salar* L.) to large numbers of *M. margaritifera* glochidia. Besides the negative effects due to the loss of host fish, host mortality decreases the amount of mussels available for propagation when encysted glochidia are lost prior to metamorphosis. The finding that no *S. trutta* within treatments C and D and only one fish infested within treatment B (350 glochidia per g fish weight) died during the first 48 h post-infestation suggests that weight-normalized glochidial loads below 350 glochidia per g fish weight had only little or no effects on host fish survival. This is supported by Treasurer et al. (2006), who found that infestation with 1393 glochidia per 5 g (279 glochidia g^{-1}) salmon had no significant effect on salmon survival and only a small effect on host growth. It is likely that the first 2 days post-infestation are the most sensitive phase for the host fish, since i) cyst formation by the host appears within the first 12 h (Nezlin et al., 1994; Rogers-Lowery and Dimock, 2006), ii) the innate immune responses of the host are being activated, iii) there is no adaptation to an impaired gas exchange due to the cyst formation around the parasite, and iv) the number of encysted glochidia on the host is highest at this time. Only 5 - 10 % of the initially attached glochidia metamorphose

successfully (Hastie and Young, 2001), suggesting generally high mortality rates of mussels during their encystment phase.

6. 5. 3. Swimming performance

Among the potential effects of parasitism on fish hosts, swimming performance was used to assess sublethal effects of *M. margaritifera* infestation on host fish. Although the ecological relevance of critical swimming speed data is limited because these data do not consider other behavioural traits which can be crucial for survival, the results of this study still provide a rough estimate of swimming ability (Plaut, 2001) and a relative comparison by which the physical status of differently infested fish can be quantified. As reported in Hastie et al. (2000), preferred water velocities for *M. margaritifera* range between 25 and 75 cm s^{-1} which indicates the necessity of host fish to withstand such velocities under natural conditions. The results of this study suggest that *M. margaritifera* infestation can have significant effects on host swimming ability. The significantly lower mean critical swimming speed of *S. trutta* infested with 900 glochidia per g fish weight compared with fish infested with 6 glochidia per g fish weight indicate that high glochidial loads decrease critical swimming speed of infested hosts while low glochidial loads had no effect. In contrast, hosts with the lowest infestation rate reveal higher mean critical swimming speeds compared with the noninfested reference group, which can be either explained by high individual variances in swimming speed of tested individuals or a beneficial effect of glochidia infestation on the host as described for Atlantic salmon after *M. margaritifera* infestation (Ziuganov, 2005). However, reduced U_{crit} of < 2.4 body length s^{-1} was only found in fish infested with more than 10,000 glochidia indicating a decreased swimming capability only after heavy glochidia infestation. If such high glochidial loads are found under natural conditions remains unclear, but infestation rates between 2,000 and 7,000 were previously recorded for wild salmon (Ziuganov, 2005). Slower sustained swimming speeds may have a number of implications for infested fish. Factors which reduce the swimming performance are considered detrimental since they can result in higher risks of predation, a reduced ability of a fish to obtain food and avoid unfavourable conditions, as well as a higher risk for a specimen to be carried downstream during peak flow events. The ability of adults to reach spawning grounds may also be limited by high infestation rates. In contrast, *M. margaritifera* may benefit from these effects if highly infested fish prefer habitats with lower velocities where the chance of settlement of excysted juvenile *M. margaritifera* can be higher compared with areas with high current speeds.

6. 6. Conclusion and implications for artificial breeding of *M. margaritifera*

Since the fate of glochidia is directly linked to the survival of the host and host mortality is negatively related to glochidia concentrations, conservation managers are interested in strategies which guarantee good condition of infested host fish to obtain high yields of juvenile mussels. In addition to the in vitro culturing of mussels (Lima et al., 2012), there are two possible scenarios for reintroduction of *M. margaritifera*, i) captive-bred juvenile mussels can be stocked in suitable habitats and ii) artificially infested fish can be released in suitable stream sections. Since infested fish should be able to withstand high velocities in streams where *M. margaritifera* is present, glochidial loads should not impair their swimming performance. Consequently, we recommend glochidial loads between 5 and 100 glochidia per g fish weight (350 – 7,000 glochidia per fish with sizes of ~ 18 cm) for infested fish stocked to reintroduce *M. margaritifera*. If high variability in swimming performance of stocked fish is desired, i. e. to ensure that hosts become widely distributed in areas of different flow conditions, host fish with different infestation rates can be stocked.

If juvenile mussels are being cultured under artificial and controlled conditions, good swimming performance is not crucial for host survival and glochidial loads can be higher than for fishes used for stocking. Our results suggest that moderate *M. margaritifera* infestation of up to 300 glochidia per g fish weight had only marginal effects on host fish used for artificial breeding of juvenile mussels. High glochidia uptakes can be obtained when using infestation baths with glochidia concentrations between 15,000 and 75,000 glochidia L^{-1} where host fish mortality was ≤ 10 %.

7. General discussion

The obligate parasitic phase on a suitable host makes the reproduction of unionoid mussels susceptible to factors that reduce the abundance and distribution of their hosts. Therefore, the knowledge about basic ecological requirements such as host fish suitability and parameters affecting host-specificity are crucial for development of effective conservation strategies for endangered freshwater mussel species (Barnhart et al., 2008; Geist, 2010).
This study identified new hosts for the endangered thick-shelled-river mussel under laboratory and natural conditions and confirmed successful encystment of *M. margaritifera* larvae on *Hucho hucho*. In addition to the differences in the specificity of various fish species for hosting *U. crassus* and *M. margaritifera*, this study clearly demonstrates that host suitability also varies considerably between different populations within the same host species and between different individuals within the same host population. The substantial interspecific variation in host suitability was found for both, infestation / excystment rates as well as development time of encysted *U. crassus* and *M. margaritifera* larvae. Furthermore, *M. margaritifera* exhibited highly variable development times of larvae encysted on the same host population. In addition, the previously postulated mandatory threshold of ≥ 15 °C could not be confirmed in this study. In a last step, the type of the interaction between *M. margaritifera* larvae and their hosts was analysed by lethal and sublethal endpoints.

7. 1. Characterization of the type of interaction - positive and negative effects of the mussel fish relationship

Taking the freshwater pearl mussel as an example, the interaction between freshwater mussels and their host fishes can be classified as a parasitic relationship, indicated by a reduced swimming performance and an elevated mortality of heavy infested hosts (Chapter 6). This host-parasite relationship between mussels and fishes can be considered as unique because the generation time of the parasite (mussel) exceeds the generation time of the host (fish) by up to the factor 10 to 20 (e.g. *M. margaritifera* and *S. trutta*) (Bauer, 1997). From an evolutionary point of view, the significant shorter generation time of the host would be advantageous for evolving defense mechanisms to the parasite, but since this host parasite system has been stable for at least 60 million years (Bauer, 1997), the negative effects of the parasite to the host seem to be of minor importance or were caunterbalanced by other factors. The parasitic character of this relationship is also supported by the high and entirely host-dependent growth rates of larvae of *M. margaritifera* during metamorphosis which indicates a nutritional flow from the host fish to the mussel larvae. In contrast, the larvae of *U.*

crassus grew only marginally during metamorphosis (Chapter 2 and 3). However, the flow of nutrients from the host to a freshwater mussel during metamorphosis was also confirmed by stable isotope analysis (Fritts et al., 2013). In addition, the dependency on components of the host for initiation and completion of metamorphosis is also supported by successful development of mussel larvae using host-fish plasma for in-vitro culture (Lima et al., 2012). Despite the fact that the mussels do gain some nutrition from their hosts, the most obvious advantage of this interaction is the dispersal of juvenile mussels, especially in upstream direction for stream dwelling species (Chapter 2 and 3).

There are also some positive effects for the hosts resulting from the co-occurrence with mussels. For example, salmonid fishes benefit from the filtering action of adult freshwater pearl mussels which reduces the eutrophication of the rivers and the creation microhabitats for juvenile fishes (Ziuganov et al., 1994; Skinner et al., 2003). In addition to these indirect positive effects for the hosts, Ziuganov (2005) found that the host could benefit directly from glochidia infestation by turning out the programme of accelerated senescence following the extension of the hosts (*Salmo salar*) life span. In Chapter 6, a low larval infestation rate was found to be beneficial for the swimming performance of *S. trutta* although the differences between the group with the lowest infestation rates and the non-infested reference were not statistical significant and the finding might also be explained by high individual variances in swimming speed of the tested individuals.

7. 2. Host-specificity of *Unio crassus* and *Margaritifera margaritifera* - two endangered freshwater mussels

Based on the different metamorphosis success and / or development times of *U. crassus* and *M. margaritifera* larvae, the results of the case studies presented in Chapter 2 to 6 clearly indicate that there are at least three different levels of host-specificity for the two assessed mussel species. A variation in host-specificity was found on i) the individual level, ii) the population level, and iii) the species level of hosts (Figure 7-1). These three levels of host-specificity complicate host-specificity analyses and universal predictions on the host use of a mussel species. In addition, ranking the suitable host species to determine which is the most important for the mussel should also consider host availability under natural conditions (Chapter 3). However, a simple characterization of host suitability is the classification into good (primary or high-quality), poor (marginal or low-quality) and no hosts as described in Chapter 2 and 3 for the species level. Since the assessment of the variation in host suitability is important to determine host quality, it is essential to consider these three levels of host-specificity during host analyses.

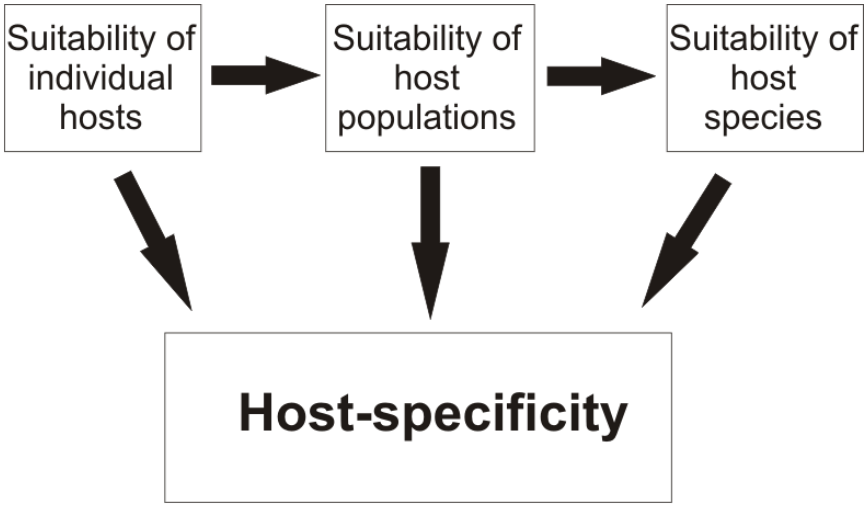

Figure 7-1: Different levels which determine host-specificity.

7. 2. 1. Species level of host-specificity

The species level is the most obvious level of host-specificity and was evident from the different suitability of various fish species for successfully hosting glochidia of *U. crassus* (Chapter 2 and 3) and the significant different encystment rates of *H. hucho* and *S. trutta* after *M. margaritifera* infestation (Chapter 4). In contrast to *M. margaritifera*, for which *S. trutta* is considered the most important or exclusive host in central Europe (Geist et al., 2006), the results of Chapter 2 and 3 indicate that *U. crassus* can use multiple host species for successful metamorphosis. Another indicator for the differences in host-specificity on the species level is the variation in development time of larvae encysted on different host species. Different duration of development resulted in different excystment times on different host species (Chapter 2 and 3, e.g. *P. phoxinus* vs. *S. cephalus*), or in different sizes of encysted larvae at the same time point (Chapter 4, *S. trutta* vs. *H. hucho*). The differences in duration of development might facilitate the distribution of juvenile mussels (Watter and O`Dee, 1999) and increase their chances of survival, because a wide distribution reduces the competition for nutrients and enhances the survival of populations under locally or temporarily unfavourable conditions.

The variability in suitability of different host species may help explain the differences in abundance and distribution of the mussel species (McNichols et al., 2011). For example, the high environmental plasticity o*f U. crassus* (Hochwald, 1997), in addition to its wide host range, enables the species to colonize different habitat types with divergent fish communities ranging from ditches and small brooks to bigger streams and lakes (Chapter 3; Douda et al., 2012). In contrast, *M. margaritifera* distribution is restricted to cool siliceous streams with low nutrient contents that are only colonized by specialized fish species, such as *Salmo trutta*,

Salmo salar (during its freshwater phase) and occasionally *H. hucho*. *M. margaritifera* can be considered as a host specialist, because in streams where *S. trutta* and *S. salar* occur sympatrically, the mussel seems to be adapted to either *S. trutta* or to *S. salar* (Karlsson et al., 2013). Although the obligate parasitic phase on a suitable host implies a host-dependent distribution area of freshwater mussels, the occurrence of host fish does not explain the distribution pattern of *M. margaritifera* and *U. crassus* (Bauer et al., 1991). The authors conclude that the low metabolic rate is an important factor limiting the distribution of *M. margaritifera* to rivers with low food availability, whereas the higher metabolic rate of *U. crassus* restricts the species to habitats with higher food supply. The salmonid hosts of *M. margaritifera* tolerate a much wider range of carbon and nutrient contents and the distribution range of suitable hosts exceeds the distribution range of the mussel (Bauer, 1992; 1997; Geist and Kuehn, 2008). The more specific habitat requirements of *M. margaritifera* compared with its salmonid hosts seems to contradict the host-dependend distribution of the mussel. Although additional factors like substratum quality and nutrient content may limit the distribution of freshwater mussels, the presence of suitable hosts is one important prerequisite for the presence of a mussel species (Stoeckl et al., 2014). Since the fish community of aquatic ecosystems is primarily dominated by habitat characteristics, the differences in the range of species serving as suitable hosts is at least one indicator that the two analysed mussel species colonize different habitats. There are only few exceptional streams in Europe, where habitat and host conditions seem to be intermediate and allow the co-occurrence of *U. crassus* and *M. margaritifera*.

7. 2. 2. Population level of host-specificity

The case studies of this work demonstrate that various populations of the same fish species exhibit significant differences in hosting larvae of *U. crassus* (three *Gasterosteus aculeatus* populations, Chapter 3) and *M. margaritifera* (three *S. trutta* populations, Chapter 4). In another more recent study, Jung et al. (2013) also detected differences in the suitability of various *S. trutta* populations for hosting *M. margaritifera*. Host-parasite interactions could result in adaptations that influence the life history of one or both interaction partners. In the case of local adaptation of the mussel to the host, it would be expected, that the sympatric host strain reveals higher encystment rates (e.g. resulting from decreased loss of encysted glochidia during parasitic phase) and / or higher growth rates (e.g. resulting from better supply with nutrients) of encysted larvae than the allopatric host strains. The fact that the generation time of the mussel exceeds the generation time of the host fishes in most cases makes a local adaptation of mussels to their hosts unlikely from an evolutionary point of view. On the other hand, host strains may develop features that reduce growth and survival of the parasitic mussel larvae. While mussels depend on successful infection of the host to

complete their life cycle, there might be only little selective pressure on the fish, because only heavy infections are harmful (see Chapter 6). In addition, mussel larvae do not reproduce on the host and the energetic costs for nutrition are slightly low, especially if larvae do not grow during their metamorphosis (Barnhart et al., 2008). In addition, it is not clear if non-host species benefit of the rejection of glochidia or if the “rejection costs” are higher than the costs for development of mussel larvae (Barnhart et al., 2008). If the infection by mussel larvae generally result in a great disadvantage for the host, it is likely that the fish species would have developed strategies to avoid the initial infection, because initial attachment of mussel larvae, which is also known for non-host species (Chapter 2) results in the first disadvantage for the fishes. However, to the authors knowledge there is no study which delivers clear evidence for local adaptation of one or both interaction partners in these host-parasite systems.

Although the causes for the differences in suitability of different host strains remain unclear, the observed differences in suitability of the studied populations might have consequences for the conservation of endangered mussel species. This is particularly true if extirpated host fish stocks should be reestablished or when mussel streams were stocked with fishes (e.g. for fishing purposes). Whilst no tested host population was considered unsuitable for hosting mussel larvae, the significant differences in suitability of various host strains may influence the recruitment success of the mussels directly by altering the survival rates of encysted larvae or indirectly by changing their growth rates. In the case of *M. margaritifera*, lower growth rates of encysted larvae on a less suitable host strain (Chapter 4) may decrease the final size of juvenile mussels entering the benthic life-stage, which in turn decreases the chances of surviving the first winter, as shown for semi artificial breeding systems (e.g. Buddensiek 1995).

7. 2. 3. Individual level of host-specificity

Besides the species and the population level, a high variability in host-specificity was found between individual hosts throughout the replicates of one host population used in the case studies (Chapter 2 to 6). A high individual variation in infection / transformation rates was also found in other studies (Kirk and Layzer, 1997; Roberts and Barnhart, 1999; Khym and Layzer, 2000; Hastie and Cosgrove, 2001). The high variability in host suitability on the individual level could either be based on i) the genetic constitution of the host fish, on ii) the current physiological status, on iii) environmental conditions, or on iv) a combination of multiple factors. Since all fish were kept under identical conditions before and during each experiment and were infected in the same infestation bath, it is unlikely that environmental conditions are responsible for the differences in individual suitability throughout this study.

The hypothesis, that the physiological status of individual hosts influences the host-specificity is supported by the finding that the weight normalized infection rate decreased with increasing host size (Chapter 2). For preferred gill-parasites like *U. crassus* and *M. margaritifera* this could be explained by differences in relative gill surface area of different sized fishes (Pauly, 1981; Rombough and Moroz, 1997). In addition, Österling and Larsen (2013) found a positive correlation between larval growth and condition of hosts. Although under natural conditions host fish size and condition were influenced by the habitat, host size and condition could easily been managed during artificial propagation of endangered mussels. For example the host size could be adapted to the available capacity in the fish holding facility by different feeding and / or temperature regimes during host breeding. The physiological effects on the host-specificity should be considered for standardization of host suitability analyses to allow the comparison of different studies into host-relationships (Taeubert et al., 2013b).

The genetic constitution of the used host fishes, especially the immunogenetic diversity of the MHC (major histocompatibility complex) was not analysed in this study but may play an important role for the individual differences in host-specificity. For example in *S. salar*, the frequency of specific MHC-molecules was correlated with the resistance to *Aeromonas salmonicida* (Langefors et al., 2001).

7. 3. Implications for conservation of large freshwater mussels

Freshwater mussels belong to one of the most imperiled groups of species worldwide (Lydeard et al., 2004; Strayer et al., 2004; Bogan, 2008) and many populations severely declined during the last decades. Despite factors that influence freshwater mussels directly, they are indirectly susceptible to any factors that reduce the abundance and distribution of suitable host fishes. From a conservation perspective, this dependence on other species along with the high habitat requirements make freshwater mussels highly susceptible to environmental changes and put them at a high risk of going extinct (Lydeard et al., 2004; Bogan, 2008; Geist, 2010). Therefore detailed knowledge about suitable host fish species and the variation in suitability between different populations of the same host species are essential for the development of effective and sustainable conservation strategies.

Conservation efforts should be focus mainly on habitat restoration and improvement of environmental conditions for the target species and their interaction partners (host fishes), e. g. by mitigating the deficits caused by anthropogenic activities like the creation of dams, flow modifications, habitat destruction, water pollution and increased fine sediment loads. In addition to restoration measures which are time consuming, cost-intensive and often not immediately successful (especially for species with long generation times like *M.*

margaritifera) there are also some host-specific conservation strategies, which immediately help mussel species in danger of extinction. For endangered mussel species, like *U. crassus* and *M. margaritifera* – two of the most threatened mussel species in Europe – the following host-specific management strategies could be used to improve the conservation measures:

1. Stocking of infested host fishes to restore remnant mussel populations.
2. Artificial breeding and stocking of juvenile mussels.
3. Management of (host) fish stocks in mussel streams.

7. 3. 1. Stocking of infested host fishes to restore remnant mussel populations

Stocking of infested host fishes is a fast and relative cost-extensive conservation measure to restore remnant mussel populations with deficits in the host assemblages or to reintroduce the species to historical mussel rivers. There are two possible scenarios for stocking infested host fishes which can be distinguished by the host origin. In the first scenario, native hosts are caught (e.g. by electro fishing), artificially infested by mussel larvae and directly released back into the stream. This measure is especially valuable when host densities are too low or host distribution does not match the distribution of the remnant mussel population following decreased probability of natural infestation. In the second scenario, captive bred hosts will be infested and stocked, which is useful when suitable hosts are completely missing or host densities are extremely low. For both possibilities, analyses of the current status of host fishes (abundance and dispersal) are needed to decide which measurement has the highest chance of success. If possible, the first scenario is recommended, because catching native host fishes and infecting them with mussel larvae has the advantage that the fishes are well adapted to the stream conditions, resulting in high survival rates of infested hosts (and thus encysted larvae). Compared with the introduction of additional hosts, the infestation of native hosts also excludes an ecological imbalance caused by over-stocking and a potential loss of genetic integrity of native host fish populations due to hybridization with introduced host stocks. If densities of suitable hosts are extremely low, remnant host fish populations can be used for artificial breeding of hosts in captivity. Later, captive bred hosts can be artificially infested and stocked. However, it should be considered that the artificial breeding of host fish can lead to genetic adaptation to captivity within a single generation (Christie et al., 2012), which might have disadvantages for the later performance of stocked fishes in the wild. If suitable hosts are completely missing in the catchment area, stocking of closely related host-populations can bridge a possible host gap (Scenario 2). Despite the significant differences in the host quality of various populations within a host species, no population was considered completely unsuitable as evident from the successful encystment and / or metamorphosis of mussel larvae (Chapter 2 to 4).

In the case of *M. margaritifera* which inhabit fast flowing streams and brooks, infested fish should be able to withstand high velocities and glochidia infestation should not impair their swimming performance. Following Chapter 6, it is recommended to use glochidial loads between 5 and 100 glochidia per g fish weight (350 – 7,000 glochidia per fish with sizes of ~ 18 cm) when infested hosts are being stocked. Such thresholds should also be established for other mussel species and their hosts. Before stocking of infested fishes the environmental conditions (especially the substratum quality) should be evaluated to ensure optimal conditions for the post-parasitic phase of the juvenile mussels.

7. 3. 2. Artificial breeding and stocking of juvenile mussels

Captive propagation of juvenile mussels is increasingly used to conserve rare populations of endangered mussel species (Thomas et al., 2010; Gum et al., 2011). After successful excystment from the hosts, juvenile mussels are collected and pre-cultured in small containers and / or transferred into cages and placed in natural rivers or semi-natural flow channels (Hruška, 1999; 2001; Thomas et al., 2010; Gum et al., 2011; Eybe et al., 2013). Since fecundity of adult mussels is often not a problem, the first critical step in artificial propagation is the parasitic phase of the mussels. To increase the output of juvenile mussels for further propagation or for stocking of freshly excysted juveniles, it is important to provide ideal conditions during the parasitic phase. In a first step, it is recommended to investigate the host-specificity for the available host species (if the mussels can use different hosts like *U. crassus*) and strains. To ensure optimum condition of infested host fishes (host mortality also decreases the amount of juvenile mussels by loosing encysted larvae prior completion of metamorphosis), the effect of environmental conditions like temperature during maintenance of infested fishes should be investigated (Taeubert et al., 2014) in a second step. In order to harvest high yields of juvenile mussels, high numbers of hosts should be infested with intermediate numbers of larvae (see Chapter 6). The use of a high number of hosts accounts for the high variability of individual host-specificity and counterbalances the presence of less suitable host individuals. The results of Chapter 6 suggest that for *M. margaritifera* moderate infestation of up to 300 glochidia per g fish weight had only marginal effects on the host fish maintained in captivity. In addition, Thomas et al. (2013) found that glochidia infestation may impose a respiratory burden to *S. trutta* and that these effects appear to be additive. Therefore, the authors dissuade from using high glochidial loads during mussel propagation programmes. Concerning environmental conditions during parasitic phase of *M. margaritifera*, infested fish can be maintained in groundwater fed hatcheries (such hatcheries most often reveal constant water temperatures below 15 °C) as proposed in Chapter 5. This approach reduces the risk of losing infested fishes by fish diseases which are regularly introduced if warm brook water is used. Following Chapter 5,

the absence of a previously postulated threshold temperature of ≥ 15 °C for successful excystment of living juveniles *M. margaritifera* clearly shows that a reintroduction of the species into cool headwaters is not limited by the temperature requirements during their parasitic phase.

7. 3. 3. Management of (host) fish stocks in mussel streams

Although a successful recruitment of freshwater mussels is not assured by high host fish densities alone, the presence of suitable hosts is an important prerequisite for sustainable recruitment of a mussel population (Chapter 2 – 6). An increase in host fish density is likely to facilitate mussel recruitment, especially in rivers where only few sites provide suitable substratum conditions for the development of juvenile mussels. For example, in *M. margaritifera* streams with ongoing recruitment, juvenile mussel density was positively related to the number of glochidia infections per stream area (Österling et al., 2008). The first prerequisite in the management of host fish stocks is the exact knowledge about host-specificity of sympatric fish species. Before fishes are introduced to streams with persisting mussel populations, it is important to study the host-quality of the used strain to ensure host-compatibility. In order to provide ideal conditions for a sustainable host recruitment, management and conservation of host fish stocks should primarily focus on stream restoration measures such as the revitalization of migration barriers, reducing fine sediment loads and improving habitat structure.

For *M. margaritifera*, two fish species (*Salmo trutta* and *Salmo salar*) with high economic importance are considered as the most suitable hosts. Therefore fishermen and anglers are also interested in the conservation of these host species and often actively supporting them by stocking and improvement of their habitats (creation of spawning grounds etc.). In contrast, the species identified as the most suitable host fishes for *U. crassus* - *Squalius cephalus, Phoxinus phoxinus, Scardinius erythrophthalmus, Gasterosteus aculeatus, Chondrostoma nasus,* and *Cottus gobio* - are not among the highly valued species in fisheries management and have occasionally even been actively removed from streams by electrofishing (e.g. *Squalius cephalus*) in order to reduce competition with species of higher value such as *S. trutta*. The results of Chapter 2 and 3 clearly show that these fish species should be actively supported in *U. crassus* catchments.

7. 4. Outlook

Since detailed knowledge on the host-specificity of several central European mussel species is still rare, future research should focus on the host-use of species like *Unio tumidus, Unio pictorum, Anodonta anatina, Anodonta cygnea* and *Pseudanodonta complanata*. An evaluation of the interaction between freshwater mussels and their hosts requires both standardized laboratory investigations, as well as analyses under natural conditions which account for the host and mussel behaviour and their distribution patterns (Chapter 3). During standardized laboratory experiments, adequate individual replicates of tested host species should be infested and maintained under identical conditions (Taeubert et al., 2013b). It is also recommended to include local subpopulations from different drainages into these analyses to resolve the complexity of the host relationships (Taeubert et al., 2013b). For analyses under natural conditions, the host suitability should be assessed by quantifiying excysted mussels from naturally infested fishes (Chapter 3) to test if the mussels utilize their different host species equally or if one host species is preferred over another.

In addition to the metamorphosis success, which is a common endpoint in suitability analyses, the post-parasitic growth and survival of excysted juveniles are probably the most important factors for the recruitment success of a mussel species. Therefore future studies into mussel-fish relationships should also consider the effects of host-specificity on the growth and survival of post-parasitic juveniles.

Additional information on factors governing host suitability will improve the understanding of host-parasite interactions and factors determining host-specificity. For example, gene transcription patterns of hosts infested with mussel larvae might be ideal to identify the factors which are responsible for the differences in host-suitability at different levels.

The highly different infestation / metamorphosis rates of the *P. phoxinus* population during different experiments (Chapter 2 and 3) indicate that glochidia viability might substantially influence the attachment and the metamorphosis success of mussel larvae. Therefore it is important to assess the factors (time point of release, water temperature, water chemistry, etc.) which influence glochidia viability. Knowledge on factors that govern the viability of glochidia may help to increase the success of artificial infestation of hosts during captive breeding programmes. Furthermore, information about glochidia viability is also relevant for host-specificity investigations. The state of larvae used for host analyses should be standardized to allow a comparison between experiments of different studies.

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9. Publication list

9. 1. Peer reviewed publications included in this thesis:

Taeubert JE, Geist J. 2013. Critical swimming speed of brown trout (Salmo trutta) infested with freshwater pearl mussel (*Margaritifera margaritifera*) glochidia and implications for artificial breeding of an endangered mussel species. *Parasitology Research* 112(4): 1607–1613.

Taeubert JE, Denic M, Gum B, Lange M, Geist J. 2010. Suitability of different salmonid strains as hosts for the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 728–734.

Taeubert JE, Gum B, Geist J. 2012. Host–specificity of the endangered thick–shelled river mussel (*Unio crassus,* Philipsson 1788) and implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 22: 36–46.

Taeubert JE, Posada Martinez AM, Gum B, Geist J. 2012. The relationship between endangered thick–shelled river mussel (*Unio crassus*) and its host fishes. *Biological Conservation* 155: 94–103.

Taeubert JE, Gum B, Geist J. 2013. Variable development and excystment of freshwater pearl mussel (*Margaritifera margaritifera* L.) at constant temperature. *Limnologica-Ecology and Management of Inland Waters* 43(4): 319–322.

9. 2. Peer reviewed publications not included in this thesis:

Jablonowski D, **Täubert JE**, Bär C, Stark MJR, Schaffrath R. 2009. Distinct subsets of Sit4 holo-phosphatases are required for inhibition of yeast growth by rapamycin and zymocin. *Eukaryotic Cell* 8: 1637–1647.

Taeubert JE, El-Nobi G, Geist J. 2014. Effects of water temperature on the larval parasitic stage of the thick-shelled river mussel (*Unio crassus*). *Aquatic Conservation: Marine and Freshwater Ecosystems*. 24: 231–237.

Taeubert JE, Gum B, Geist J. 2013. Towards standardization of studies into host relationships of freshwater mussels. *Biological Conservation*.

Stoeckl K, **Taeubert JE**, Geist J. 2014. Fish species composition and host fish density in streams of the thick-shelled river mussel (*Unio crassus*) - implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*. DOI: 10.1002/aqc.2470

9. 3. Oral contributions related to this thesis:

Taeubert JE, Geist J. 2010. Training on species determination. *Slovak-German Expert Workshop on Methodological competence of experts on river / species assessment (according to European WFD)*. Banská Bystrica, Slovakia, February 2010

Taeubert JE, Gum B, Geist J. 2011. Eignung verschiedener Fischarten als Wirt für die Bachmuschel (*Unio crassus*). *1. Münchner Angler Club*. Munich, Germany, May 2011

Taeubert JE, Geist J. 2011. Host-specificity of the endangered thick-shelled river mussel (*Unio crassus*). *BaCaTec International Summer School „Life Sciences in the 21st Century with a Focus on Water“*. Freising, Germany, July 2011

Taeubert JE, Gum B, Geist J. 2011. Eignung verschiedener Fischarten als Wirt für die Bachmuschel (*Unio crassus*). *Jahrestagung der Deutschen Gesellschaft für Limnologie*. Freising, Germany, September 2011

Taeubert JE. 2012. Gewässermanagement und Fischbesatz. *Kreisfischereiverein Freising*. Freising, Germany, April 2012

Taeubert JE, Geist J. 2012. The relationship between endangered thick-shelled river mussel (*Unio crassus*) and its host fishes. *International Meeting on Biology and Conservation of Freshwater Bivalves*. Bragança, Portugal, September 2012

Taeubert JE. 2013. Die Bachmuschel (*Unio crassus*), ihre Wirtsfische und deren Management. Bachmuschelaktionstag im Haus im Moos. Karlshuld, Germany, June 2013

Taeubert JE. 2014. Fischereiliche Hege in Muschelgewässern. *Muschelschutz im Spannungsfeld zwischen Gewässernutzung und Klimawandel – Herausforderungen in der Naturschutzpraxis.* Freising, Germany, March 2014

10. References

Ansteeg O. 2006. Erfassung des Muschelbestandes (Bachmuschel: *Unio crassus*) im Mooshamer Weiherbach (Ausfluß des Mooshamer Weihers), Gemeinde Egling - Moosham, Landkreis Bad Tölz-Wolfratshausen. *Gutachten im Auftrag des Bayerischen Landesamtes für Umwelt*, Augsburg, Germany.

Barnhart MC, Haag WR, Roston WN. 2008. Adaptations to host infection and larval parasitism in Unionoida. *Journal of the North American Benthological Society* 27: 370–394.

Bauer G. 1979. Studies on the reproduction biology of the Freshwater Pearl Mussel (*Margaritina margaritifera*) in the Fichtelbirge. *Archiv für Hydrobiologie* 85: 152–165.

Bauer G. 1987a. The parasitic stage of the freshwater pearl mussel (Margaritifera margaritifera L.) III. Host relationship. *Archiv für Hydrobiologie* 76: 413–423.

Bauer G. 1987b. Reproductive strategy of the freshwater pearl mussel *Margaritifera Margaritifera*. *Journal of Animal Ecology* 56: 691–704.

Bauer G. 1987c. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) II. Susceptibility of brown trout. *Archiv für Hydrobiologie* 76: 403–412.

Bauer G. 1991. Plasticity in Life History Traits of the Freshwater Pearl Mussel - Consequences for the Danger of Extinction and for Conservation Measures. *In* A. Seitz and V. Loeschke (eds), *Species Conservation: A Population-Biological Approach.* Birkhäuser Verlag, Basel: 103–120.

Bauer G. 1992. Variation in the life span and size of the freshwater pearl mussel. *Journal of Animal Ecology* 61: 425–436.

Bauer G. 1994. The adaptive value of offspring size among freshwater mussels (Bivalvia; Unionoidea). *Journal of Animal Ecology* 63: 933–944.

Bauer G. 1997. Host relationships at reversed generation times: Margaritifera (Bivalvia) and salmonids. *Ecological Studies* 130: 69–79.

Bauer G, Vogel C. 1987. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) I. Host response to Glochidiosis. *Archiv für Hydrobiologie* 76: 393–402.

Bauer G, Hochwald S, Silkenat W. 1991. Spatial distribution of freshwater mussels: the role of host fish and metabolic rate. *Freshwater Biology* 26: 377–386.

Bauer G, Wächtler K, (eds). 2001. Ecology and Evolution of the Freshwater Mussels Unionoida. *Ecological Studies 145*. Springer Verlag, Berlin-Heidelberg, Germany.

Beasley CR. 1996. The distribution and ecology of the freshwater pearl mussel, *Margaritifera margaritifera* L. 1758, in county Donegal, Ireland and implications for its conservation. Unpublished PhD thesis, Queen's University, Belfast, Northern Ireland.

Bednarczuk J. 1986. Untersuchungen zu Wirtsfischspektrum und Entwicklung der Bachmuschel *Unio crassus*. PhD thesis, Tierärztliche Hochschule Hannover, Germany.

Bell WH, Terhune LDB. 1970. Water tunnel design for fisheries research. *Fisheries Research Board of Canada Technical Report No. 195*, Nanaimo, British Columbia.

Blažka P, Volf M, Cepela M. 1960. A new type of respirometer for the determination of the metabolism of fish in an active state. *Physiologia Bohemoslovenica* 9: 553–560.

Bogan AE. 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): A search for causes. *American Zoologist* 33: 599–609.

Bogan AE. 2008. Global diversity of freshwater mussels (Mollusca: Bivalvia) in freshwater. *Hydrobiologia* 595: 139-147.

Bohl E, Kleisinger H, Leuner E. 2003. Rote Liste gefährdeter Fische (Pisces) und Rundmäuler (Cyclostomata) Bayerns. In: *Rote Liste gefährdeter Tiere Bayerns* Bayerisches Landesamt für Umweltschutz 166: 52–55.

Bouchet P, Falkner G, Seddon M. 1999. Lists of protected land and freshwater molluscs in the Bern Convention and European Habitats Directive: are they relevant to conservation? *Biological Conservation* 90: 21–31.

Brett JR. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board Canada* 21: 1183–1225.

Buddensiek V. 1995. The culture of juvenile fresh-water pearl mussels *Margaritifera margaritifera* L. in cages – a contribution to conservation programs and the knowledge of habitat requirements. *Biological Conservation* 74: 33–40.

Christie MR, Marine ML, French RA, Blouin MS. 2012. Genetic adaptation to captivity can occur in a single generation. *Proceedings of the National Academy of Sciences* 109(1): 238–242.

Costello MJ, Moorkens EA, Larkin M, Kurz I, Dowse J. 1998. Management priorities for the river Nore (Ireland) to conserve the pearl mussel *Margaritifera durrovensis* Phillips, 1928. *Journal of Conchology Special Publication* 2: 257–264.

Council of the European Communities. 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. *Official Journal of the European Communities* L206: 7–50.

Dodd BD, Barnhart MC, Rogers-Lowery CL, Fobian T, Dimock RV. 2005. Cross-resistance of largemouth bass (*Micropterus salmoides*) to glochidia of different species of unionid mussels. *The Journal of Parasitology* 91: 1064–1073.

Douda K, Horký P, Bílý M. 2012. Host limitation of the thick-shelled river mussel: identifying the threats to declining affiliate species. *Animal Conservation* 15: 536-544.

Engel H. 1990. Untersuchungen zur Autökologie von *Unio crassus* (Philipsson) in Norddeutschland. PhD thesis, Tierärztliche Hochschule Hannover, Germany.

Engel H, Wächtler K. 1989. Some peculiarities in developmental biology of two forms of freshwater bivalve *Unio crassus* in northern Germany. *Archiv für Hydrobiologie* 115: 441–450.

Eybe T, Thielen F, Bohn T, Sures B. 2013. The first millimetre – rearing juvenile freshwater pearl mussels (*Margaritifera margaritifera* L.) in plastic boxes. *Aquatic Conservation: Marine and Freshwater Ecosystems* 23: 964–975.

Fritts MW, Fritts AK, Carleton SA, Bringolf RB. 2013. Shifts in stable-isotope signatures confirm parasitic relationship of freshwater mussel glochidia attached to host fish. *Journal of Molluscan Studies* 79(2): 163–167.

Fustish CA, Millemann RE. 1978. Glochidiosis of salmonid fishes. II. Comparison of tissue response of coho and chinook salmon to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae). *American Society of Parasitologists* 64: 155–157.

Galbraith HS, Spooner DE, Vaughn CC. 2010. Synergistic effects of regional climate patterns and local water management on freshwater mussel communities. *Biological Conservation* 143: 1175–1183.

Geist J. 2010. Strategies for the conservation of endangered freshwater pearl mussel (*Margaritifera margaritifera* L.): a synthesis of Conservation Genetics and Ecology *Hydrobiologia* 664: 69–88.

Geist J. 2011. Integrative freshwater ecology and biodiversity conservation. *Ecological Indicators* 11: 1507–1516.

Geist J, Kuehn R. 2005. Genetic diversity and differentiation of central European freshwater pearl mussel (*Margaritifera margaritifera* L.) populations: implications for conservation and management. *Molecular Ecology* 14: 425–439.

Geist J, Porkka M, Kuehn R. 2006. The status of host fish populations and fish species richness in European freshwater pearl mussel (*Margaritifera margaritifera*) streams. *Aquatic Conservation: Marine and Freshwater Ecosystems* 16: 251–266.

Geist J, Auerswald K. 2007. Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (*Margaritifera margaritifera*). *Freshwater Biology* 52: 2299–2316.

Geist J, Kuehn R. 2008. Host-parasite interactions in oligotrophic stream ecosystems: the roles of life-history strategy and ecologial niche. *Molecular Ecology* 17: 997–1008.

Geist J, Kohlasa M, Gum B, Kuehn R. 2009. The importance of genetic cluster recognition for the conservation of migratory fish species: the example of the endangered European huchen *Hucho hucho* (L.). *Journal of Fish Biology* 75: 1063–1078.

Geist J, Söderberg H, Karlberg A, Kuehn R. 2010. Drainage-independent genetic structure and high genetic diversity of endangered freshwater pearl mussels (*Margaritifera margaritifera*) in northern Europe. *Conservation Genetics* 11: 1339–1150.

Graf DL, Cummings KS. 2006. Palaeoheterodont diversity (Mollusca: Trigonioida + Unionoida): what we know and what we wish we knew about freshwater mussel evolution. *Zoological Journal of the Linnean Society* 148: 343–394.

Graf DL, Cummings KS. 2007. Review of the systematics and global diversity of 451 freshwater mussel species (Bivalvia: Unionoida). *Journal of Molluscan Studies* 73: 291–314.

Gum B, Lange M, Geist J. 2011. A critical reflection on the success of rearing and culturing juvenile freshwater mussels with a focus on the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems* 21: 743–751.

Haag WR, Buttler RS, Hartfield PW. 1995. An extraordinary reproductive strategy in freshwater bivalves: prey mimicry to facilitate larval dispersal. *Freshwater Biology* 34(3): 471–476.

Haag WR, Staton JL. 2003. Variation in fecundity and other reproductive traits in freshwater mussels. *Freshwater Biology* 48(12): 2118–2130.

Hastie LC, Boon PJ, Young MR. 2000. Physical microhabitat requirements of freshwater pearl mussels, *Margaritifera margaritifera* (L.). *Hydrobiologia* 429: 59–71.

Hastie L, Cosgrove P. 2001. The decline of migratory salmonid stocks: a new threat to pearl mussels in Scotland. In *Freshwater Forum* 15: 85–96.

Hastie LC, Young MR. 2001. Freshwater pearl mussel (*Margaritifera margaritifera*) glochidiosis in wild and farmed salmonid stocks in Scotland. *Hydrobiologia* 445: 109–119.

Hastie LC, Young MR. 2003a. Timing of spawning and glochidial release in Scottish freshwater pearl mussel (*Margaritifera margaritifera*) populations. *Freshwater Biology* 48: 2107–2117.

Hastie LC, Young MR. 2003b. Conservation of the Freshwater Pearl Mussel I: Captive Breeding Techniques. *Conserving Natura 2000 Rivers* Conservation Techniques Series No. 2. English Nature, Peterborough.

Hastie LC, Cosgrove PJ, Ellis N, Gaywood MJ. 2003. The threat of climate change to freshwater pearl mussel populations. *Ambio* 32(1): 40–46.

Hochwald S. 1997. Das Beziehungsgefüge innerhalb der Größenwachstums- und Fortpflanzungsparameter bayerischer Bachmuschelpopulationen (*Unio crassus* Phil. 1788) und dessen Abhängigkeit von Umweltparametern. PhD thesis, Universität Bayreuth, Germany.

Hochwald S. 2001. Plasticity of life-history traits in *Unio crassus*. *In* G. Bauer and K. Wächtler (eds), Ecology and Evolution of the Freshwater Mussels Unionoida. *Ecological Studies* 145. Springer Verlag, Berlin-Heidelberg: 127-141.

Hochwald S. 2008. Kartierung ausgewählter Bestände der Bachmuschel (*Unio crassus*) in Bayern, *unveröffentlichtes Gutachten im Auftrag des Bayerischen Landesamtes für Umwelt*, Augsburg, Germany.

Hochwald S, Bauer G. 1990. Untersuchungen zur Populationsökologie und Fortpflanzungsbiologie der Bachmuschel *Unio crassus* (PHIL.) 1788. *Schriftenreihe Bayerisches Landesamt für Umweltschutz Heft* 97: 31–49.

Hochwald S, Ansteeg O. 2009. Kartierung ausgewählter Bestände der Bachmuschel (*Unio crassus*) in Bayern, *unveröffentlichtes Gutachten im Auftrag des Bayerischen Landesamtes für Umwelt*, Augsburg, Germany.

Hruška J. 1992. The freshwater pearl mussel in South Bohemia: evaluation of the effect of temperature on reproduction, growth and age structure of the population*. Archiv für Hydrobiologie* 126: 181–191.

Hruška J. 1999. Nahrungsansprüche der Flussperlmuschel und deren halbnatürliche Aufzucht in der Tschechischen Republik*. Heldia* 4(6): 69–79.

Hruška J. 2001. Experience of semi-natural breeding program of freshwater pearl mussel in the Czech Republic*. Die Flussperlmuschel in Europa: Bestandssituation und Schutzmaßnahmen. Kongressband. WWA Hof, Albert-Ludwigs Universität: Freiburg*, 69–75.

Israel W. 1910. Die Najaden des Weidagebietes. Beiträge zur Kenntnis der mitteleuropäischen Najaden. *Beilage zum Nachrichts-Blatt der Deutschen Malakozooloischen Gesellschaft* 4: 49–56.

Israel W. 1913. Biologie der europäischen Süßwassermuscheln. K.G. Lutz Verlag, Stuttgart: 44–47.

Jäckel SH. 1962. Ergänzungen und Berichtigungen zum rezenten und quartären Vorkommen der mitteleuropäischen Mollusken. In: *Die Tierwelt Mitteleuropas 2*, P. Brohmer, P. Ehrmann and G Ulmer (eds), Quelle and Meyer, Leipzig: 206–207.

Jones JW, Neves RJ. 2011. Influence of life-history variation on demographic responses of three freshwater mussel species (Bivalvia: Unionidae) in the Clinch River, USA. *Aquatic Conservation: Marine and Freshwater Ecosystems* 21(1): 57–73.

Jung M, Scheder C, Gumpinger C, Waringer J. 2013. Habitat traits, population structure and host specificity of the freshwater pearl mussel *Margaritifera margaritifera* in the Waldaist River (Upper Austria). *Biologia* 68(5): 922–931.

Karlsson S, Larsen BM, Hindar K. 2013. Host-dependent genetic variation in freshwater pearl mussel (*Margaritifera margaritifera* L.). *Hydrobiologia* 1–12. DOI 10.1007/s10750-013-1679-2

Karna DW, Millemann RE. 1978. Glochidiossis of salmonid fishes. III. Comparative susceptibility to natural infections with *Margaritifera margaritifera* and asssociated histopathology. *Journal of Parasitology* 64: 528–537.

Khym JR, Layzer JB. 2000. Host fish suitability for glochidia of *Ligumia recta*. *The American Midland Naturalist* 143(1): 178–184.

Kirk SG, Layzer JB. 1997. Induced metamorphosis of freshwater mussel glochidia on nonhost fish. *Nautilus* 110(3): 102–106.

Langefors Å, Lohm J, Grahn M, Andersen Ø, von Schantz T. 2001. Association between major histocompatibility complex class IIB alleles and resistance to Aeromonas salmonicida in Atlantic salmon. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268: 479–485.

Lasiak T, Dye A. 1989. The ecology of the brown mussel *Perna perna* in Transkei, Southern Africa: Implications for the management of a traditional food resource. *Biological Conservation* 47(4): 245–257.

Lima P, Lima ML, Kovitvadhi U, Kovitvadhi S, Owen C, Machado J. 2012. A review on the “in vitro” culture of freshwater mussels (Unionoida). *Hydrobiologia* 691(1): 21–33.

Lòpez MA, Altaba CR, Rouault T, Gisbert E. 2007. The European sturgeon *Acipenser sturio* is a suitable host for the glochidia of the freshwater pearl mussel *Margaritifera auricularia*. *Journal of Molluscan Studies* 73: 207–209.

Lydeard C, Cowie RH, Ponder WF, Bogan, AE, Bouchet P, Clark SA, Cummings KS, Frest TJ, Gargominy O, Herbert DG, Hershler R, Perez KE, Roth B, Seddon M, Strong EE, Thompson FG. 2004. The global decline of nonmarine molluscs. *Bioscience* 54: 321–329.

Maaß S. 1987. Untersuchungen zur Fortpflanzungsbiologie einheimischer Süßwassermuscheln der Gattung Unio. PhD thesis, Tierärztliche Hochschule Hannover, Germany.

McIvor A, Aldridge D. 2008. The cultivation of the freshwater pearl mussel, *Margaritifera margaritifera*. CCW Contract Science Report No: 849, Countryside Council for Wales / Environment Agency, Bangor.

McNichols KA, Mackie GL, Ackerman JD. 2011. Host fish quality may explain the status of endangered *Epioblasma torulosa rangiana* and *Lampsilis fasciola* (Bivalvia: Unionidae) in Canada. *Journal of the North American Benthological Society* 30(1): 60–70.

Meyers TR, Millemann RE. 1977. Glochidiosis of salmonid fishes. I. Comparative susceptibility to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae)*. *The Journal of Parasitology* 63: 728–733.

Mollusc Specialist Group. 1996. *Margaritifera margaritifera*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 12 February 2014.

Moorkens, E.A., Costello, M.J., 2004. Survival study of the freshwater pearl mussel *Margaritifera margaritifera* after opening with mussel tongs. *Journal of Conchology Special Publication* 3: 91–94.

Mutvei H, Westermark T. 2001. How environmental information can be obtained from naiad shells. *In* G. Bauer and K. Wächtler (eds), Ecology and Evolution of the Freshwater Mussels Unionoida. *Ecological Studies* 145. Springer Verlag, Berlin-Heidelberg: 367–379.

Nelson JA, Gotwalt PS, Reidy SP, Webber DM. 2002. Beyond Ucrit: matching swimming performance tests to the physiological ecology of the animal, including a new fish drag strip. *Comparative Biochemistry and Physiology A* 133: 289–302.

Nelson JA, Gotwalt PS, Snodgrass JW. 2003. Swimming performance of blacknose dace (*Rhinichthys atratulus*) mirrors home–stream current velocity. *Canadian Journal of Fisheries and Aquatic Sciences* 60: 301–308.

Nezlin LP*,* Cunjak RA*,* Zotin AA*,* Ziuganov VV. 1994. Glochidium morphology of the freshwater pearl mussel (*Margaritifera margaritifera*) and glochidiosis of the Atlantic *salmon* (*Salmo salar*)*: a study by scanning electron microscopy. Canadian Journal of Zoology* 72*:* 15–21.

Ondračková M, Dávidová M, Pečínková M, Blažek R, Gelnar M, Valová Z, Černý J, Jurajda P. 2005. Metazoan parasites of *Neogobius* fishes in the Slovak section of the River Danube. *Journal of Applied Ichthyology* 21: 345–349.

Österling ME, Greenberg LA, Arvidsson BL. 2008. Relationship of biotic and abiotic factors to recruitment patterns in *Margaritifera margaritifera. Biological Conservation* 141: 1365–1370.

Österling ME, Arvidsson BL, Greenberg LA. 2010. Habitat degradation and the decline of the threatened mussel *Margaritifera margaritifera*: influence of turbidity and sedimentation on the mussel and its host. *Journal of Applied Ecology* 47: 759–768.

Österling ME, Larsen BM. 2013. Impact of origin and condition of host fish (*Salmo trutta*) on parasitic larvae of *Margaritifera margaritifera*. *Aquatic Conservation: Marine and Freshwater Ecosystems* 25: 564–570.

Pauly D. 1981. The relationships between gill surface area and growth performance in fish: a generalization of von Bertalanffy`s theory of growth. *Berichte der Deutschen Wissenschaftlichen Kommission für Meeresforschung* 28: 251–282.

Plaut I. 2001. Critical swimming speed: its ecological relevance. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 131(1): 41–50.

Preston SJ, Keys A, Roberts D. 2007. Culturing freshwater pearl mussel *Margaritifera margaritifera*: a breakthrough in the conservation of an endangered species. *Aquatic Conservation: Marine and Freshwater Ecosystems* 17: 539–549.

Ricciardi A, Neves RJ, Rasmussen JB. 1998. Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion. *Journal of Animal Ecology* 67(4): 613–619.

Richardson WB, Bartsch LA. 1997. Effects of zebra mussels on food webs: interactions with juvenile bluegill and water residence time. *Hydrobiologia* 354: 141–150.

Roberts AD, Barnhart MC. 1999. Effects of temperature, pH, and CO_2 on the transformation of glochidia of *Anodonta suborbiculata* on fish hosts and invitro. *Journal of the North American Benthological Society* 18: 477–487.

Rogers–Lowery CL, Dimock RV. 2006. Encapsulation of attached ectoparasitic glochidia larvae of freshwater mussels by epithelial tissue on fins of naive and resistant host fish. *Biological Bulletin* 210: 51–63.

Rombough PJ, Moroz BM. 1997. The scaling and potential importance of cutaneous and branchial surfaces in respiratory gas exchange in larval and juvenile walleye *Stizostedion vitreum*. *The Journal of Experimental Biology* 200: 2459–2468.

Santos HDE, Pompeu PD, Martinez CB. 2007. Swimming performance of the migratory neotropical fish *Leporninus reinhardti* (Characiformes: Anostomidae). *Neotropical Ichthyology* 5: 139–146.

Schmidt C, Vandré R. 2010. Ten years of experience in the rearing of young freshwater pearl mussels (*Margaritifera margaritifera*). *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 735–747.

Schneider H. 2012. Habitatcharakterisierung funktionaler Bachmuschelvorkommen (*Unio crassus*) in Fließgewässern Bayerns. Master thesis, Technische Universität München, Germany.

Schwartz ML, Dimock RV. 2001. Ultrastructural evidence for nutritional exchange between brooding unionid mussels and their glochidia larvae. *Invertebrate Biology* 120: 227–236.

Skinner A, Young M, Hastie L. 2003. Ecology of the Freshwater Pearl Mussel. *Conserving Natura 2000 Rivers* Ecology Series No. 2 English Nature, Peterborough.

Smith DG. 1976. Notes on the biology of *Margaritifera margaritifera* (Lin.) in Central Massachusetts. *The American Midland Naturalist* 96: 252–256.

Stoeckl K, Taeubert JE, Geist J. 2014. Fish species composition and host fish density in streams of the thick-shelled river mussel (*Unio crassus*) - implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*. DOI: 10.1002/aqc.2470

Strayer DL, Caraco NF, Cole JJ, Findlay S, Pace ML. 1999. Transformation of freshwater ecosystems by bivalves. *BioScience* 49(1): 19–27.

Strayer DL, Downing JA, Haag WR, King TL, Layzer JB, Newton TJ, Nichols SJ. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience* 54(5): 429–439.

Taeubert JE, Geist J. 2013. Critical swimming speed of brown trout (*Salmo trutta*) infested with freshwater pearl mussel (*Margaritifera margaritifera*) glochidia and implications

for artificial breeding of an endangered mussel species. *Parasitology Research* 112(4): 1607–1613.

Taeubert JE, Denic M, Gum B, Lange M, Geist J. 2010. Suitability of different salmonid strains as hosts for the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 728–734.

Taeubert JE, Gum B, Geist J. 2012a. Host–specificity of the endangered thick–shelled river mussel (*Unio crassus,* Philipsson 1788) and implications for conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 22: 36–46.

Taeubert JE, Posada Martinez AM, Gum B, Geist J. 2012b. The relationship between endangered thick–shelled river mussel (*Unio crassus*) and its host fishes. *Biological Conservation* 155: 94–103.

Taeubert JE, Gum B, Geist J. 2013a. Variable development and excystment of freshwater pearl mussel (*Margaritifera margaritifera* L.) at constant temperature. *Limnologica-Ecology and Management of Inland Waters* 43(4): 319–322.

Taeubert JE, Gum B, Geist J. 2013b. Towards standardization of studies into host relationships of freshwater mussels. *Biological Conservation*.

Taeubert JE, El-Nobi G, Geist J. 2014. Effects of water temperature on the larval parasitic stage of the thick-shelled river mussel (*Unio crassus*). *Aquatic Conservation: Marine and Freshwater Ecosystems* 24: 231–237.

Tankersley RA, Dimock RV. 1993. Endoscopic visualization of the functional morphology of the ctenidia of the unionid mussel *Pyganodon cataracta. Canadian Journal of Zoology* 71: 811–819.

Thomas GR, Taylor J, Garcia de Leaniz C. 2010. Captive breeding of the endangered freshwater pearl mussel *Margaritifera margaritifera*. *Endangered Species Research* 12: 1–9.

Thomas GR, Taylor J, Garcia de Leaniz C. 2013. Does the parasitic freshwater pearl mussel *M. margaritifera* harm its host? *Hydrobiologia* 1–11. DOI 10.1007/s10750-013-1515-8

Timm H. 1994. Big clams of the Estonian freshwaters: comparison of the age, shell length, and shell weight indifferent species and populations. *Proceedings of the Estonian Academy of Sciences: Biology* 43: 149–159.

Treasurer JW, Hastie LC, Hunter D, MacLean C, Duncan F, Treasurer CM. 2006. Effects of (*Margaritifera margaritifera*) glochidial infection on performance of tank–reared Atlantic salmon (*Salmo salar*). *Aquaculture* 256: 74–79.

Tudorancea C, Gruia L. 1968. Observations on the *Unio crassus* Philipsson population from the Nera River. *Travaux du Muséum d'Histoire Naturelle „Grigore Antipa"* 8: 381–394.

Van Damme D. 2011. *Unio crassus*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 12 February 2014.

Vaughn CC, Hakenkamp CC. 2001. The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology* 46(11): 1431–1446.

Vaughn CC, Nichols SJ, Spooner DE. 2008. Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society* 27(2): 409–423.

VDFF. 2000. Fischereiliche Untersuchungsmethoden in Fließgewässern. *Schriftenreihe des Verbandes Deutscher Fischereiverwaltungsbeamter und Fischereiwissenschaftler e.V.* 13: 1–52.

Vicentini H. 2005. Unusual spurting behaviour of the freshwater mussel *Unio crassus. Journal of Molluscan Studies* 71: 409–410.

Wächtler K, Dreher-Mansur MC, Richter T. 2001. Larval Types and Early Postlarval Biology in Naiads (Unionoida). *In* G. Bauer and K. Wächtler (eds), Ecology and Evolution of the Freshwater Mussels Unionoida. *Ecological Studies 145*. Springer Verlag, Berlin-Heidelberg: 93–125.

Watters GT, O'Dee SH. 1999. Glochidia of the freshwater mussel *Lampsilis* overwintering on fish hosts. *Journal of Molluscan Studies* 65: 453–459.

Young MR. 1991. Conserving the freshwater pearl mussel (*Margaritifera margaritifera* L.) in the British Isles and continental Europe. *Aquatic conservation: Marine and Freshwater Ecosystems* 1: 73–77.

Young MR, Williams J. 1984a. The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* (Linn.) in Scotland - II. Laboratory studies. *Archiv für Hydrobiologie* 100: 29–43.

Young MR, Williams J. 1984b. The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* (Linn.) in Scotland - I. Field studies. *Archiv für Hydrobiologie* 99: 405–422.

Zettler ML, Jueg U. 2007: The situation of the freshwater mussel *Unio crassus* (PHILIPSSON, 1788) in northeast Germany and its monitoring in terms of the EC Habitats Directive. *Mollusca* 25(2): 165–174.

Zimmerman LL, Neves RJ. 2002. Effects of temperature on duration of viability for glochidia of freshwater mussels (Bivalvia: Unionidae). *American Malacological Bulletin* 17(1/2): 31–36.

Ziuganov VV. 2005. A long–lived parasite extending the host life span: the pearl mussel *Margaritifera margaritifera* elongates host life by turns out the program of accelerated senescence in salmon *Salmo salar. Doklady Biological Sciences* 403: 291–294.

Ziuganov VV, Nezlin LP. 1988. Evolutionary aspects of the symbiosis of pearl mussels and salmonid fishes. In The problems of Macroevolution. Moscov. *Nauka*: 110–111.

Ziuganov V, Zotin A, Nezlin L, Tretiakov V. 1994. The freshwater pearl mussels and their relationship with salmonid fish. *VNIRO* 3: 3–104.

Ziuganov V, Miguel ES, Neves RJ, Longa A, Fernandez C, Amaro R, Beletsky V, Popkovitch E, Kaliuzhin S, Johnson T. 2000. Life span variation of the freshwater pearl shell: a model species for testing longevity mechanisms in animals. *AMBIO: A Journal of the Human Environment* 29(2): 102–105.

Zwanziger G. 1920. Über die Verbreitung der Najaden im Gebiet der sächsischen Saale bei Hof. *Archiv für Molluskenkunde* 52: 14–33.