PARASITE HOST INTERACTION BETWEEN THE FRESHWATER PEARL MUSSEL (*MARGARITIFERA MARGARITIFERA*) AND BROWN TROUT (*SALMO TRUTTA*) – THE IMPACT FROM GLOCHIDIA LARVAE ON THE HOST

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Parasite host interaction between the freshwater pearl mussel (*Margaritifera margaritifera*) and brown trout (*Salmo trutta*) – the impact from glochidia larvae on the host

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Abstract

Parasites can modulate the physiology and behavior of the hosts to enhance their chances to complete their life cycle. The numerous freshwater bivalves of the order Unionoida all have a parasitic larval stage, known as glochidia, parasitizing fish hosts. The freshwater pearl mussel *Margaritifera margaritifera* is a host specialist and their glochidia can only metamorphose on salmonid fish (Salmonidae), and in Europe the glochidia has only been shown to develop into juvenile mussels on Atlantic salmon *Salmo salar* and brown trout *S. trutta.* In this thesis, the interaction between the parasitic freshwater pearl mussel and its salmonid host, the brown trout have been studied in five papers. The overall aim was both to investigate if host behavior can increase the risk of being infected by glochidia (**paper I**), to what extent glochidia infection alters the behavior and physiology of the host (**paper II, III** and **IV**) and finally how infection in a natural stream correlates with movement patterns, growth, and habitat use (**paper V**). My results show that more active trout had an increased risk of being infected, competitive ability decreased with elevated infection intensity, prey handling time were longer for infected fish and growth rate was lower in infected fish. Standard and maximum metabolic rate as well as levels of hematocrit was elevated in infected fish compared to non-infected fish. In a natural stream infected fish was smaller than non-infected fish, utilized different habitats, and covered larger areas than non-infected fish suggesting that they are inferior competitor and not able to defend a territory. In summary, all the results suggest that the glochidia infection is a burden to the fish host that will prevent the individual to compete for resources on equal terms as non-infected fish. These patterns have also been seen in previous studies performed in laboratory with hatchery reared and artificially infected fish. The glochidia is a potent parasite, and at high numbers, they cause a disease state to its host. The symptoms can be seen both physiological and behavioral but subdued behavioral symptoms seem to be seen only when the infection intensity is high. I would like to see more studies investigating behavioral and physiological effects from glochidia infections on the fish host. In my future work with propagating freshwater pearl mussels, I will have the opportunity to test new hypothesis derived from this thesis.

Svensk sammanfattning

Parasiter kan påverka både sin värds fysiologi och beteende och därigenom öka chansen att fullborda sin livscykel. Alla sötvattensmusslorna av ordningen Unionoida har ett parasitiskt larvstadium (glochididier) på fisk. Flodpärlmusslan *Margaritifera margaritifera* är en värdfiskspecialist där dess glochidier bara kan utvecklas till musslor på laxfiskar (Salmonidae), och i Europa kan glochidierna bara utvecklas till musslor på atlantlax *Salmo salar* och öring *S. trutta*. Denna avhandling om fem artiklar beskriver interaktionen mellan flodpärlmussla och öring. Det övergripande syftet var att undersöka om öringens beteende kan öka risken att bli infekterad av glochidier (**artikel I**), i vilken utsträckning glochidiainfektion förändrar fiskens beteende och dess fysiologi (**artikel II, III och IV**) och slutligen hur infektion i en naturlig bäck korrelerar med rörelsemönster, tillväxt och användning av olika habitat (**artikel V**). Mina resultat visar att mer aktiv öring hade en ökad risk att bli infekterad, konkurrensförmågan minskade med förhöjd infektionsintensitet, hanteringstiden av byten var längre för infekterad fisk och tillväxthastigheten var lägre hos infekterad fisk. Ämnesomsättningen och mängden röda blodkroppar (hematokrit) var också förhöjd hos infekterade fiskar jämfört med icke-infekterade fiskar. I ett naturligt vattendrag var öringen som blev infekterad mindre än icke-infekterad öring, det stämmer väl överens med andra studier. Den infekterade öringen rörde sig också över större områden och utnyttjade andra habitiat än den icke-infekterade öringen vilket kan tolkas som att den infekterad öring inte är lika konkurrenskraftig och sämre på att försvar sitt revir. Sammanfattningsvis tyder alla resultat på att glochidieinfektionen är en belastning för värdfisken som hindrar den infekterade öringen att konkurrera om resurser på lika villkor som en icke-infekterad öring. Detta mönster har också observerats i tidigare studier utförda på laboratorier med odlad öring och artificiellt infekterade öringar. Glochidier är potenta parasiter och i höga antal orsakar de ett sjukdomstillstånd för sin värdfisk som kan orsaka både fysiologiskt och beteendemässiga förändringar. Ett förändrat beteende tycks dock endast ske när infektionsintensiteten är hög. Jag skulle vilja se fler studier som undersöker beteendemässiga och fysiologiska effekter av glochidieinfektion på musslornas värdfiskar. I mitt framtida arbete med att odla flodpärlmusslor kommer jag att ha möjlighet att testa många nya hypoteser som härrör från denna avhandling.

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List of papers

Paper I Wengström, N., Wahlqvist, F., Näslund, J., Aldvén, D., Závorka, L., Österling, M. E & Höjesjö, J. 2016. Do individual activity patterns of brown trout (*Salmo trutta*) alter the exposure to parasitic freshwater pearl mussel (*Margaritifera margaritifera*) larvae? Ethology 122, 769–778.

Paper II Filipsson, K., Petersson, T., Höjesjö, J., Piccolo, J. J., Näslund, J., Wengström, N & Österling, M. E. 2016. Heavy loads of parasitic freshwater pearl mussel (*Margaritifera margaritifera* L.) larvae impair foraging, activity and dominance performance in juvenile brown trout (*Salmo trutta* L.). Ecology of freshwater fish 27, 70–77.

Paper III Wengström, N., Höjesjö, J., Filipsson, K., Loeb, L., Kvarnliden, H & Österling, M. 2022. The influence of freshwater pearl mussel (*Margaritifera margaritifera*) glochidia infection on brown trout (*Salmo trutta*) prey consumption and growth. Manuscript.

Paper IV Filipsson, K., Brijs, J., Näslund, J., Wengström, N., Adamsson, M., Závorka, L., Österling, M. E. & Höjesjö, J. 2017. Encystment of parasitic freshwater pearl mussel *(Margaritifera margaritifera*) larvae coincides with increased metabolic rate and haematocrit in juvenile brown trout (Salmo trutta). Parasitology research 116, 1353 – 1360.

Paper V Wengström, N., Milic, L. J. M., Näslund, J., Eriksson, H & Höjesjö, J. 2022. Migration patterns between glochidia infested and non-infested brown trout in two streams inhabited by freshwater pearl mussels. Manuscript.

The following papers were published during my doctoral studies but are not included in the dissertation.

Von Proschwitz, T & Wengström, N. 2020. Zoogeography, ecology, and conservation status of the large freshwater mussels in Sweden. Hydrobiologia, doi.org/10.1007/s10750-020-04351-6.

Wengström, N., Söderberg, H., Höjesjö, J & Alfjorden, A. 2019. Mass mortality events in freshwater pearl mussel (*Margaritifera margaritifera*) populations in Sweden: an overview and indication of possible causes. Freshwater Mollusk Biology and Conservation 22, 61–69.

Závorka, L., Brijs, J., Wengström, N., Lovén Wallerius, M., Näslund, J., Koeck, B., Lassus, R., Höjesjö, J., Johnsson, J. I., and Cucherousset. 2019. Laboratory captivity can affect scores of metabolic rates and activity in wild brown trout. Journal of Zoology, 307: 249-255.

Závorka, L., Larranaga, N., Lovén Wallerius., Näslund, J., Koeck, B., Wengström, N., Cucherousset, J & Johnsson, J. I. 2019. Within-stream phenotypic divergence in head shape of brown trout associated with invasive brook trout. Biological Journal of the Linnean Society, http://dx.doi.org/10.1093/biolinnean/blz192/5682636

Näslund, J., Wengström, N., Wahlqvist, F., Aldvén, D., Závorka, L & Höjesjö, J. 2018. Behavioral type, in interaction with body size, affects the recapture rate of brown trout Salmo trutta juveniles in their nursery stream. Integrative Zoology 13:604-611.

Österling, M. E. & Wengström, N. 2015. Test of the host fish species of a unionoid mussel: A comparison between natural and artificial encystment. Limnologica 50, 80 – 83.

Introduction

This thesis describes the parasite host interaction between the freshwater pearl mussel (FPM) (*Margaritifera margaritifera*) and its host the brown trout (*Salmo trutta*) from five papers focusing on the impact the mussel larvae have on the behavior and physiology of the host. I find it very fascinating how prevalence (number of fish being infected in the population) and intensity (number of parasites in the infected individual) of glochidia can differ so much, in and between streams and years. Why are not every trout in a small stream inhabited by FPM infected with hundreds or thousands of glochidia when every female FPM can release 2-4 million glochidia each year?

Parasitism

Parasitism is a life form where one species takes advantage of another species to gain benefits and improve fitness (Doebeli & Knowlton, 1998). The diversity of parasites is overwhelming and only the number of known metazoan parasites exceeds more than 100 000 species (Poulin & Morand, 2000). The most widely accepted definition of a parasite is the one by Dogiel (1947), "*Parasites are animals which use other organisms as a food source and environment, at the same time partly or completely imposing on their hosts a task of regulation of their relations with the environment*". Although that definition implies that parasites are restricted to the animal kingdom, it is also valid for all kinds of parasites such as, all viruses, many groups of bacteria, fungi, protists, and multicellular plants (Balashov, 2011). A similar definition as Dogiel (1947) but with an addition of a new terminology "harm" was proposed by Anderson & May (1978) who classified an animal as parasitic if three conditions where fulfilled: "utilization of the host as a habitat", "nutritional dependence" and "causing harm" to its host. This is also an accepted definition to describe an animal parasite (Poulin, 2007).

There are no clear definitions to the concept "harm to its host" according to Barber & Dingemanse (2010) and parasites can impair the health and fitness of the host through a wide variety of mechanisms such as costly immune responses, increased susceptibility to secondary infections and reduced nutritional status. Harm is also associated with the virulence of the parasite (Ewald, 1993) and is linked with a negative cost on the host fitness or at least with a negative impact on proxies for fitness, e.g., growth. The virulence can be described as a continuum that goes from low virulence to death and that the parasite causing this must survive the initiated response from the host, i.e., virulence also comes with a cost for the parasite unless the parasite is a parasitoid (adapted to survive the death of the host).

Parasites have most likely evolved from free-living ancestors and the origin of parasites can be as old as several million years, as indicated by fossil evidence (Morris, 1981). A common step towards a more permanent parasitic lifestyle is phoresy, a phenomenon when small invertebrates attach to the external surface of larger animals (Poulin, 2007). The evolutionary processes leading to parasitic life cycles are natural selection where the reproductive success as a parasite must be greater than its success as a free-living animal (Poulin, 2007). When an interaction is said to be parasitic only one of the involving parts gain benefits of the interaction, if both parts would gain benefits from the interaction, it would be a mutualistic relationship (Doebeli & Knowlton, 1998). To become a parasite the organism must have some pre-adaptations for survival, feeding, and/or reproduction on (ecto) or inside (endo) the host (Poulin, 2007).

Parasites have either a direct or an indirect lifecycle depending on how many hosts they need for their transmission and to complete their life cycle (Barber et al., 2000). Parasites with a direct strategy use one definite host whereas parasites with an indirect or complex strategy use at least one intermediate host (Barber et al., 2000). Furthermore, one parasite species can use several different hosts, referred to as host range and depending on how many hosts a parasite can use for the transmission it can be referred to as host generalist or host specialist (Poulin, 2007). Parasites can be divided into two classes depending on their size: micro and macro parasites. Micro parasites (viruses, bacteria, protozoan) are characterized by their small size, short generation times, extremely high rates of direct reproduction

within the host, and a tendency to induce immunity to reinfection in those hosts that survive the infection (Anderson & May, 1979a). Macro parasites (parasitic helminths, arthropods, and unionids) tend to have longer generation times than micro parasites, and direct multiplication within the host is either absent or occurs at a low rate. The immune responses caused by macro parasites are generally shorter and depend on the intensity of parasites in each host (Anderson & May, 1979a).

Interaction between parasites and host

Parasites can regulate animal populations in a similar way as a predator or a resource limitation, affecting growth of plants and animal populations (Anderson & May, 1979a). For example, the sporozoan parasite *Adelina* reduced a population of flour beetle *Triboliumcasteneum* in a laboratory experiment and the main cause of death among bighorn sheep populations in North America is probably infection by the lungworm *Protostrongylus stilesi* which predisposes the host to pathogens causing pneumonia. It is likely that the interplay between the pathogenicity of viral, bacterial, protozoan or helminth infections and the nutritional state of the host contributes to the density-dependent regulation of natural populations, with the parasites amplifying the effects of low levels of nutrition (Anderson & May, 1979a). Further, parasites have been suggested to play an import role in shaping behavior and the evolution of animal personality (Barber & Dingemanse, 2010) where a disease state caused by the infection will alter the development of individual personalities (Lehmann, 1993; Barber & Dingemanse, 2010). Certain behavioral traits like boldness, exploration, and aggressiveness are associated with fitness consequences, e.g., bold individuals exhibit higher reproductive success than shy ones, whereas explorative individuals do not exhibit the same success (Smith & Blumstein, 2008). Explorative individuals have been suggested to have an increased risk of being exposed to a wider range of parasites, active individuals exhibit the same risk. The behavior of an individual can also decrease the exposure to parasitic infections using selective foraging and elimination behavior, e.g., avoiding areas where the risk is high for getting an infection (Hart, 1990).

Fish serve as hosts for a range of taxonomically different parasites (Barber et al., 2000). In fish, parasite infections have been known to reduce foraging behavior in sticklebacks *Gasterosteus aculeatus* infected by the flatworm *Schistocephalus solidus* (Barber & Huntingford, 1995), and foraging efficiency caused by trematode metacercaria infection (Holmes & Zohar, 1990). An increased risk of predation caused by an altered behavior has been found in killifish parasitized by the trematode *Euhaplorchis californiensis* (Lafferty & Morris, 1996). The nematode uses the killifish as an intermediate host, and the behavioral modification of the killifish increased parasite transmission to its final host (bird) (Lafferty & Morris, 1996). More than ten species of parasitic worms (Helminths) have been found in brown trout from Norway, Corsica, and Germany (Hartvigsen & Halvorsen, 1992; Quilichini et al., 2007; Unger & Palm, 2016). Brown trout is also a functional host to the freshwater pearl mussel *Margaritifera margaritifera* in Europe, but it can also be infected by glochidia from other mussel species like *Unio crassus*, *U. tumidus*, *Anodonta anatina* and *Pseudanodonta complanata* (Larsson, 2015; Jonsson & Bertilsson, 2016).

However, little is known about the parasitic group of freshwater bivalves know as unionids and the effect they may have on their hosts.

Freshwater mussels Unionoida and their role as parasites

In the order Unionoida there are more than eight hundred species (Graf & Cummings, 2007), that all have their own life history with traits that will affect their role as parasites (Bauer & Wächtler, 2001). All species in the families Unionidae, Margaritiferidae and Hyriidae (from now on mussels) have a similar type of parasitic larvae called glochidia (Pfeiffer & Graf, 2015). The glochidium is a small (50 – 350 µm) calcified bivalve parasite with a single adductor muscle and lack a visceral mass and foot

(Arey, 1924; Pfeiffer & Graf, 2015). There are five different types of glochidia: (I) unhooked; (II) hooked with basal spines; (III) S-shaped hooked; (IV) axe-head shaped; (V) bilaterally asymmetrical (Pfeiffer & Graf, 2015). Glochidia with hooks can attach to body, fins, and gills on the fish, whereas glochidia without hooks can only attach to the gills (Blažek & Gelnar, 2006; Jansen et al., 2001).

The life cycle of mussels includes four stages, egg, larval (e.g., glochidium), juvenile and the adult mussel stage (Kat, 1984). Age of sexual maturity within and between species varies with a range from 0+ to 20 years (Bauer, 1987a; Haag & Staton, 2003; Reis & Araujo, 2016; Wu et al., 2018). Mussel species have different sexual strategies, and they can be dioecious (separate sex) (e.g., *Unio crassus*) or hermaphroditic (self-fertilized), (e.g., *Anodonta cygnea*) and there is also a plastic strategy that shifts between dioecious and hermaphroditic (e.g., *M. margaritifera*) (Bauer & Wächtler, 2001). The number of glochidia produced by females have a range between 8000 and 4 million (Wächtler et al., 2001). Fertilized eggs are kept in special structures on the gills called marsupia and they can occupy all four gills or just part of the gills (Lefevre & Curtis, 1910). The brooding time varies between a few days (short term breeders, tachytictic) to several months (long term breeders, bradytictic) before mature glochidia can be released (Jansen et al., 2001). Mature glochidia are released individually and/or in loosely bound mucus that dissolves in the water, and some species release them in conglutinates (Jansen et al., 2001). The timing of the release is temperature driven and the duration of a release can last for a few days to several months (Hastie & Young, 2003; Hochwald, 2001; Culp et al., 2011).

Glochidia are mainly parasitizing fish and the host range is variable depending on if the mussel species is a host specialist (*Margaritifera* sp.) or a host generalist (*Unio* sp.) (Kat, 1984; Watters, 1992; Bauer, 2001). The glochidia have no ability to choose which host they attach to, but their mothers may have adopted special morphological features that may lure the fish to attack her, and when the fish attacks, she releases the glochidia (Haag & Warren, 1999). There is a wide variety of fish lures that has evolved over time and the most spectacular are found in the tribe Lampsilini (Figure 1) (Zanatta & Murphy, 2006).

Figure 1. Mantle display of the species Lampsilis fasciola. Photo: Tim Lane, The Department of Wildlife Resources - Aquatic Wildlife Conservation Center, Virginia, U.S.A.

The parasitic stage varies in time, from a few weeks (*Anodonta anatina*) to several months (*M. margaritifera*). After the metamorphose from glochidia to a juvenile mussel, the juvenile mussel falls off the fish.

The virulence of mussel glochidia is not fully understood but we can assume that it is increasing in correlation with the infection intensity (Anderson & May, 1979a). Glochidia infections are often described as phoretic (phoresy; when a species attaches itself on another species for the purpose of dispersal (White et al., 2017)) (Modesto et al., 2017). However, recent studies indicate that glochidia infections are more harmful than just being phoretic. Chub *Squalius cephalus* infected by *A. anatina* glochidia were less active in laboratory and in the field, and they dispersed less far upstream than non-infected chub (Horký et al., 2014). Common carp *Cyprinus carpio* infected by *Sinanodonta woodiana* glochidia showed higher energy costs in movement compared to non-infected fish (Slavik et al., 2017).

The interaction between the freshwater pearl mussel and its salmonid fish host

My work has focused on the interaction between the freshwater pearl mussel (*M. margaritifera*) and brown trout (*Salmo trutta*), one of the fish hosts to the freshwater pearl mussel. In the sections below I will give an overview of the current knowledge about this exciting relationship.

The freshwater pearl mussel and its life cycle

The freshwater pearl mussel (FPM) belongs to the class Bivalvia, order Unionoida and the family Margaritiferidae (Bogan, 2008; Graf & Cummings, 2015). It has a Holarctic distribution and can be found in northern and central Europe, northern Russia and in the north-east part of North America (Araujo & Ramos, 2001). In Sweden, the species is distributed almost throughout the entire country, from the province of Skåne in the south to northernmost Lapland (von Proschwitz & Wengström, 2020). The earliest fossils of molluscan pearls are from the Triassic period (>200 million years ago) (Morris 1981). The family Margaritiferidae is probably monophyletic and derived from a putative ancestor in the Silesunionidae with a likely origin from Asia spreading along the Tethys margin and crossed the Atlantic in the late Triassic or early Jurrasic (Araujo et al., 2017).

Figure 2. Glochidia from one FPM collected during a propagation project in the county of Västerbotten. Notice how glochidia are bound to each other in large yellow clumps. Photo: Daniel Wendesten, Swedish Anglers Association.

The FPM has separate sexes, and the reproductive season starts with the production of gonads in June - July (Bauer, 1987a). Males release their sperm into the water current and females inhale the sperm through the inhalant aperture. A female FPM produces 2-4 million eggs that are kept in specialized brood chambers (marsupia) on both gill pairs. The eggs get fertilized internally, and they develop into tiny larvae (glochidia). The female carries the glochidia until they mature and are ready to be released in a synchronized spat that may occur between June and September (Hastie & Young, 2003; Wächtler et al., 2001; Scheder et al., 2011). The FPM glochidia are released in a mucus that dissolves in water (Figure 2).

Glochidia are incapable of active locomotion, and they only survive a few days unless they attach to a fish host (Jansen et al., 2001). The parasitic stage lasts for 10-11 months and during this period the glochidia grow from 0.07 to 0.5 mm (Taeubert et al., 2010). When the metamorphose is completed juvenile mussels fall off the host and bury themselves into the bottom substrate of the stream and stay buried for 4-8 years (Söderberg et al., 2008). Individuals of the FPM can be more than two hundred years old and are believed to start reproducing at an age of 20 years (Bauer, 1987a), although there are reports of an earlier start, at an age of 12-13 years (Young & Williams, 1984). Once they are sexually mature, they reproduce throughout their remaining lifespan (Bauer, 1987a).

The FPM is a host fish specialist and the glochidia larvae can only metamorphose on the gill filaments and gill rakers of salmonid fish. In Europe, the hosts are Atlantic salmon (*Salmo salar*) and/or brown trout (*S. trutta*) (Taeubert et al., 2010; Karlsson et al., 2014; Salonen et al., 2016; Österling & Wengström, 2015) and in North America the hosts are brook trout (*Salvelinus fontinalis*), Atlantic salmon, coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*) (Smith, 1976; Meyers & Milleman, 1977; Cunjak & McGladdery, 1991). Generally, the FPM only uses one of the salmonid species as a host (Larsen et al., 2000), but there are reports from Scotland and Norway that FPM larvae have been found on both species in the same drainage system (Hastie & Young 2001; Karlsson et al., 2014). This has been seen on rare occasions also in the southwestern part and the northern parts of Sweden (own unpublished data and data from the County Board of Norrbotten).

The brown trout and its life cycle

The brown trout is a teleost fish that belongs to the family Salmonidae. It has a native distribution range from Iceland, northern Scandinavia, and Russia in the north, to the northern coastline of the Mediterranean Sea in the south. The western limits are the European coastline, and the eastern limits are probably the Ural Mountains, Caspian Sea, and the upper reaches of the Orontes River in Lebanon (Elliott, 1994). Outside their native range brown trout has been successfully introduced in at least 24 countries outside Europe (Elliott, 1994). The natural distribution of brown trout coincides very well with the distribution of the FPM.

All salmonids breed in freshwater and in the northern hemisphere brown trout has been found to breed from October to March, but the usual time in most populations is November and December (Elliott, 1994; Klemetsen et al., 2003). The spawning takes place on clean gravel in running water. The female digs a depression in the gravel using her body to create currents to move gravel. She lays her eggs in the depression and at the same time the male sheds his sperm. Thereafter she moves upstream and uses her body again to move gravel over the eggs. She does this procedure until all her eggs are laid in a series of redds. A female produces 100-2000 eggs per reproductive season depending on her body size (Jonsson, 1985; Elliott, 1984).

In the redd, the eggs develop and hatch after approximately 400-degree days in February or early March (Crisp, 1981). The alevins (hatched fish with yolk sac), stay in the redd for 5-6 weeks until the yolk sac is depleted, and they emerge from the gravel bed as fry (a short transition stage when the trout emerge from the gravel) (Elliott, 1984). The parr (juvenile trout with a fully absorbed yolk sac) stays

in the stream 2-4 years, before being transformed into smolt (involving a physiological and morphological adaptation to seawater, i.e., “smoltification”), or its whole life, depending on life-history strategies (Jonsson, 1985; Elliott, 1994; Nevoux et al., 2019). Sexual maturity is reached at an age of 3-5 years, and they can spawn several times (iteroparous) during their lifespan (Jonsson, 1985; Elliott, 1994; Klemetsen et al., 2003).

Habitat requirements for freshwater pearl mussel and brown trout

Generally, the habitat should not only provide shelter and food but also support the ability to reproduce. The quality of the habitat is of immense importance for an animal with limited mobility such as mussels (Strayer, 2008; Schwalb et al., 2011). Clearly, habitat quality plays an important role also for mobile animals, like the brown trout that migrates to different habitats for shelter, food, and spawning (Klemetsen et al., 2003). The habitat requirements of FPM involve both abiotic and biotic factors such as hydro morphology, water chemistry, food availability and fish host densities (Hastie et al., 2000; Hastie et al., 2003; Hastie et al., 2004; Geist & Auerswald, 2007; Österling et al., 2008; Strayer, 2008). In general, and important to highlight, the early life stages of brown trout share the same habitat requirements as the FPM (Figure 4).

In the rivers Kerry and Spey, the highest densities of adult and juvenile FPM were found in areas 1-3 m from the nearest bank, at a water depth of 0.3-0.4 m and at a water velocity between 0.25-0.75 ms^{-1}. Both adults and juveniles preferred boulder (63 – 200 mm) dominated substrate (Figure 3), but high densities of FPM could also be found in other types of coarse substrates, but not in silt (Hastie et al., 2000). Mussel densities were positively associated with coarse river substrate, fast flowing water, riparian woodlands, and river bends (Hastie et al., 2004). When Geist and Auerswald (2007) investigated 26 streams in 7 countries, they found that streams with good juvenile recruitment

Figure 3. Freshwater pearl mussel sitting in a mix of substrate ranging between sand and boulders. Photo: Niklas Wengström

had coarser and better sorted substrate with significantly lower quantities of fine sediment than streams without or poor juvenile recruitment. In agreement, Österling et al. (2008), found that turbidity was four times higher in streams that lacked recruitment compared to streams with recent recruitment.

According to the Swedish conservation program (Henrikson & Söderberg, 2020), the FPM in Swedish and Norwegian systems require streams with pH close to neutral, low concentrations of inorganic aluminum and low concentrations of nutrients (Table 1) (Degerman et al., 2009, 2013).

Table 1. Water chemistry requirements of the FPM in Swedish streams (Degerman et al. 2009;2013).

pH	>6,2	Min
Inorganic aluminum	<30 µg/l	Max
Total phosporus	<10 µg/l	average
Nitrate, NO_3	<125 µg/l	Median

Freshwater mussels feed on phytoplankton, zooplankton, and particulate detritus (Allen, 1914;1921) as well as suspended bacteria (Silverman et al., 1997; Nichols & Garling, 2000), fungal spores (Bärlocher & Brendelberger, 2004) and sedimented organic matter (Yeager et al., 1994).

Brown trout has been found to spawn at sites with a water velocity range of 10.8 – 80.2 cm s^{-1} and at depths between 6 – 82 cm (Witzel & MacCrimmon, 1983; Shirvell & Dungey, 1983). The bottom substrate at spawning sites has a range of 8-128 mm and it correlates with the size of the fish. The, a mean depth in gravel of egg burial is 15.2 cm, but smaller fish (<30 cm) dig to depths of 10 cm while bigger fish can dig deeper than 30 cm (Crisp & Carling, 1989). The alevin requires a habitat with high oxygen concentration 7-10 mg O_2/l and a substrate firm enough to resist flooding conditions, i.e., stones with a size-range between 5-81 mm (Rubin & Glimsäter, 1996; Maret et al., 2000; Näslund, 1992). The fry that emerges from the redd needs a nursery habitat that provides shelter from predators and aggressive competitors but also a space to establish a territory for foraging, during the first summer as parr (Höjesjö et al., 2004; Fausch, 1984; Armstrong et al., 2003; Kalleberg, 1958). These shelters can be structures like boulders, large dead wood, and fine dead wood (Enefalk & Bergman, 2015). Suitable habitats for parr have been found in streams with discharge between <15-25 cm s^{-1} (Heggenes & Traen, 1988; Crisp & Hurley, 1991) and a bottom substrate dominated by gravel of the size 50-70 mm (Heggenes, 1988).

Brown trout is territorial, and it defend its territories from intruders, and habitat preferences and time holding the territory seem to be of value when brown trout invest in territorial defense (Johnsson et al., 2000; Johnsson & Forser, 2002), but social rank or dominance can also influence trout position in a stream (Alanärä et al., 2001).

Fish host densities and the importance of cohorts

To the FPM, host fish density is an important feature in the habitat and has been measured in several streams in Europe. The overall rule of thumb is that viable populations of FPM have host fish densities above 5 individuals per 100 m^2 (Geist et al., 2006; Arvidsson et al., 2012; Degerman et al., 2013). Arvidsson et al. (2012) showed that brown trout densities were positively correlated with juvenile recruitment, but surprisingly Geist et al. (2006) found that viable FPM populations had significantly lower host fish densities than non-viable populations. The underlying mechanisms to these contradictions may be different experimental set ups and need to be further investigated. When 56 streams in the county of Västernorrland, Sweden, were investigated the results showed that below 8 mg L^{-1} total phosphorus and above 5 0+ trout per 100 m^2 were the best predictors for finding viable populations of FPM (Degerman et al., 2013). Similarly, in a study by Tamario & Degerman (2017), the abundance of young of the year (0+) trout was positively correlated with the presence of very small (<20 mm) FPM.

The general assumption is that young of the year (0+) brown trout are better hosts to the FPM glochidia than older (>0+) conspecifics due to a lack of an acquired immune response caused by previous infections (Bauer, 1987b). However, there are few studies showing a difference in prevalence and intensity between cohorts (0+ and >0+) (Geist et al., 2006). In an experiment with artificially infected brown trout Taeubert et al. (2010) found that a sympatric trout strain within the FPM distribution range had both higher infection intensity and higher glochidial growth compared with two allopatric trout strains outside the FPM distribution range. Their results suggest that sympatric trout strains should be more suitable hosts than allopatric trout strains (Taeubert et al., 2010). Contrary to Taeubert et al. (2010), Österling & Larsen (2013) showed that allopatric trout had higher infection intensity and larger glochidia compared to a sympatric strain of trout. The two studies used different methods to infect the fish, which may explain the opposite outcome of the results. In theory, sympatric brown trout and FPM should adapt to each other over evolutionary time (Poulin, 2007). The FPM would have adjusted its virulence to enhance the transmission and at the same time the brown trout should have adopted defense mechanisms to avoid or cope with the infection (Poulin, 2007). When species are in allopatry, the parasite should be in favor when entering a new environment with naïve fish hosts (Weis & Sol, 2016). If certain conditions are fulfilled and the transport, introduction, and establishment phase is clear, the spread of the FPM in the new environment can start (Chapple & Wong, 2016). But still, the question whether sympatric or allopatric trout strains are better hosts or not needs to be better investigated before any reliable conclusions can be drawn.

Many FPM populations show signs of recruitment failure, but this seems not to be related with glochidia intensity on the host (Österling et al., 2008). In a study performed over two years, a huge variation in both prevalence and intensity on salmon and brown trout was shown (Hastie & Young, 2001). The variation between years may help to explain the reproductive success of the FPM, but we need longer time series.

Conservation status of the freshwater pearl mussel and brown trout

Red list category

The FPM is endangered throughout its distribution range (IUCN 2017), has declined in all countries, and is probably extinct in Belarus, Denmark, Lithuania, and Poland (Lopes-Lima et al., 2017). The Swedish populations are listed as *endangered* (EN). Further, the species is protected by the EU habitats directive and the Bern convention. There are more than 600 populations in Sweden, the approximate numbers of individuals are 39 million, and 46 % of the populations show signs of juvenile recruitment (individuals <50mm) (Henrikson & Söderberg, 2020).

The brown trout is listed as *least concern* (LC) in the IUCN red list of threatened species, but the anadromous parts of populations have in many cases declined because of pollution and habitat degradation. In Sweden, the species is also listed as *least concern* but there are regions in the north of the Baltic Sea where the status is decreasing (Artdatabanken, 2017). Brown trout is used as a key species indicating a well-functioning ecosystem when assessing water bodies in the water frame directive. There are very few water courses in Sweden that are classified as having *good ecological status*, and one underlying reason is fragmentation, where various barriers hinder the fish to migrate (see figure 5 for an example). There are several projects in Sweden that have improved the habitat, removed barriers, or constructed fish ways, which will probably improve the status according to the water frame directive, e.g., LIFE-ReBorN, LIFE-ReMiBar, LIFE-Connects.

Threats

The threats towards brown trout and FPM are similar. Dudgeon et al. (2006) listed five major threats to freshwater biodiversity: over exploitation, water pollution, flow modification, habitat degradation

and species invasion. All of these have a negative impact on brown trout and FPM. For example, flow modification and habitat degradation, which are associated threats, have a negative impact on brown trout spawning, nursery, and rearing habitats (Armstrong et al., 2003).

The effects of climate change on aquatic environments have been suggested to include both drought (Sousa et al., 2018) and floods (Hastie et al., 2001), two events that have been recognized to kill FPM. A severe decline of FPM was caused by pollution and river channel alteration in Portugal (Reis, 2003), and in Sweden, the most widely accepted hypothesis about current threats to the FPM is increased sedimentation due to forestry and agriculture activities (Österling et al., 2008). The effect of land-use and climate change on sediment yields in the streams may also have a negative effect on the early life stages of brown trout, since clogging of gravel beds reduce oxygen supply (Scheurer et al., 2009). A recent GIS-analysis revealed that the presence of dams had a negative effect on the recruitment in FPM populations and the presence of lakes had a positive effect on the recruitment in FPM populations (Tamarino & Degerman, 2017). Dams and habitat fragmentation may influence brown trout as local adaptation and evolutionary potential (juvenile recruitment) can be negatively affected (Hansen et al., 2014). The acidification of streams in Sweden have had a negative effect on the recruitment of brown trout and FPM (Degerman et al., 2015; Wengström & Höjesjö, 2021). The introduction of brook trout *S. fontinalis* and the signal crayfish *Pacifastacus leniusculus* are also potential threats with a negative effect on FPM. Brook trout is not a functional host to FPM in Europe. It can still be infected but the glochidia will fall off a few days after infection (Salonen et al., 2016; own unpublished data). Brook trout has also been shown to have a negative effect on brown trout growth (Öhlund et al., 2008; Závorka et al., 2017). The signal crayfish has been found to predate on juvenile FPM in Portugal; Sousa et al., 2019). A “new”, perhaps overlooked threat to FPM is pathogens (viruses and parasites) causing enigmatic mass mortality events. This have been reported from Sweden where the causative agent seems to be a micro parasite (protist) belonging to the genus *Nematopsis* (Wengström et al., 2019; Alfjorden, 2021). Similar events have happened in the U.S.A. and results suggest that a densovirus is causing the mussels to die in high numbers (Richard et al., 2020).

Figure 4. The picture is a good example of how a brown trout, nursery and rearing habitat might look like. The picture is taken in Hultabäcken, a tributary to Lärjeån. Lärjeån is a stream on the West coast of Sweden, and it is inhabited by FPM, brown trout, and Atlantic salmon. Photo: Niklas Wengström

The effects of freshwater pear mussel glochidia on brown trout

In the FPM literature, many different terms are used to describe the infection and number of glochidia on the individual fish or the proportion of host infected in the population. Terms like glochidiosis (Karna & Milleman, 1978; Meyers & Milleman, 1977), infestation (Filipsson et al., 2016; Marwaha et al., 2016), infection rate (Taeubert et al., 2010); infestation rate, prevalence rates and peak prevalence (Strayer, 2008), mass normalized encystment (Österling, 2015), and glochidia load (Österling et al., 2008) are used. The variation in terms makes it difficult to understand what is measured and the need of a common language is obvious. In this thesis, I use common parasitology terminology, as follows: The proportion of infected individuals in a population is called *prevalence* and the number of parasites on the individual host is called *intensity* (Barber et al., 2000). Instead of using the term infestation I use *infection*, which is common terminology describing a parasitic infection. I am aware that I have used other terms in the **papers I-V**.

It has been under debate whether the interaction between the two species can be considered parasitic (Karna & Milleman, 1978) or mutualistic (Ziuganov et al., 1994). The hypothesis of a parasitic relationship is supported by the high growth rates of glochidia during metamorphoses (Taeubert & Geist, 2013), and the trophic relationship between FPM and its host (Denic et al., 2015). The mutualistic hypothesis is supported by the fact that rivers with populations of FPM reveal a higher resistance to eutrophication and that they provide better microhabitats to juvenile salmonids (Ziuganov et al., 1994). In recent years the understanding of the relationship between the species has grown

(Österling et al., 2008; Taeubert et al., 2010; Arvidsson et al., 2012; Taeubert & Geist, 2013), especially the knowledge of the effects of glochidia infection on the host. The latest news is that the glochidia infection can increase survival from *Flavobacterium* disease, perhaps as an effect of an enhancement of the fish immune system (Chowdhury et al., 2021).

The effect on the fish host when being infected by FPM glochidia has been described in a few papers. When the infection of FPM on coho and chinook salmon was investigated, blood samples showed elevated levels of hematocrit, erythrocyte numbers and the mean corpuscular volume in infected chinook salmon and coho salmon (Meyers et al., 1980). In a study on Atlantic salmon and their performance in seawater while infected by FPM, the results suggest that infected salmon were less well-adapted to seawater than non-infected salmon (Treasurer & Turnbull, 2000). In an experiment with tank-reared Atlantic salmon, no significant differences could be detected in condition factor and lactate levels (an indicator of stress) between infected and non-infected fish (Treasurer et al., 2006). In contrast, in a study of the physiological response of juvenile brown trout to the infection of FPM glochidia, spleen enlargement and thicker and longer gill lamellae were found in infected fish, and infected fish took 6 hours longer to reach basal ventilation rate than controls (Thomas et al., 2013). Further, brown trout with high intensity of FPM showed reduced swimming performance and if the intensity was too high (>900 glochidia per g fish) it caused mortality (Taeubert & Geist, 2013). In an experimental set up, infected brown trout also grow significantly lower than non-infected fish (Chowdhury et al., 2019).

Swedish Anglers Association and their work with brown trout and freshwater pearl mussel

I am employed at the Swedish Anglers Association (SAA), and we have a contract with the University of Gothenburg since 2013 making it possible for me to do a third-cycle education within my post.

The SAA is a non-governmental organization working to improve aquatic environments. At the office in Gothenburg, we are specialized in mussel propagation, and we have just started a breeding facility to support FPM in an EU funded LIFE project called ECOSTREAMS for LIFE (LIFE19 NAT/SE/000333). In addition to the work with FPM, we work with Unio and Anodonta species, mainly to investigate their host fish range.

The SAA at the Gothenburg office works all over Sweden with FPM propagation (biological restoration) using a method influenced by the work in the River Lutter in Germany (Altmüller & Dettmer, 2006). With this method we artificially infect fish, using fish and mussels from the same basin, and when the fish are infected, we release them back into the stream they were taken from. There are two methods that we use to infect the fish. (I) When the glochidia are not ripe, fish and mussels are put together in flowthrough containers that are placed in the stream. When the glochidia are released, the fish will automatically get infected and thereafter we investigate prevalence and intensity and release the fish. (II) When glochidia are released by the females in our presence, we check viability of the glochidia using the so called NaCl method (Zale & Neves, 1982) (when glochidia are put in water (1mL) together with a few salt grains, viable glochidia close their valves in contact with the salt). We then collect fish from the stream and put fish and glochidia (100 000 $\cdot$ L^{-1}) in a bucket with water and stir it for 15 - 30 min. Thereafter, the fish is placed in flowthrough containers for 1–24-hour, prevalence and intensity are documented, after which the fish is released. All our projects have been foreseen by habitat surveys and habitat restorations.

The method of releasing infected fish is relatively cheap and not very time consuming. The problem with the method is that it is difficult to know where the fish will settle down after we have released them, and the time it takes to finally see a juvenile FPM can be very long. In one of our oldest

projects, we have been working in a stream trying to reintroduce FPM for more than ten years. The stream is called Kvarnabäcken and it has a history of being populated with FPM as late as the 1950's when the population was eradicated by pearl fishing. The stream was restored by SAA in 2011. In the summer of 2021, we found five juvenile mussels in areas where the stream was restored. Most likely the juveniles are the results of our efforts during the first years of the project. This project is still running, but it was paused in 2014 (Paper I) and 2017 (Paper IV).

In the future SAA will be part of two LIFE-projects, ECOSTREAMS for LIFE and LIFE REVIVES, which both work with FPM propagation along with stream restoration. We are also working in close collaboration with the University in Gothenburg, investigating the impact of the invasive brook trout on brown trout and FPM.

Aim and purpose of the thesis.

My main hypothesis is that the parasitic glochidia of FPM have a negative impact on its host. Together with my colleagues, I have tested in **paper I** if interindividual differences in activity of brown trout alter the exposure to parasitic freshwater pearl mussel glochidia. In **paper II**, we investigated if brown trout infected with glochidia would have a reduced competitive ability towards non-infected fish. In **paper III**, we hypothesized that prey consumption time and growth would be negatively affected by glochidia infection. In **paper IV**, we hypothesized that metabolism and haematology were negatively affected in glochidia infected brown trout. In **paper V**, we investigated to what extent glochidia infection affects the spatial distribution of brown trout in a stream.

Method

Here I give a short description of all the methods used in the thesis. For a more detailed description see the relevant method section of each paper.

Study location and experimental animals

We have used juvenile wild brown trout in all studies. The fish have been caught with electrofishing gear (LR-20B, Smith-Root, USA) from Slereboån (**Paper II**, **III** & **V**) (Lat 58.0098, Long 12.3454), Kvarnabäcken (**Paper I** & **V**) (Lat 57.8125, Long 12.1838) and Lindåsabäcken (**Paper IV**) (Lat 57.6700, Long 13.0628) (Figure 5). In Slereboån and Kvarnabäcken the brown trout is resident and/or sea migrating and in Lindåsabäcken the brown trout is only resident. The FPM population in Slereboån is calculated to contain ~5000 mussels and there are no signs of recruitment (Wengström et al., 2022). In Lindåsabäcken the population is calculated to contain ~7000 mussel and 6% of the population are juveniles (<50mm) (Andersson, 2018). In Kvarnabäcken, FPM was translocated with 50 gravid adults taken from Slereboån in 2014, as part of the experimental design of **paper I.** There are signs of recruitment in Kvarnabäcken, as we found five juveniles in 2021. In 2016, an additional set of 100 mussel was translocated from Slereboån to Kvarnabäcken.

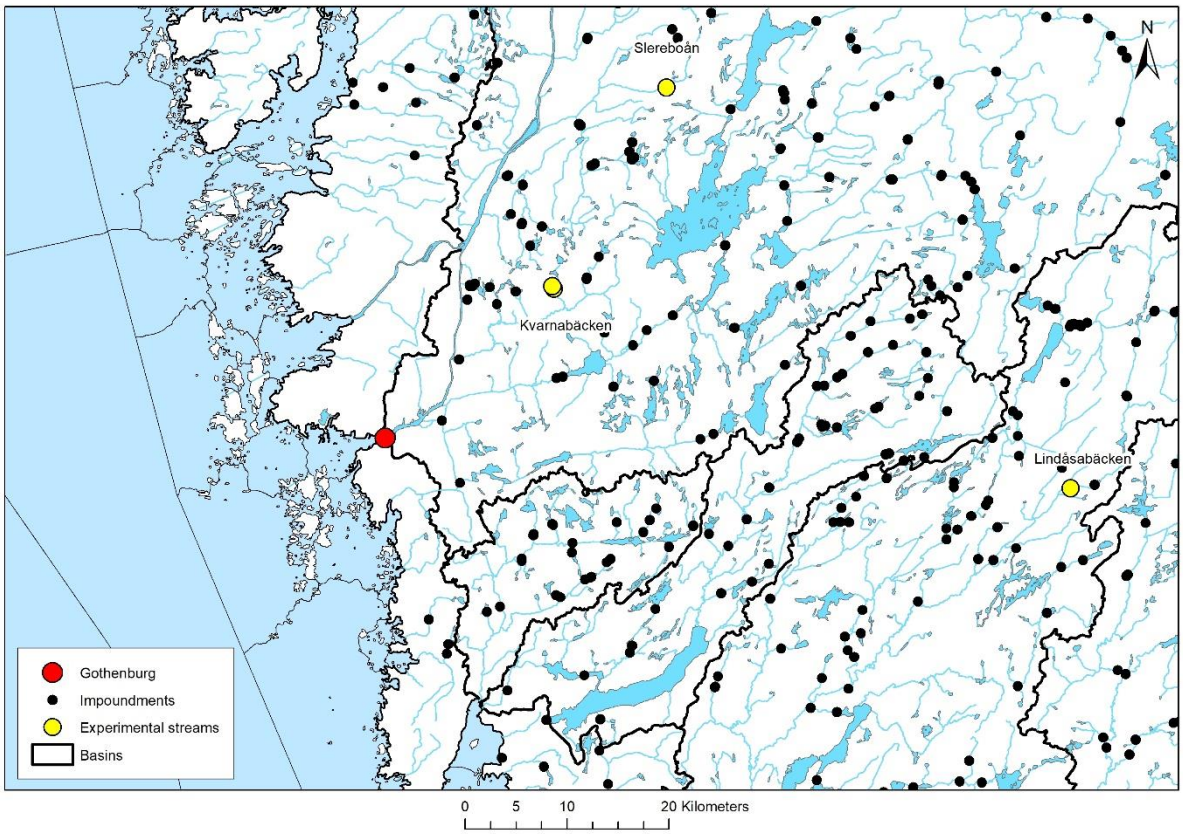

Figure 5. Geographical locations of the streams used in papers I-V. Basin borders and impoundments are shown as line and points. All experimental sites are located on the Swedish West coast, close to Gothenburg.

Scoring of behavior

In **paper I** and **V** I have used a forced open field test to measure behavioral variation between individuals of brown trout (Carter et al., 2013). With this method the individual fish was put in a novel environment (white plastic container 30 x 60 x 22 cm,) and its behavior recorded by a video camera. The arena was filled with 5 cm of water that was exchanged between trials. Each fish was filmed for 30 minutes and analyzed using a motion tracking software (LoliTrack v. 4, Loligo Systems ApS, Tjele, Denmark). The motion tracker uses the background contrast to detect the fish and the system analyses the following behavioral parameters: (1) average swimming velocity when active (cm/s), (2) active time (%), and (3) distance moved (cm). Given an acute stress response in brown trout, which commonly lasts for at least two hours (Pickering et al., 1982), the fish were trialed in a stressed state. Previous studies on juvenile brown trout have shown that activity in this type of test is repeatable within individuals (Adriaenssens & Johnsson, 2013; Näslund and Johnsson, 2015), and covaries positively with behaviors in the wild, such as movement activity after displacement (Závorka et al., 2015) and diurnal activity (Závorka et al., 2016).

In **paper II** I studied 14 pairs of wild caught brown trout, 7 pairs were young of the year (0+) and the other 7 were at least 1 year old (>0+). The fish in the pairs were size matched but with different infection intensity, measured as number of glochidia per infected trout. The study was conducted in a stream tank of the size 1.4 x 0.6 x 0.2 m and with an average water velocity of 0.2 m/s. The study arena had the bottom covered with gravel (0.5-22 mm) and in the middle of the arena we placed one stone (9 x 6 x 3 cm) to serve as focal point. Fish were fed every 15 second for 20 minutes with one red chironomid. The pairs were observed every 15 second and behavior such as initiated aggressive

interactions, position in the tank, activity, foraging attempts, number of food item caught, and coloration of the fish were noted in a protocol.

Prey handling time and growth

In **paper III** I studied prey handling time and growth of infected and non-infected wild caught brown trout. In the prey handling time experiment 14 infected and 14 non-infected brown trout were placed individually in a 40-L aquarium. For 8 days the fish were fed one live maggot *Calliphoria vomitaria* and one live amphipod crustacean *Gammarus pulex*, one in the morning and one in the afternoon. In this experiment, time was recorded from the time the fish took the prey and until the prey was swallowed. All foraging trials were video recorded and when the experiment was ended all trout were measured for length and mass and glochidia infection.

In the growth experiment, eight groups consisting of six size matched brown trout (three infected and three non-infected) were put in a 100-L aquarium. All brown trout were tagged with a 12 mm PIT-tag. A PIT-tag is a Passive Integrated Transponder that is implanted into the body cavity of the fish, the PIT-tag gives the individual fish a unique number. Trout were fed 1.25 % of their body mass with minced calf liver, two times every weekday (Monday - Friday) for 9 weeks. After 3, 6 and 9 weeks, measurements on length and mass were taken (fork length to mm and body mass to 0.01 grams), and their gills were controlled for glochidia infection intensity.

Standard metabolic rate and haematocrit measurements

In **paper IV** I studied standard metabolic rate and levels of haematocrit in FPM glochidia infected brown trout and non-infected brown trout. In this experiment we caught juvenile brown trout from Lindåsabäcken in April, approximately eight months post FPM infection. After starvation for nine days, brown trout were anaesthetized in water containing 0.5 mL L^{-1} 2-phenoxyethanol (Aldrich Chemistry, Germany) and measured for mass. Thereafter they were put in cylindrical, intermittent flow-through respirometers (volumes of 0.584 or 1.112 L depending on fish size). Standard metabolic rate (SMR) was calculated for each fish as the mean of the lowest 10% of ln-transformed whole-animal oxygen uptake measurements during the ~21-h period when the fish were left undisturbed after respirometer entry.

Haematocrit levels were measured from 0.1 mL blood drawn from 34 infected brown trout and 24 non-infected brown trout. Haematocrit (Hct) was determined as the fractional red cell volume upon centrifugation of a subsample of blood in 80-µL microcapillary tubes at 10,000 rpm for 5 min.

Field scanning

In **paper V** I studied behavioral patterns of brown trout in relation to FPM infection in Slereboån and Kvarnabäcken, two streams on the Swedish West coast (Figure 5). Brown trout were caught with electrofishing (LR-20B, Smith-Root, USA) approximately 1 – 2 weeks after infection by FPM glochidia. In Slereboån we caught 169 brown trout and 99 salmon and in Kvarnabäcken we caught 345 brow trout, mixed cohorts from both streams. All fish were tagged with 12 mm PIT-tags. Thereafter, the trout were tested individually in a forced open field test as mentioned above. After the open field test, fish were measured for length, mass and glochidia infection intensity. All fish were released in the same spot in the stream they came from. Thereafter, trout were scanned with a portable RFID-antenna (Oregon RFID, Portland, OR, USA) to detect individual movement between October 2017 and April 2018. Both streams were divided into 10 m stretches and every stretch was numbered. Every detection was registered using the exact stretch number. Fish were recaptured after 30 weeks and again measured for length, mass, and glochidia prevalence and infection intensity.

Habitat scoring

In **paper V** I made a habitat mapping based on a method developed by the county board in Jönköping, Sweden (Gustafsson, 2017). We mapped all the stretches, that is, 30 ten-meter stretches in each stream. An assessment was made for every 10-meter stretch describing dominant bottom substrate (>50% cover of the bottom surface), water flow, average depth, average width, and amount of dead wood. The data were later combined with movement patterns in the field, to establish habitat preference, and correlated to individual behavior in the laboratory and to glochidia infection intensity.

Infection intensity – Glochidia load

In **paper I** and **V** I classified the glochidia infection intensity using four different encystment categories (EC): EC0 = zero glochidia, EC1 = 1 to 10 glochidia, EC2 = 11 to 100 glochidia, and EC3 = more than 100 glochidia (Figure 3). This nondestructive method is similar to the method described by Salonen & Taskinen (2017) using five scale classification to assess infection intensity (Figure 6). To determine which category the fish belongs to, we anesthetize the fish and with the help of a binocular microscope observe all eight gill arches by gently lifting the operculum. The method has been proven fast once the fish is anesthetized. It generally takes less than a minute to check both sides of the fish and to determine which category the fish belongs to (Wengström, 2016).

In **paper II** the fish was anesthetized and the number of glochidia on every gill arch were counted using a stereo microscope. Glochidia load was standardized by fish wet weight, which has been a common method to calculate and present glochidia load in previous studies (Österling et al., 2014; Taeubert & Geist, 2013).

In **paper III** and **IV** infection intensity was counted as precisely as possible for each anesthetized fish. However, the number of glochidia on heavily infected fish could not be estimated to an exact number using this method. Thus, trout with more than 100 glochidia encysted on their gills were set to have 100 glochidia when analyzing the data (Figure 6).

Figure 6. This is how it might look when a brown trout is infected with freshwater pearl mussel larvae (glochidia, the white spots on the gills) when the glochidia intensity is high, with more than 100 glochidia per fish. Photo: Hampus Kvarnliden

Results and discussion

Summary of results

Paper I

In paper I I investigated if different individual personalities in brown trout can affect the risk of being infected by parasitic freshwater pearl mussel larvae (glochidia). Our result suggests that active individuals run a higher risk of being infected than passive individuals. I suggest that space use and/or high ventilation rates could be possible causal explanations to our findings, as a higher activity could be correlated with these traits in the wild.

Our result indicated that the individuals which got infected with glochidia over the summer had a higher general open-field activity prior to infection in June than individuals that were not infected ($p = 0.041$). After infection, infected fish also had higher open-field activity in the October trials (overall effect: $p = 0.003$), but here was only average swimming velocity significant among the tests of between-subject effects ($p = 0.016$).

Tests of between-subject effects (individual linear models for each dependent variable) showed that average swimming velocity and distance moved drove this effect, whereas the percent of time spent active did not seem to have any effect.

All three activity variables (average velocity, % active, and distance moved) were repeatable over the summer, at similar levels as previous fish studies (Bell et al., 2009), with correlation coefficients (r) of 0.38–0.41 (all $p < 0.028$), showing that individual fish were consistent in their behavior.

Paper II

In **paper II**, I investigated if infection intensity of glochidia had an impact on performance ability in brown trout using pairs consisting of one infected fish and one uninfected fish. From our results, I conclude that high infection intensity has an impact on performance, as individuals with high infection intensity made fewer interaction attempts, foraged less, and had an overall lower activity than individuals without infection.

There was also a pattern that higher glochidia load had a significant negative effect on the total number of chironomids captured by the infected fish (Spearman's $\rho = -0.61$, $p = 0.02$, $n = 14$), and that activity of the infected fish decreased with higher glochidia loads (Spearman's $\rho = -0.70$, $p = 0.005$, $n = 14$). Further, the number of initiated aggressive interactions decreased with higher glochidia loads for the infected fish (Spearman's rank correlation test: $\rho = -0.72$, $p = 0.004$, $n = 14$). The number of displays initiated by the infected fish was also observed to correlate negatively with increasing glochidia load, and highly infected fish did not display at all (Spearman's rank correlation test $\rho = -0.66$, $p = 0.01$, $n = 14$). Within pairs, infected trout showed darker coloration than non-infected fish (Sign test, $p < 0.001$). This pattern was observed in all pairs except one, where the two fish were scored to have the same coloration.

Paper III

In **paper III**, glochidia-infected trout generally took longer time to consume a maggot (mean ± se, 24 ± 1.3 s) compared to non-infected trout (17.5 ±1.9 s, Wald $\chi 2 = 4.37$, df = 1, $p = 0.037$). In contrast, the time to consume a *Gammarus* did not differ between glochidia infected trout (11.1 ± 0.8 s, and non-infected trout took (10.9 ± 1.1 s), (Wald $\chi 2 = 0.01$, df = 1, $p = 0.93$) (Figure 7).

Infected brown trout had lower growth rate (0.046 ± 0.046) than non-infected brown trout (0.169 ± 0.061, $F_{1, 16.6} = 6.39$, $p = 0.022$). A similar trend was observed for relative growth rate in length ($F_{1, 29.7} = 3.57$, $p = 0.069$) where infected trout had a relative growth rate of 0.027 ± 0.011 compared with non-infected trout that had a growth rate of 0.061 ± 0.016. Relative growth rate for length was positively correlated with the number of glochidia that trout sloughed off during the experiment ($r = 0.45$, $p = 0.046$).

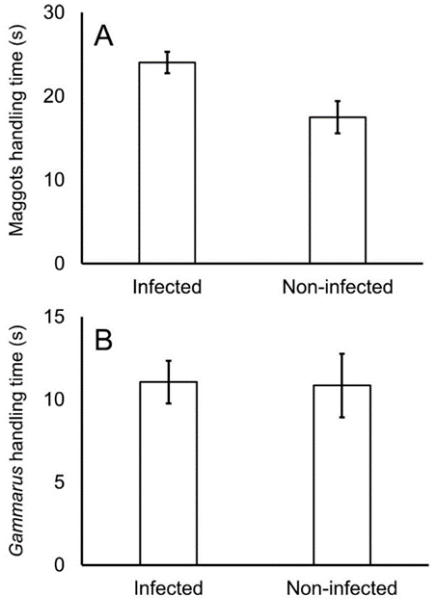

Figure 7. Prey-handling time in seconds of juvenile brown trout (Salmo trutta) infected and non-infected with freshwater pearl mussel (Margaritifera margaritifera) glochidia. Graphs show consumption times for maggots (Calliphoria vomitaria) (a) and Gammarus pulex (b).

Paper IV

In **paper IV**, brown trout infected with glochidia had a 26% higher standard metabolic rate (SMR) than non-infected fish ($F_{1,59} = 7.26$, $p = 0.009$). Similarly, maximum metabolic rate (MMR) was on average 11% higher in infected trout compared to non-infected trout (F1,59 = 4.27, p = 0.043) (Figure 8).

Hematocrit levels were 2.5% higher in infected brown trout compared to non-infected brown trout ($F_{1,55} = 4.50$, $p = 0.038$), and there was no significant difference between high or low infection intensity ($p = 0.66$).

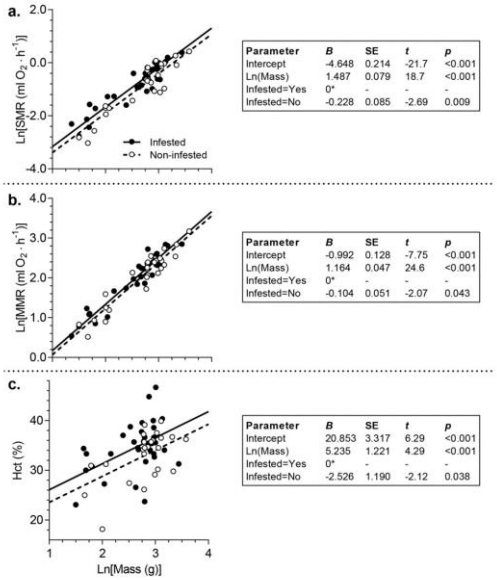

Figure 8. Linear relationships between mass (ln-transformed) and a standard metabolic rate (SMR) (ln-transformed), b maximum metabolic rate (MMR) (ln-transformed) and c haematocrit (Hct), for naturally infested and uninfested brown trout. Parameter estimates (B)

Paper V

In **paper V**, the results indicate that body size (mass) of the host fish increases the probability of being infected (ANODEV: $\chi 2 = 73.63$, $p < 0.001$), with small fish being more likely to be infected (Figure 9).

The infected trout covered a larger range in the field than non-infected individuals ($F_{1,86} = 4.99$, $p = 0.028$). There was also a difference in habitat use concerning bottom substrate in the autumn ($p = 0.046$), and flow conditions in autumn ($p = 0.042$) and in spring ($p = 0.002$). However, this pattern was only seen in one of the streams.

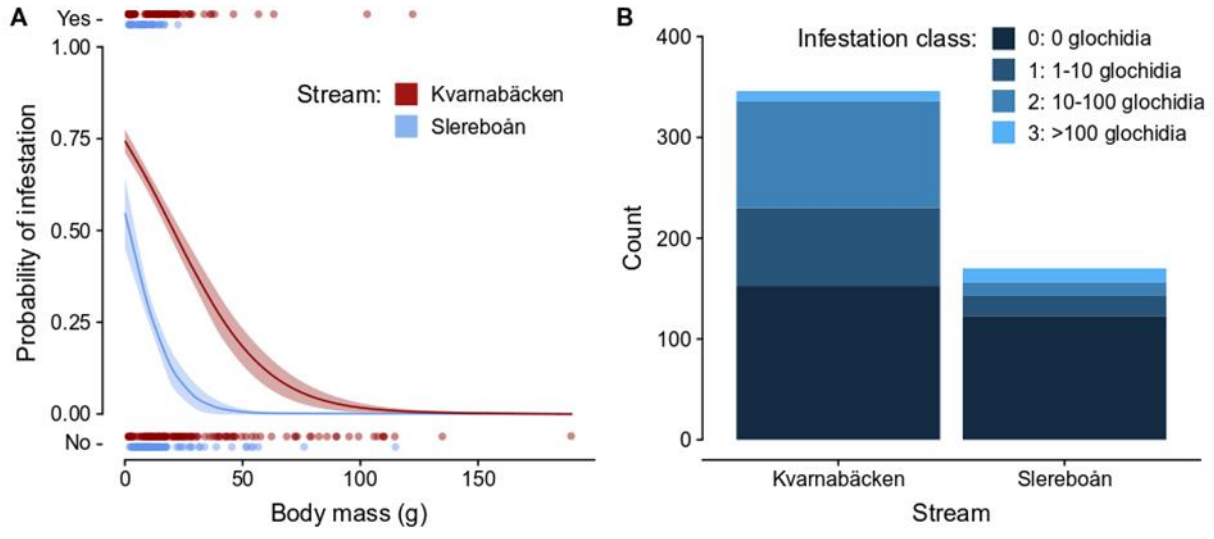

Figure 9. Infestation status in the two experimental streams. A) Probability of infested as predicted by body size. Regression line show the predicted risk of being infestation and shaded areas around the lines show 95% confidence bands. Dots at the top and bottom of the graph show data distribution for infestation ('Yes') and non-infestation ('No') trout. B) Number of fish in each infested class for the two streams.

Discussion

To summarize my work, I suggest that the FPM glochidia can be a potent parasite causing health problem to its host while infected. It is most evident when studying the behavior between infected and non-infected fish, and when the physiology of the fish is compared between infected and non-infected trout. However, there are some peculiar results that need further investigations, e.g., why do trout with high infection intensity show similar standard metabolic rates as non-infected trout, what is happening with the trout that manage to resist and even reduce the infection intensity, and following compensation growth, will the rapid growth enhance the risk of predation?

The question if an individual's behavioral type could potentially affect the risk of glochidia infection was tested in **paper I**, and the results suggest that it might, at least under the circumstance when the FPM are reintroduced in low numbers into a stream. Both activity and size measures differed between infected and non-infected brown trout and the traits were repeatable before and after infection, showing that individual behavior can affect the risk of being infected. Both activity and size have been shown to increase the risk of getting a parasitic infection, e.g., in pumpkinseed sunfish *Lepomis gibbosus* and brook trout *Salvelinus fontinalis* (Wilson et al., 1993; Poulin et al., 1991). If it is true that glochidia infections do not affect the individual behavior, as seen in this paper, this could be a

valuable tool for future studies investigating individual behavior between infected and non-infected fish host. Growth was not affected by the infection, probably because the infection intensity was very low (<10 glochidia per trout). Macro-parasites are suggested to be of low virulence unless the intensity is high (Anderson & May, 1979a). The low prevalence (14%) and intensity can also be explained by the low numbers of FPM being translocated to the study site (n=50), as FPM densities have been found to correlate positively with an increased prevalence (Österling et al., 2008). All mussels were gravid when we released them into Kvarnabäcken, but we do not know how many of them released mature and vital glochidia, and short-term breeders like FPM are known to release immature glochidia if stressed (Devers & Paterson, 2018).

In **paper II** we tried to answer the question if brown trout infected with glochidia would have a reduced competitive ability towards non-infected fish. When infected and non-infected brown trout was compared, regardless the intensity of their infection, there were no significant differences between the groups. However, when the intensity of infection was accounted for, foraging, activity, and interactions decreased with increasing intensity. Low intensity did not seem to affect the behavior of the trout, which was also seen in **paper I**. The results are in line with a previous study performed in a laboratory environment using artificially infected brown trout (Taeubert & Geist, 2013), where a high intensity of larvae increased the negative impact on the fish host (Taeubert & Geist, 2013). In **paper II** the infected fish were also darker colored, relative the dominant fish, which is a sign of subdominance in Atlantic salmon and brown trout (O'Connor et al., 1999; Höjesjö et al., 2011). However, we do not know if the infected trout were subdominant before the infection, and the potential change in social hierarchy is something I would like to investigate in the future.

In **paper III** the results suggest that infected brown trout have a decreased foraging efficiency and a reduced growth compared to non-infected trout, similar results have been found in recent studies (Österling et al., 2014; Chowdhury et al., 2019). The results also indicate a compensatory growth for those fish that were able to decrease the parasite intensity. The difference in prey handling time between the two food items presented may be explained by a novel prey effect of maggots (Sundström & Johnsson, 2001) but perhaps it is more likely that the size and texture of the maggot caused the longer handling time. The results from the growth experiment where infected fish had a decreased growth in mass compared to non-infected fish are in line with previous studies describing the differences in growth and competitive ability between infected and non-infected brown trout (Chowdhury et al., 2019; Filipsson et al., 2016). This will most likely be a disadvantage in nature for the infected fish. However, the decrease in intensity over time and the compensation growth observed in the study may reduce the negative impact from the infection.

In **paper IV** we investigated standard (SMR) and maximum (MMR) metabolic rate as well as levels of hematocrit (Hct) in glochidia infected and non-infected brown trout. Infected fish had significantly higher SMR and MMR and elevated levels of Hct compared to non-infected fish. Fish with a low infection intensity had the highest levels of SMR, MMR and Hct. Surprisingly, fish with high intensity had SMR levels like non-infected fish. We hypothesize that other physiological effects involved in energy utilization may cause this phenomenon, however, this needs to be further investigated. It is also interesting how even a low infection intensity can have a negative effect on SMR, MMR and Hct, as macro parasites in general are of low virulence and the immune response depends on the intensity (Anderson & May, 1979a). It seems like Hct levels may increase regardless of intensity levels, but this effect has not been detected before in FPM – brown trout interactions and it needs further investigations (Thomas et al., 2013). In chub *Leuciscus cephalus* parasitized by ecto- and endo parasites, levels of Hct were elevated when the intensity of the infection was elevated during periods before and after spawning (Rohlenová & Šimková, 2010).

In **paper V**, the results indicate that the probability of being infected by glochidia was higher for smaller brown trout and the risk was higher in Kvarnabäcken than in Slereboån. The results are in contradiction to the results of **paper I** where there was no significant difference in size between

infected (101.0 ± 7.0 mm) and non-infected (95.0 ± 3.2 mm) fish (Wengström et al., 2016). However, the results are in line with another previous study, which suggested that smaller fish are better host to FPM since they have not experienced any previous glochidia infections (Bauer, 1987c). However, that argument can be true for any trout of any size as long as they have not experienced any previous FPM infections (Geist et al., 2006). Further, there was a significant stream and infection status interaction throughout the experimental period where the difference in mass and length between infected and non-infected fish was larger in Kvarnabäcken than in Slereboån. A three-way interaction revealed a difference in condition between infected and non-infected fish at the last capture but not at the two previous times. The difference in condition between infected and non-infected fish was larger in Kvarnabäcken than in Slereboån and infected fish from Kvarnabäcken had better condition than non-infected fish in Slereboån. There was a trend that infected fish grew slower in both streams, but the difference was not significant. This is somewhat contrary to the results from Chowdhury et al. (2019) who found that growth was negatively affected by glochidia infection. However, the forced open field test in **paper IV** did not find any significant differences between infected and non-infected brown trout from Kvarnabäcken, but the scanning of where the different individuals were detected in the field revealed that the infected fish in Kvarnabäcken moved around more and covered a wider range than non-infected fish. The wider range covered in the stream by infected fish may be explained by a reduced competitive ability in the infected fish. As seen in **paper II** the competitive ability of infected brown trout goes down with an increased infection intensity, and if the intensity is high enough the infected fish do not compete for assets but are more likely to avoid interactions. In Kvarnabäcken the non-infected fish did not cover as large range as the infected fish, whereas this pattern could not be seen in Slereboån.

The immune response caused by macro parasites generally depends on the intensity of parasites in each host and it seems to be over a brief period (Anderson & May, 1979a). In **paper II** our results indicate that an increased glochidia load influenced brown trout behavior and competitive ability towards conspecifics, and the effect on the fish was most likely a response to the glochidia infection and particularly the glochidia intensity. The effect could not be seen before we looked at the intensity on the individual fish. In the wild we can assume that interactions take place between infected and non-infected brown trout and depending on the infection intensity the fights for resources like habitat or food will be relatively short or if the intensity is high, there will be no fights at all. This may force infected brown trout with high loads of glochidia into low quality habitats. We can assume that the effect from glochidia is constant during the infection as we have data from both fall (**Paper II**) and spring (**Paper V**) indicating a disturbance on infected brown trout. We do not know if the effect remains post infection, but probably not, as indicated in **paper III,** the effect from intensity on growth decreased as glochidia were sloughed off during the experiment.

Conclusions

In summary of all papers, I conclude that the parasitic phase in the life cycle of the FPM means a burden on the fish host as seen in both behaviour and physiology. The burden seems related to the intensity of the infection. However, I do not see any spectacular behaviour alteration caused by the glochidia infection. It seems more like the glochidia infection sets the host in a disease state that lowers activity of the infected fish rather than manipulates the behaviour. The reduction in competitive ability, decreased metabolic rate and growth rate are all signs of a health problem probably caused by the infection. This will most likely decrease host fitness.

In line with previous investigations performed in a laboratory environment with artificially infected fish hosts and in relation with what we have discovered with naturally infected fish hosts, I conclude that what has been seen on lab can also be found in nature. The use of wild and naturally infected fish can have both negative and positive effects on the results that can bias the conclusions in any

direction. On the negative side there is the lack of control, as in the wild the glochidia prevalence and infection intensity cannot be controlled and it is difficult to get equal numbers of fish with and without glochidia, and it is even more difficult to get infected fish with various levels of infection intensity. Further, I cannot know if the infected fish are bad competitors or of a lower social rank from the beginning. On the positive side, I have investigated fish that have been naturally infected for different reasons and in my opinion the results from these studies reflect a more accurate picture of what is happening in the wild than I would have gained if I had used hatchery reared fish. Further, I believe that we have added important knowledge to the question whether the FPM is parasitic or not.

Application for management

The knowledge about which effects the FPM has on its fish host can be used in propagation projects. All propagation projects aim to have as many juvenile FPM as possible to survive and to achieve that it is necessary to raise the intensity of the infection on every single fish. According to our results I would suggest that the intensity of the infection should be as high as possible but without killing the fish. We have used a concentration of 100 000 glochidia L^{-1} with good prevalence and infection intensity, and without mortality. In comparison, Taeubert & Geist (2013) recommended concentrations between 15 000 and 75 000 glochidia L^{-1}. A low intensity of the infection seems to give more stress to the fish than a high intensity, as SMR, MMR and Hct levels were higher in fish with low intensity (**Paper IV**). The willingness to interact with conspecifics did also go down in fish with high intensity of the infection (**Paper II**). It might be of interest to consider the size and origin of food when feeding fish in propagation projects as seen in **paper III**.

Future recommendations

I think individual behavior is interesting, and I would like to see more Before-After-Control-Impact (BACI) studies to investigate if individual behavior plays a role in which fish gets infected. Other things that I find interesting to investigate are the effect of glochidia on the different types of fish host defenses, parasite avoidance, the immune response, and the tolerance to manage an infection, and these kinds of investigation would be great for a BACI design. The trophic relationship between *Unio crassus* and its fish host indicates interesting new stories (Denic et al., 2015), and as *U. crassus* glochidia have a relatively short development time and a relatively fast growth it would be possible to investigate fitness effects on the mussel in correlation to different fish host traits, and fitness effects on the fish hosts. It would also be interesting to investigate if glochidia from other mussel species have similar effects on behavior and physiology of their fish hosts as the FPM has.

There are some interesting new hypotheses regarding the role of parasites as drivers of ecosystem processes, that would be interesting to investigate with focus on the effects from Unionids.

Even if I have not written much about diseases in mussels in this thesis, I think this also is a very interesting subject and something we need to get greater knowledge of as climate changes, giving new possibilities for pathogens to spread into new areas. We are just in the beginning to understand the pathogens threatening freshwater mussels.

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