Behavior and metabolic rate of brown trout infested with parasitic larvae of the freshwater pearl mussel

Karl Filipsson
Degree project for Master of Science in Biology

Animal Ecology, 60 hec, ht 2014-vt 2015
Department of Biological and Environmental Sciences
University of Gothenburg

Supervisor: Johan Höjesjö
Examiner: Lotta Kvarnemo
Abstract

The freshwater pearl mussel *Margaritifera margaritifera* is a Holarctic bivalve, endangered throughout its native range. The species life cycle includes an obligate parasitic larval phase (glochidia) on the gills of a salmonid host. This study aims to investigate the relationship between glochidia infestation, activity and dominance in brown trout. Dominance is often linked to fitness, and a decrease in fitness of the host may influence survival and dispersal of the mussels. Paired juvenile trout, one infested and one uninfested, were fed chironomid larvae in a flow-through stream tank while activity, agonistic behavior and coloration of the fish were observed. No differences were found in activity or number of initiated interactions between infested and uninfested fish. However, trout with lower glochidia loads were significantly more active and initiated more interactions than fish with higher loads. Infested trout had significantly darker color than uninfested, which may be a sign of subordinance. To examine if the observed differences may be an effect of impaired oxygen consumption for the infested trout, a complementary study was performed where infested and uninfested trout were put in respirometry chambers. No difference was found in metabolic rate between infested and uninfested trout. However, decreasing metabolic rates was observed to be correlated to higher glochidia loads. These results indicate that infestation of *M. margaritifera* glochidia relate to lower activity, competiveness and metabolic rate in brown trout. Infestation rate varied from one or a few glochidia which most likely do not affect the fish, to several hundred which may have detrimental effects. This may be the reason why no differences were observed between infested and uninfested fish, but differences were observed between fish with high and low infestation rates. To increase knowledge further it is suggested that studies are carried out using more fish with higher glochidia loads when investigating differences between groups. It is also suggested that activity and other parameters are examined prior infestation, to investigate whether glochidia affect the host or if fish with certain traits are more susceptible to glochidia encystment.
Contents
Introduction .......................................................................................................................... 3
  Parasitic glochidia and trout foraging behavior .......................................................... 5
  Parasitic glochidia and trout activity and dispersal .................................................. 5
  Juvenile trout agonistic behavior ............................................................................. 6
Aim and hypotheses .............................................................................................................. 6
Methods ............................................................................................................................. 7
  Behavior study ............................................................................................................. 7
  Metabolism study ....................................................................................................... 11
Results ................................................................................................................................. 13
  Glochidia load .............................................................................................................. 13
  Ventilation rate .......................................................................................................... 14
  Activity ......................................................................................................................... 16
  Interactions .................................................................................................................. 17
  Coloration .................................................................................................................... 18
  Standard metabolic rate ............................................................................................. 18
  Aerobic scope .............................................................................................................. 18
  Hematocrit ................................................................................................................... 18
Discussion ........................................................................................................................... 19
  Parasitic glochidia and brown trout behavior ......................................................... 19
  Parasitic glochidia and brown trout metabolic rate ................................................. 20
  Implications and suggestions for future research ................................................... 21
  Implications for mussel-host fish ecology and management .................................. 22
Acknowledgements .......................................................................................................... 24
References .......................................................................................................................... 24
Introduction

Freshwater pearl mussels (Bivalvia: Unionoida) are among the most endangered aquatic organisms in the world (Lydeard et al. 2004; Strayer et al. 2004). The freshwater pearl mussel *Margaritifera margaritifera* is a Holarctic bivalve belonging to the family Margaritiferidae and the order Unionoida. The species distribution ranges throughout Europe, Canada and northern USA. It has a maximum lifespan of over 150 years, making it one of the most long-lived and slowest growing invertebrates known (Ziuganov et al. 2000; Anthony et al. 2001). *Margaritifera margaritifera* is listed as an endangered species throughout its native range (Moorkens 2011), and it is the freshwater pearl mussel species that has suffered the greatest population declines (Young et al. 2001; Hastie et al. 2003). Causes for the decline of *M. margaritifera* populations are habitat degradation, pollution, increased siltation, reductions of host fish populations, pearl fishing and construction of dams. All known causes for the declines are attributed to human activities (Watters 1996; Vaughn & Taylor 1999; Cosgrove et al. 2000; Morales et al. 2004; Thomas 2011; Arvidsson et al. 2012). Over half of the world’s reproductive populations of *M. margaritifera* occur in Scotland. Large populations also occur throughout Scandinavia (Cosgrove et al. 2000; Young et al. 2001).

The life cycle of *M. margaritifera* includes an obligate parasitic phase on the gills of a salmonid host (figure 1); Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, in Europe (Young & Williams 1983; Ziuganov et al. 1994; Hastie & Young 2001), and Atlantic salmon and brook trout, *Salvelinus fontinalis*, in North America (Smith 1976; Cunjak & McGladdery 1991). Also, artificial infestation in captivity has led to encystment on arctic char, *Salvelinus alpinus*, (Thomas 2011). However, the most common host fish species for *M. margaritifera* is brown trout. The mussel larvae, called glochidia, are released in summer by gravid females and reach their hosts passively via the water current. After inhalation by the fish, the glochidia attach to the gills and get encysted by epithelial cells on the host’s gills (Young & Williams 1984; Hastie & Young 2003). While encysted, the glochidia obtain nutrients from the tissue of the host fish (Rogers-Lowery & Dimock Jr 2006). During encystment the glochidia grow 6-10 times in size and metamorphose into juvenile mussels (Ziuganov 2005). The juvenile mussels excyst from the host fish after approximately 10 months to bury into a suitable cobble and gravel streambed (Young & Williams 1983, 1984; Dillon 2000). However, the time from encystment to encystment on the host fish may vary with surrounding factors, such as the ambient water temperature (box 1).

![Diagram of the life cycle of the unionoid freshwater pearl mussel *M. margaritifera*](image.png)

**Figure 1** Life cycle of the unionoid freshwater pearl mussel *M. margaritifera* in Sweden. A) Fertilization of female mussels occurs during early summer. B) The glochidia are released in late summer, and infect the gills of brown trout or Atlantic salmon. C) Juvenile mussels excyst from the gills of the host fish in late spring or early summer the year after encystment and become benthic.
BOX 1. The effect of water temperature on M. margaritifera glochidia

One of the main factors influencing the time period M. Margaritifera spend on their host’s gills as parasites is the ambient water temperature. Hruska (1992) found that M. margaritifera require a mean water temperature of ≥ 15 °C for at least 4 days at the end of the parasitic phase for successful excystment. However, this observation has never been empirically verified (Taeubert et al. 2013). Taeubert et al. (2013) examined how many day degrees M. margaritifera required for completion of metamorphosis under constant water temperatures between 11 and 12 °C. The sum of day degrees from excystment to completion of metamorphosis ranged between 1700 and 3440. However, at the beginning, and the end of the period, few mussels excysted, and more than 80 % of all mussels excysted over a 73 day period between 2220 and 3080 day degrees. The peak daily excystment with 503 mussels (2.6 % of total excysted mussels) was found at 2530 day degrees (217 days) after infestation. However, the start of excystment for M. margaritifera appears highly variable (Hruska 1992; Ziuganov et al. 1994; Thomas et al. 2010; Taeubert et al. 2013). Hruska (1992) observed start of excystment after 1300 day degrees under constant water temperatures between 15.5-17 °C. This indicates that the excystment period of the glochidia is shorter in water with higher temperatures (Hruska 1992; Hastie et al. 2003). This may be beneficial for the glochidia since it decreases the time period of exposure to the host’s immune response, as well as it reduces risk that the host gets predated upon. High water temperatures may increase the fish’s immune response as the metabolic rate of the fish increase (Clarke & Johnston 1999; Tocher 2003). Thus, higher water temperatures may enhance the trout’s immune response targeting the glochidia. However, high water temperatures can have deleterious effects on brown trout (Geist et al. 2006), which makes it rather unlikely that the trout should disperse toward warmer water. Infested fish may disperse to lower water temperatures than uninfested fish in order to access more oxygen from the water since oxygen is dissolved to a higher extent in water with lower temperatures (Allan & Castillo 2007).

Taeubert and Geist (2013) showed with artificially infested brown trout that high loads of M. margaritifera glochidia reduced swimming capability of the fish and also increased host mortality. Trout mortality started to occur at infestation rates of ~350 glochidia per gram fish weight, and was highest at infestation rates of ~900 glochidia per gram fish weight. However, it should be noted that these densities of glochidia are significantly higher than any glochidia density found on naturally infested fish. Additionally, Thomas et al. (2014) found that gill lamellae of brown trout encysted with glochidia were significantly thicker and longer than those of uninfested trout, which inhibited the trout’s respiratory capacity. Consistent with this, they found that infested trout took longer to reach basal ventilation rates than uninfested fish, suggesting that the glochidia pose a respiratory burden for the trout. These results indicate a parasitic relationship between M. margaritifera and its host. In the same study mean blood hematocrit of infested and uninfested fish was examined. No significant difference was found in mean blood hematocrit between infested and uninfested fish.

Encystment of glochidia on the gills of brown trout also results in an immune response by the fish targeting the glochidia. On a definite host, i.e. a fish species which functions as a host for M. margaritifera 28-100 % of the glochidia survive on the host’s gills (Bauer & Vogel 1987; Dodd et al. 2005). The rest of the glochidia are excysted from the fish when cysts formed by the fish’s immune response are sloughed off the gills, which often sheds a large number of glochidia (Hastie & Young 2003). This immune response by the fish also indicates a parasitic relationship between the glochidia and its host (Thomas 2011). Thomas (2011) also noticed a significantly enlarged spleen in infested brown trout 31 days post-exposure, which is also an effect of increased immune response. In the case of M. margaritifera, brown trout young-of-the-year (YÖY) fish often has the largest densities of glochidia encysted to their gills. This may be explained by that the immune response of YÖY trout are not as strong and fully
developed as in larger fish (Bauer & Wächtler 2001; Hastie & Young 2001; Österling et al. 2008).

Parasitic glochidia and trout foraging behavior
Brown trout inhabiting streams obtain much of their food through drift feeding, where they hold a position in the stream and capture invertebrates as they drift past the trout’s position, often called the focal point (Fausch 1984; Hughes et al. 2003). The net energy intake of the trout depends on how much energy it lose while capturing prey in relation to the energy intake, which is dependent on the size and abundance of prey items drifting downstream. The focal point is often at locations where the trout’s net energy intake is optimized, for example in depressions behind stones where the fish is not affected by the water current but still has good visibility over down-drifting prey (Hughes & Dill 1990). Many different factors can affect the foraging behavior of brown trout. Foraging efficiency of trout has been observed to decrease with lower water temperatures, as an effect of reduced metabolism (Elliot 1994; Watz & Piccolo 2011). Other factors which may affect the foraging efficiency are distance to the prey (Fausch 1984; Hughes & Dill 1990), water depth and velocity (Piccolo et al. 2007, 2008a, b). Food intake can also be dependent on biotic factors such as predation risk or concurrence over valuable resources such as good focal points. These factors may lead to that trout choose foraging locations which are not the best in terms of net food intake (Hughes & Dill 1990). Parasites is another biotic factor which can influence its host negatively and thus reduce the trout’s net energy intake (Sadava et al. 2008).

Very little is known about how parasitic glochidia of M. margaritifera affect foraging behavior of brown trout, only one study is published where this has been investigated. Österling et al. (2014) found that uninfested trout had a significantly higher drift foraging rate than infested trout. The uninfested trout also caught more prey items further away from the focal point. The reduced foraging efficiency of the infested trout was primarily dependent upon their failure to catch prey items far away from the focal point. These results suggest that reduced foraging capability of infested trout may be caused by poorer energetic status. However, the physical effects of glochidia on trout prey handling time as glochidia encystment may impair foraging ability by reducing the efficiency of gill raker functioning, could not be ruled out in the experiment (Österling et al. 2014).

Parasitic glochidia and trout activity and dispersal
One of the main selective advantages of parasitism for freshwater pearl mussels is probably transportation on the host, resulting in dispersal of the mussels (Watters 2001). This may be beneficial regarding the highly limited dispersal capacity of the adult mussels (Horký et al. 2014). Dispersal of the larvae may lead to avoidance of inbreeding and kin competition. However, to increase fitness of the mussels the benefits of dispersal must be higher than the costs (Ranta & Kaitala 2000; Bowler & Benton 2005). Otherwise costs related to settlement, finding suitable habitats, and increased predation risk could outweigh the benefits of dispersal (Milner-Gulland et al. 2011). Research about whether parasitic freshwater bivalves such as M. margaritifera affect host activity and dispersal has gathered little attention. Regarding the critical conservation status of M. margaritifera, as well as other freshwater mussels, it could be of interest to investigate if the parasites affect dispersal of the host fish and thereby dispersal of itself. Few studies have been conducted in this field, and the studies performed indicates that encystment of freshwater mussel glochidia reduce activity and dispersal of the host (Crane et al. 2011; Thomas 2011; Taeubert & Geist 2013; Horký et al. 2014). Thus, these results do not support the hypothesis that mussels benefit from transportation on the host. However, a study conducted on various species of freshwater mussels and host fish
species in Ontario, Canada, showed that the host fish were an important factor shaping the distribution of the mussels (Schwalb et al. 2013).

Thomas (2011) found that if the scent of European otter (*Lutra lutra*) was added to the water, risk taking behaviors and activity of the trout significantly decreased, regardless if the trout were encysted with glochidia or not. These results indicate that the glochidia of *M. margaritifera* do not impair predator recognition by the trout and that it instead may increase host survival overall by reducing activity and dispersal of the host, and thereby limiting contact with predators.

**Juvenile trout agonistic behavior**

In organisms competing by interference, socially dominant individuals are often more successful than subordinates in defending resources such as food, shelter and mates (Johnsson & Björnsson 1994). Dominance is therefore often regarded a reliable indicator of fitness in these cases. Also, dominant individuals are known for being more active, offensive and for having a higher food intake than subordinate individuals (Huntingford & Turner 1987). Juvenile trout are known for having a wide array of agonistic behaviors for establishing dominance (Höjesjö et al. 2002). There is a series of agonistic signals and aggressive behaviors, such as displays, circling behaviors, chases, attacks and biting (Stuart 1953; Kalleberg 1958). In hierarchies of juvenile brown trout, the fish test their rank by display behaviors and the contest do not often escalate any further into physical interactions between the two combatants (Keenleyside & Yamamoto 1962). The subordinate individual turns dark and dull in coloration and folds its fins to signal its lower rank, whereas the dominant fish displays with bright coloration and erect fins (O'Connor et al. 1999, 2000). However, physical interactions between two opponents may escalate when their fighting ability is similar (Jakobsson et al. 1979; Leimar & Enquist 1984; Leimar et al. 1991).

Forming stable social hierarchies may allow familiarity to develop between neighboring juvenile brown trout. This can give fish the ability to distinguish between conspecifics. Thus, if an individual can memorize the fighting ability of an opponent, costly fighting and escalated disputes can be avoided. This can decrease the number of interactions with neighboring conspecifics which can be physically harmful for the fish, which also means that escalating disputes arise more often between unfamiliar fish (Höjesjö et al. 1998). This is commonly known as the dear enemy effect, and is supported by game-theory based hypotheses which explain familiarity as an effect of this phenomenon (Höjesjö et al. 1998; Piper 2011; Alcock 2013). To this date no studies have been published which investigate how glochidia of *M. margaritifera* affect competition behavior and dominance in juvenile salmonids. The same holds for studies on other parasitic mussels and host fish species, where no information seems to have been gathered.

**Aim and hypotheses**

The overall aim with this thesis is to increase knowledge of freshwater mussel biology and conservation through studies of the interaction between *M. margaritifera* and its host brown trout. I want to investigate how brown trout infested or uninfested with glochidia differ in foraging and dominance behavior.

This thesis will cover the activity and competition sections of the study, whereas the parts on foraging behavior will be presented in the master thesis written by Tina Petersson at Karlstad University. I hypothesize that there is a difference in (1) activity, (2) competition capability and (3) coloration between infested and uninfested trout. The prediction is that juvenile trout
with *M. margaritifera* glochidia on their gills are not as active and will have reduced competition capabilities when compared to uninfested fish. I also predict that fish with lower glochidia loads are more active and initiate more interactions than fish with higher glochidia loads. Regarding coloration I predict that infested trout will show subordinance towards uninfested fish by displaying dull and dark coloration, as an effect of poorer energetic status and reduced fighting capabilities caused by the glochidia.

In order to gather further knowledge on how glochidia of *M. margaritifera* affect its host, a complementary study on metabolic rate and hematocrit of infested and uninfested brown trout was performed. Here I hypothesize that there is a difference between infested and uninfested fish in metabolic rate. The prediction is that fish infested with parasitic glochidia will have lower metabolic rates than uninfested individuals because of the impaired oxygen uptake as the parasites physically inhibit respiration. Thus, I also predict that fish with higher glochidia loads will have lower metabolic rates than fish with lower glochidia loads. Regarding hematocrit I do not hypothesize that there is a difference between infested and uninfested fish, since no previous studies investigating this aspect have shown any relationship between glochidia encystment and the amount of erythrocytes in the blood (Thomas *et al.* 2014).

**Methods**

**Behavior study**

**Experimental Procedures**

Juvenile brown trout were collected October 1-2, 2014, from the stream Slersboån in the Göta Älv catchment area in southwestern Sweden 40 km NE of Gothenburg, using standardized electrofishing methods (Bohlin *et al.* 1989). The *M. margaritifera* population size in Slersboån is estimated to be approximately 10,000 individuals, with low recruitment of new mussels (personal communication, Niklas Wengström, 15 April 2015). The trout were visually assessed in the field while anesthetized (2-phenoxyethanol, 0.5 ml/l), to determine if they were infested or not. While sedated, fork length of each individual trout was measured. Trout fork length was plotted in a histogram to enable differentiation between year classes (figure 2).

A total of 118 trout were caught for the experiments (9 infested and 67 uninfested 0+, and 9 infested and 33 uninfested 1+). The fish were kept in containers within the stream until they were transported in well-oxygenated tanks to the Department of Biology, Karlstad University, on October 3rd, 2014. The fish were put in six 200-liter aquaria which were 1 m long, 0.5 m wide, and 0.4 m deep. Infested fish were kept in one aquarium and uninfested in the remaining five, with approximately the same number of trout in each aquarium.

![Figure 2](image-url)  
**Figure 2** Frequency distribution over length of juvenile brown trout caught in Slersboån first and second October 2014 (n= 161).
The water temperature in the aquaria was held constant at 12°C. The specified temperature was chosen as it was the same temperature as measured in Slereboån at the time when the fish were collected. The light regime was 9.5 hours daylight, 1 hour dusk, 12.5 hours darkness and 1 hour dawn each day. The light intensity was approximately 100 lux at water surface level during the daylight period. These measures approximate the natural light regime for October an overcast day in the region the trout originated from. The aquaria were constantly filtered (Eheim 2217), and 25 % of the water in each aquarium was changed 1 time each week. Thawed, previously frozen bloodworms (Chironomidae sp.), were given to the fish as food. The fish were fed approximately 2 % of their body weight two times each week.

The experiments started October 17th, 2014. Seven infested and seven uninfested trout from each year class were chosen for the experiments. Trout were anesthetized (Benzocaine, 0.1 g/l) and their length and weight was measured. The fish were paired, one infested and one uninfested from the same year class. Each pair was size-matched to control that size did not affect the outcome of the study (table 1). No significant differences in fork length (Mann-Whitney U test, U=95 p=0.89) or weight (Mann-Whitney U test, U=92.5, p=0.80) were observed between the infested and uninfested fish. One fish in each pair (half of the infested and half of the uninfested) were randomly chosen and marked by adipose fin-clipping to enable differentiation of the fish in each trial. Earlier studies have shown that removal of the adipose fin does not affect behavior of juvenile trout (Sundström et al. 2003), and this is now a common procedure for marking juveniles (Höjesjö et al. 2011). The remaining four infested trout were not included in the experiments, as it was not able to adequately size-match them with any of the uninfested fish.

The influence of glochidia encystment was investigated in the upstream section of 7 meter long stream tanks. One side of the tank had a glass window for enabling observations and video recording of the experiments. The other surfaces of the tank were painted in a light gray color. Arenas were constructed in the upstream section of the stream tanks and were 1.4 m long, 0.61 m wide, and 0.2 m deep and demarcated from the rest of the stream tank with stainless steel mesh grids (mesh size 5.35 mm, thread 1.0 mm, 71 % open area). The bottom of the stream tanks was covered with gravel (0.5-20 mm), which was arranged to form a slight incline at both ends. A single larger stone (9 cm long, 6 cm wide and 3 cm high) were placed in the middle to serve as a focal point. A small depression was made behind the stone (figure 3). The mean water velocity in the stream tanks was approximately 0.2 m•s\(^{-1}\), which approximates the velocity at which juvenile brown trout are known to hold a drift feeding station (Österling et al. 2014). The water temperature and light regimes were the same as in the holding tanks. The fish were starved at least 24 hours prior the experimental trials. One pair of fish consisting of one infested and one uninfested size-matched trout from the same year class were netted from the aquaria into a stream tank. The trout were allowed to acclimatize for 15 minutes in the stream tank prior the experiments.

<table>
<thead>
<tr>
<th>Pair</th>
<th>Infested</th>
<th>Uninfested</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>59</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
<td>66</td>
<td>2.7</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>61</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
<td>58</td>
<td>2.1</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>58</td>
<td>2.3</td>
</tr>
<tr>
<td>7</td>
<td>86</td>
<td>84</td>
<td>6.3</td>
</tr>
<tr>
<td>8</td>
<td>99</td>
<td>101</td>
<td>9.3</td>
</tr>
<tr>
<td>9</td>
<td>98</td>
<td>95</td>
<td>9.2</td>
</tr>
<tr>
<td>10</td>
<td>101</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>110</td>
<td>110</td>
<td>13.5</td>
</tr>
<tr>
<td>12</td>
<td>98</td>
<td>96</td>
<td>9.8</td>
</tr>
<tr>
<td>13</td>
<td>106</td>
<td>107</td>
<td>10.8</td>
</tr>
<tr>
<td>14</td>
<td>111</td>
<td>110</td>
<td>11.9</td>
</tr>
</tbody>
</table>
This acclimatization period was chosen because during pilot studies prior to the experiments longer acclimatization lead to that the trout established dominance by aggressive interactions before the foraging trials, which resulted in no conflict during the experiments. It was also seen during the same pilot studies that the trout started to look for food after a couple of minutes in the stream tanks.

During the experimental trials fish were fed one thawed red chironomid larvae (less than 10 mm long) each 15 seconds for 20 minutes. The food items were delivered through a plastic tube which entered the stream tank in the middle of the upstream cross-section of the stream tank at 10 cm water depth. The tube was attached to a grid so that it would hold its position. The grid also separated the fish from where the feeding apparatus was located, and also from where water entered the stream tank where a lot of turbulence and back eddies occurred. For each fish position in the tank, activity, initiated aggressive interactions, coloration, number of foraging attempts and number of food items caught were noted every 15 seconds. Three hours after the foraging trials the fish were again monitored for 10 more minutes. During the latter monitoring session position, activity, initiated aggressive interaction and coloration were noted every 15 seconds. The purpose of the last monitoring session was to control that the trout were acclimatized to the stream tanks and behaved the same way as during the foraging trials. Ventilation rate was noted for each fish before and after the foraging trial and at the monitoring session after three hours of acclimatization. All the experiments were video recorded (Canon XA10) to simplify and verify collection of data. During the video recording, verbal notes regarding each fish position, activity, color, aggressive behavior and foraging behavior was recorded. After the experiments all fish (except the infested trout, see below) were released back into Slereboån.

**Figure 3** A schematic sketch of the stream tanks used for the experiments. Prey were delivered through a plastic tube into the tank and drifted through the experimental arena. A rock was placed in the middle of the tank to serve as a focal point. The experiments could be observed and video recorded through a glass window on the side of the stream tank.

The treatment of data and statistical analysis involved estimating the glochidia load when all experimental trials had been performed the infested fish were euthanized and put in 95% ethanol to assess the number of glochidia on their gills. The glochidia were counted using a stereo microscope and by gently lifting each fish’s gill arches. The number of glochidia was counted on each gill arch, and was thereafter summarized for each fish. After assessment the glochidia were stored in plastic tubes filled with ethanol. The glochidia were stored in one separate tube for each fish.
Glochidia load was standardized by fish body weight, which is the most common method to analyze the effect of and present glochidia load in scientific studies (Taeubert & Geist 2013; Österling et al. 2014). Thus, this standardization was used for enabling comparison with other studies. Since fish with low infestation rates were more abundant than fish with high infestation rates, the standardized measure of glochidia load was transformed by base 10-logarithms to get a better continuum of the data and an adequate normal distribution

Ventilation rate
Ventilation rate was estimated three times for each trout as a measure of stress and metabolic rate (see Millidine et al. 2008). It was measured directly when the trout were put into the stream tanks, after approximately one hour, and three hours after the trout initially were put into the stream tanks. The number of opercular beats of each fish was counted using the zoom-in function on the video camera. During each video recording the number of opercular beats from each fish was counted during one to four 15 second periods, from which a mean ventilation rate was estimated as number of opercular beats per minute. The number of 15 second periods varied with how many representative sessions that could be filmed for each fish. Some fish lay with its head away from the camera or was swimming around rapidly most of the time, which inhibited counting of opercular beats. Differences in ventilation rate over time for both infested and uninfested trout were examined using one-way Anova and post-hoc analyses (IBM SPSS Statistics 20, the statistical software used for all tests in this study). Differences in ventilation rate between infested and uninfested fish were examined using a paired t-test. Pearson’s correlation was conducted to look for relationships between glochidia load and ventilation rate.

Differences in ventilation rate between year classes were examined by subtracting ventilation rate of the uninfested fish with ventilation rate of the infested fish for each pair. These values were thereafter compared between year classes using independent samples t-tests. This procedure was conducted to test if the pairwise effect of glochidia was more pronounced in any of the year classes. To directly compare ventilation rate between infested fish from different year classes would not take the influence of the uninfested fish into account, therefore this procedure was chosen. Hence, this procedure was subsequently used in all further analyses where differences between year classes were examined. As the data for ventilation rate fulfilled the properties of a normal distribution, parametric tests were used.

Activity
During the monitoring session a measure of fish activity was taken each 15 seconds. These measures were either 1 for lying on the bottom, 2 for standing still in the water column, 3 for cruising through the aquarium, and 4 for actively swimming. All 80 measures from these 15 second periods over the total 20 minute session were added together, which meant activity was summarized in each fish’s total activity score. Difference in activity between infested and uninfested fish was tested with a paired t-test. Pearson’s correlation was used to investigate relationships between activity and glochidia load for the infested fish. Difference in activity between year classes was examined using a Mann-Whitney U test. Parametric tests were chosen when difference in activity was examined between infested and uninfested fish and fish with varying glochidia loads as the data fulfilled the properties of a normal distribution. Non-parametric tests were used when difference in activity was investigated between year classes, as the data did not fulfill the parametric criteria.
Interactions
During the monitoring session every interaction initiated by each fish was noted. The following interactions were observed: (1) Display, either lateral or frontal, where the fish erects all its fins (2) Circling, where the two fish circle around each other (3) Attack, where one fish charges at the other (4) Bite, and (5) Hunt, where one fish pursues the escaping combatant (Höjesjö et al. 2002). The number of interactions for each fish was summarized.

Differences between infested and uninfested fish were tested with a Wilcoxon signed rank test. Spearman’s correlation was used to investigate relationships between the total number of initiated interactions performed and glochidia load, as well as the number of initiated displays and glochidia load. When examining differences between year classes, a Mann-Whitney U test was used. Non-parametric tests were used because data did not fulfill the properties of a normal distribution.

Coloration
Differences in coloration were examined using a three category color scoring (see O’Connor et al. 1999, 2000), where fish with light coloration were scored 1, fish with intermediate coloration 2, and fish with dark and dull coloration 3. Differences in coloration between infested and uninfested trout were examined using a Sign test, where the fish in each pair with the lightest coloration was scored 0 and the one with darkest coloration 1. Differences in coloration between year classes were examined using a Mann-Whitney U test. Non-parametric tests were used because ordinal data was used when investigating differences in coloration.

Metabolism study
Experimental Procedures
Juvenile brown trout were collected April 17 2015, from the stream Lindåsabäcken, Häggån catchment area, in southwestern Sweden approximately 60 km east of Gothenburg, using standardized electrofishing methods. The population size of M. margaritifera in Lindåsabäcken is estimated to hold 10 000-20 000 individuals (personal communication, Niklas Wengström, April 15, 2015). Lindåsabäcken was chosen as the probability of capturing infested fish was expected to be higher there compared to Slerboån. A total of 62 brown trout were caught, 35 infested and 27 uninfested. The trout were visually assessed in the field while anesthetized (MS-222, 5 ml/l), to determine if they were infested with parasitic glochidia or not. No differentiation between year classes was carried out for this study, as results gathered the previous fall showed no difference between year classes.

The trout were transported to the zoology building at the department of biological and environmental sciences, Gothenburg University, the same day as they were caught. The fish were put in one 120-liter aquarium which was 0.64 m long, 0.48 m wide, and 0.4 m deep, where they were allowed to acclimatize for 9 days. The water temperature in the aquarium was kept at approximately 12 °C. The light regime was 12 hours daylight and 12 hours dark. The light intensity was approximately 100 lux at water surface level during the daylight period. The trout were not fed prior the respirometry experiments to allow gut evacuation. As the experiments started, individual trout were carefully netted from the holding tank, anesthetized (2-phenoxyethanol, 0.5 ml/l), weighted, and thereafter placed into cylindrical, intermittent flow-through respirometers (volumes of 0.584 or 1.112 liters depending on fish size), which were submerged in a reservoir bath containing flow-through, aerated freshwater (10 °C). The fish were left undisturbed in the respirometers for ~21 hours while oxygen
consumption was measured. A black lid was put over the respirometry chambers to reduce stress in the fish. Water was continuously circulated through each respirometer using an in-line submersible pump within a recirculation loop, and the oxygen concentration of the water in the respirometers was measured continuously at 0.5 Hz using a FireSting O₂ system (PyroScience, Aachen, Germany) calibrated in accordance with the supplier’s manual. Automated flush pumps refreshed the water in the respirometers for 5 minutes in every 20 minute period, ensuring that oxygen levels in the respirometers always remained above 90% air saturation. Oxygen consumption was calculated from the decline in oxygen concentration in the respirometers during each 15 minute period between flush cycles.

Following this period, fish were individually removed from their respirometers and subjected to an exhaustive exercise protocol (Clark et al. 2013) consisting of a 3 minute period of manual chasing around a circular tank (diameter 0.6 m, water depth 0.2 m) containing approximately 50 liters of 10 °C, aerated freshwater. All individuals were visibly exhausted by the end of the 3 minute exercise period as highlighted by a lack of response to an experimenter tapping the caudal fin. Immediately following the exercise protocol, fish were returned to their individual respirometers within 10 seconds, whereupon respirometers were sealed for 5 minutes and maximum metabolic rate was taken as the steepest 3 minute slope during this time. When the exhaustive exercise protocol had been performed, the fish were taken out from the respirometers and put separately in smaller aquaria, each holding an approximate water volume of 40 liters.

Thereafter, blood samples were taken from the fish in order to measure hematocrit, i.e. the proportion of erythrocytes in the blood. Blood taken from the caudal veins of anesthetized (2-phenoxyethanol, 0.5 ml/l) trout were drawn into capillary tubes and centrifuged at 3000g for 5 minutes and the total packed red blood cell volume was read from a hematocrit graduated scale.

Treatment of Data and Statistical Analysis

Estimation of glochidia load
Glochidia load was estimated for each fish after they had been in the respirometers. Trout were carefully netted from the small aquaria and put in anesthetics (2-phenoxyethanol, 0.5 ml/l). While sedated, fork length and weight of each fish was measured. Glochidia load was examined by gently lifting the operculum and gill arches using pincers and estimating the number of glochidia encysted on the fish’s gills. The number of glochidia were counted as precisely as possible. However, as the trout were not euthanized glochidia load of heavily infested fish could not be estimated to an exact number. Thus, trout with more than 100 glochidia encysted to their gills were set to have 100 glochidia while analyzing the data. As previously mentioned, glochidia load was standardized by fish body weight and transformed by base 10-logarithms. While sedated, the trout were marked with passive integrated transponder (pit) tags. When all procedures had been performed, the trout were released back into Lindåsabäcken.

Standard metabolic rate
Standard metabolic rate (SMR) was calculated for each fish as the mean of the lowest 10% of oxygen consumption measurements taken during the ~21 hour period where the fish were left undisturbed after respirometer entry (excluding a total of 12 outliers distributed over 12 of the fish, which were considered to be > 2 sd. below the mean of the lowest 10% of values.). SMR was calculated as mg of oxygen consumed per 1000 gram fish per hour. Differences in SMR between infested and uninfested fish were measured using independent samples t-test.
For investigating relationships between SMR and glochidia load Pearson’s correlation was carried out. Parametric tests were used as the data was normally distributed.

Aerobic scope
Aerobic scope (AS) was calculated for each individual trout by subtracting maximum oxygen consumption by minimum oxygen consumption for the steepest 3 minute slope after the exhaustive exercise, and presented as mg of oxygen consumed per 1000 gram fish per hour. Differences in AS between infested and uninfested fish were measured using independent samples t-test. For investigating relationships between AS and glochidia load, Pearson’s correlation was carried out. Parametric tests were used as the data was normally distributed.

Hematocrit
Differences in hematocrit between infested and uninfested fish was measured using independent samples t-test as the data fulfilled the properties of a normal distribution. For investigating relationships between hematocrit and glochidia load, Pearson’s correlation was carried out.

Results

Glochidia load

Slereboån
Infested trout from Slereboån used in the behavior study showed a high variation in glochidia load, ranging from one to several hundreds. The 1+ trout had significantly higher glochidia loads than the 0+ fish. (Mann-Whitney U Test, $U=8.5, p=0.04$). However, no statistically significant difference was detected between year classes when glochidia load was standardized by fish weight (Mann-Whitney U Test, $U=14, p=0.18$), or fork length (Mann-Whitney U Test $U=14, p=0.09$). The largest fish were observed to have the highest glochidia loads (figure 4).

Lindåsabäcken
High variation in glochidia load was also noted for the infested fish from Lindåsabäcken used in the metabolism study, ranging from one to several hundreds of glochidia encysted on the trout’s gills. Although no relationship was observed between either glochidia load and fork length (Spearman’s correlation (36) = 0.06, $p=0.71$), or glochidia load and weight (Spearman’s correlation (36) = - 0.02, $p=0.92$), larger trout tended to have more glochidia encysted to their gills than smaller individuals (figure 5).
Ventilation rate
A decrease in ventilation rate was observed over time for both infested trout (one-way Anova: $F_{2, 37}=9.28, p=0.001$) and uninfested trout (one-way Anova: $F_{2, 37}=5.68, p=0.007$) (figure 6). Post-hoc analyses using Tukey’s HSD indicated that ventilation rate was significantly higher when the trout were initially put into the stream tanks, than after both one hour ($p = 0.002$), and three hours ($p < 0.001$). However, ventilation rate did not differ significantly between one and three hours in the stream tanks ($p = 0.14$).

There were no significant differences in ventilation rate between infested and uninfested fish (Paired samples t-test, $t (35) =0.7, p=0.52$). No relationship was observed between glochidia load and ventilation rate (Pearson’s correlation, $r = -0.47, p=0.81$). No significant difference in ventilation rate was detected between year classes (Independent samples t-test, $t (38) =0.72, p=0.48$).
Figure 6 Ventilation rate (beats per minute) before the foraging trial (n=26) (A), after 1 hour (n=26) (B) and after 3 hours (n=28) (C) for the infested and uninfested trout in each pair standardized by glochidia load of the infested fish. The number of glochidia for all control fish is 0.
Activity

Body size was not observed to have any general relation to activity, neither when tested by fork length (Pearson’s correlation, (28) = -0.34, p=0.08) or weight (Pearson’s correlation, (28) = -0.34, p=0.07) (figure 7). No significant difference in activity was found between infested and uninfested fish (Paired samples t-test, \( t(13) = -0.34, p=0.74 \)). Activity of the infested fish was seen to decrease with increasing glochidia loads (Pearson’s correlation, (14) = -0.7 p=0.005) (figure 8). No significant difference in activity was detected between year classes (Mann-Whitney U test, U=24, p=0.96).

![Figure 7](image1.png)  
*Figure 7* Neither fork length (A) or weight (B) was observed to have any general effect on fish activity (n=28).

![Figure 8](image2.png)  
*Figure 8* Activity of the infested fish was observed to decrease with higher glochidia loads (n=14).
**Interactions**

The total number of interactions between all fish in the study was 108, of which 51 were initiated by the infested fish (47.2 %), and 57 by the uninfested fish (52.8 %). The most common interactions were displays. Displays represented 39.8% of all interactions, followed by chases (34.3 %), attacks (12 %), circling (7.4 %), and biting (6.5 %).

No significant difference in initiated interactions was found between infested and uninfested fish (Wilcoxon signed rank test, Z=0.54, p=0.59). However, the number of initiated interactions decreased with higher glochidia loads for the infested fish (Spearman’s correlation (14) =-0.72 p=0.004) (figure 9). The number of displays initiated by the infested and uninfested fish in each pair was highly correlated (Spearman’s correlation, (14) =-0.987 p= < 0.001) (figure 10). Also, the number of displays initiated by the infested fish was observed to negatively correlate with increasing glochidia loads, and highly infested fish did not display at all (Spearman’s correlation (14) =-0.66 p=0.01) (figure 11). No significant difference in the total number of initiated interactions was detected between year classes (Mann-Whitney test, U=20.5, p=0.6).

![Figure 9](image9.png) **Figure 9** The total number of interactions initiated by the infested fish decreased with higher glochidia loads (n=14).

![Figure 10](image10.png) **Figure 10** Number of displays performed by the infested fish and the uninfested fish (control) in pairs where display behaviors were noted was highly correlated (n=8).

![Figure 11](image11.png) **Figure 11** Number of displays performed by the infested fish decreased with higher glochidia loads (n=14).
**Coloration**
Infested trout showed darker coloration than the uninfested (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 (figure 12). No significant difference in coloration was detected between year classes (Mann-Whitney U test, \( U=14, p=0.11 \)).

**Standard metabolic rate**
No significant difference in standard metabolic rate (SMR) was found between infested and uninfested fish (Independent samples t-test, \( t (60) = 1.79, p=0.08 \)). However, SMR for the infested fish was found to be correlated to glochidia load (Pearson’s correlation \( (35) = -0.38, p=0.03 \)) (figure 13).

**Aerobic scope**
No significant difference in aerobic scope (AS) was found between infested and uninfested fish (Independent samples t-test, \( t (60) = 1.47, p=0.15 \)). No relationship was found between glochidia load and AS (Pearson’s correlation, \( (35) = 0.02, p=0.90 \)).

**Hematocrit**
No difference in hematocrit was detected between infested and uninfested fish (Independent samples t-test, \( t (56) = -1.38, p=0.19 \)). No significant correlation between glochidia load and hematocrit was observed (Pearson’s correlation, \( (34) = -0.15, p=0.41 \)).

![Figure 12](image12.png)
*Figure 12* The majority of the infested fish showed darker coloration than their uninfested combatant. The lines connect the uninfested (control) and infested fish in each pair. The numbers next to each line indicates in how many pairs each relation was observed (n=14).

![Figure 13](image13.png)
*Figure 13* Standard metabolic rate (SMR) was observed to decrease with higher glochidia loads (n=35).
Discussion

Parasitic glochidia and brown trout behavior

The aim with this part of the thesis was to investigate how parasitic glochidia of the freshwater pearl mussel *M. margaritifera* influence activity and competitive behavior in juvenile brown trout while drift feeding. The results suggest that glochidia of *M. margaritifera* can have a negative influence on juvenile brown trout if the trout are heavily infested with glochidia. Although no difference was seen in activity or initiated aggressive interactions between infested and uninfested fish, significant relationships were observed where activity and initiated interactions decreased with increasing glochidia loads. This indicates that the glochidia may affect the fish negatively, as highly infected fish were less active and behaved more subordinately than fish with lower infestation rates. The reason for the lack of difference between infested and uninfested fish may be that some of the infested fish only had very few glochidia encysted on their gills. It is likely that the burden of a few glochidia is so insignificant that it does not have any influence on the host fish. There are to my knowledge no studies which have shown that such low glochidia loads would affect salmonids negatively. All studies reviewed in the preparation of this thesis have used fish with glochidia loads ranging from 25-900 Glochidia g\(^{-1}\) body weight (Taeubert & Geist 2013; Österling et al. 2014), and glochidia loads that high have been observed in fish less active and with poorer foraging capabilities than uninfested fish. Thus, pursuing a study of this sort where a difference between infested and uninfested fish as two separate groups can be expected, higher glochidia loads than those used in this study may be necessary. Also, to detect significant differences between the two groups, the variation in glochidia load should be kept as small as possible. It should also be taken into consideration that the behavior study was conducted during autumn, when the fish becomes less active in general and forage less than during spring and summer. Therefore, it could also be of interest to perform a similar study during spring instead of autumn, as it would be expected of the fish to be more active and have higher foraging intensity. Also, the glochidia will have grown larger in the spring, and may therefore have greater influence on the host fish.

However, the results from this study show significant negative correlations between glochidia load and activity, where trout with high glochidia loads were less active than those with low infestation rates. This supports the hypothesis that trout are affected by *M. margaritifera* glochidia, and these findings indicate that a difference in activity occur between uninfested trout and trout with high infestation rates. Thus, this study is consistent with previous studies regarding the hypothesis that the glochidia of *M. margaritifera* have a negative influence on juvenile brown trout. This study also shows that low glochidia loads do not have any severe impact on juvenile trout regarding activity and dominance behavior, whereas higher glochidia loads may have very detrimental effects on the host fish. Thus, it can be of interest to investigate how different glochidia loads affect the host, to determine which levels of infestation that negatively affect juvenile trout.

In this study it was observed that the largest fish had the highest glochidia loads. This relationship has been observed earlier, both regarding *M. margaritifera* and juvenile brown trout (Thomas et al. 2014) and other species (Poulin 2000). The reason why larger fish have more glochidia encysted has previously been explained simply by that larger fish have larger gill area, and thus a larger area available for encystment of parasitic glochidia (Poulin 2000; Thomas et al. 2014). This may be the reason for why this trend was observed in this study as well. Also, this may be the reason that activity of the fish tended to decrease with fish size, the
correlation showed nearly significant results ($p=0.08$ for the relationship between fork length and activity, and $p=0.07$ for the relationship between weight and activity).

No statistical differences were observed between year classes, which indicate that the influence of parasitic glochidia does not differ between 0+ and 1+ brown trout. Therefore, the difference between year classes was not investigated in the metabolism study, as it did not seem to have any effect in the behavior study. However, the lack of significant differences between year classes may depend on weak power of the tests used when examining differences between groups. This pattern was observed for all statistical tests, where continuous correlative tests had far greater statistical power than tests investigating differences between groups.

It was observed that infested fish had significantly darker coloration than uninfested. As mentioned earlier, dark and dull coloration is a sign of subordinance in salmonids (O'Connor et al. 1999, 2000). Far from all infested fish in this study behaved subordinately, but many of them still had darker coloration than the infested fish in the same pair. It was not expected of the fish to turn to darker coloration if they did not behave subordinately over all. However, some other mechanism influenced by the glochidia may have turned the infested trout’s coloration more dark and dull.

**Parasitic glochidia and brown trout metabolic rate**

Although no statistical difference in metabolic rate was observed between infested and uninfested fish, SMR was negatively correlated to glochidia load. One could hypothesize that trout with higher infestation rates should have higher oxygen consumption and metabolic rate because of the stress caused by the parasites. However, another explanation may be that the glochidia simply cover a significant area of the trout’s gills, which physically inhibits respiratory capacity. This has also been suggested from conclusions drawn in previous studies (Thomas et al. 2014). With this in mind, it seems reasonable that trout with higher glochidia loads should have lower oxygen consumption and metabolic rate than trout with lower infestation rates. The reason for why no difference in metabolic rate was observed between infested and uninfested fish is likely dependent on the large variation in glochidia load on the infested fish. Thus, this is the same pattern as observed in the behavior study.

In agreement with previous studies, hematocrit was not shown to have any relation to glochidia load (Thomas et al. 2014). The number of erythrocytes in the blood may affect oxygen uptake and metabolic rate. However, if highly infested fish have lower oxygen consumption because of that a large area of the gills are covered in glochidia, then it is reasonable that hematocrit of the infested fish do not differ from uninfested fish, as other factors are influencing metabolic rate. Also, prior taking the blood samples the fish had been starved for over two weeks, they had been anesthetized several times and gone through the respirometry procedure. Thus, the large amount of handling and experimentation on the fish prior taking the blood samples may well affect the composition of the blood for all fish going through the treatment. Therefore data collected from the blood samples should be analyzed with consideration that it may not be fully reliable. However, all fish subjected to the blood sampling had gone through the same treatment.
Implications and suggestions for future research

Fish collection
During the collection of juvenile brown trout in Slereboån fewer infested fish were caught than expected. Also, the observed glochidia load on the infested fish was lower than expected. The reason for the low recruitment of *M. margaritifera* in Slereboån during 2014 can only be speculated upon for now, but the high water temperature, discharge, and water levels during the summer may have had its impact. However, the low densities of infested fish resulted in fewer fish than planned being caught, 9 infested of each year class instead of 25, and the fish generally had lower glochidia loads than expected. The variance in glochidia load on the trout was also far greater than expected, thus it was problematic to treat the infested fish as a separate group because of the large variation in glochidia load.

Another factor which affected the number of replicates was the difficulty to estimate glochidia load in the field. Although fish were anesthetized while infestation was controlled and a loupe was used, it may be difficult to estimate glochidia load during certain light conditions. Generally it can be difficult to estimate glochidia load in overcast weather because of the absence of direct sunlight, whereas it often is more effective to estimate glochidia load during strong sunlight. In this study glochidia load was controlled for both in the field and prior the trials in the stream tanks. During the latter control prior the trials fish were sedated and glochidia load was estimated under strong light using pincers. During this control fewer fish with glochidia were found than what was gathered in the field. The fish that had high glochidia loads had the same loads during the control prior the trials. However, not all fish that were assessed to have only a few glochidia in the field were found infested during the control prior the trials. It is not likely that the glochidia had released from the fish’s gills, as they often do so in late spring and not during autumn. Also, if the glochidia had released or been sloughed off by the fish’s immune response, traces of glochidia encystment would probably have been seen on the trout’s gill lamellae. Thus, the reason why fewer infested fish were found prior the trials than what was collected in the field was most probably that some trout were thought to be infested when in fact they were not. It may look like the fish is infested with one or a few glochidia in the field, but when infestation is controlled for more detailed in the laboratory it can be observed that those fish are in fact uninfested. However, none of the fish classified as uninfested in the field were found to be infested when glochidia load were examined in detail in the laboratory. Thus, the probability of not finding any glochidia on infested fish seems rather unlikely.

Standardizing glochidia count
In this study glochidia load is standardized by fish weight, simply because this is the method used for assessing glochidia load in earlier work covering similar issues (Taeubert & Geist 2013; Österling *et al.* 2014). Thus, this standardization method was used for this study as well to enable comparison of results between studies. However, very few studies have been performed which investigate these aspects of fresh water pearl mussel ecology, and therefore it is questionable if standardization by fish body weight can be referred to as a standard even though it is the measurement most often used. One could argue that fork length may be a better measure than weight to standardize glochidia load. When a fish gets access to lot of high quality food it will grow and increase in both length and weight. However, if the fish thereafter gets limited access to food it may get starved and lose weight, but in this case the length of the fish will not decrease.
Regarding fresh water pearl mussel parasitism on brown trout, it is important to take into account that glochidia encysts to the gill of the host fish. Thus, glochidia abundance standardized by accessible gill area may be another representative way to estimate the influence of parasitic glochidia. It is likely that the negative influence of parasitic glochidia is caused by the inhibited respiratory capacity of the fish as the gill area available for respiration decrease with higher glochidia loads. Therefore gill area may be an elegant measure to standardize glochidia load by.

However, fish weight can still be an important factor to take into account while standardizing glochidia abundance. If two fishes have the approximately same length but one weigh more than the other, the one which weighs more is most likely the one to be in best condition. Fish that weigh more are likely healthy and successful foragers, and also successful in finding and defending good foraging locations. Fish that weigh less may have poorer foraging capabilities, and may also not be capable of defending good foraging points. Underweight fish may be suffering from parasites and other pathogens, and therefore be in poorer health condition. Thus, fish that weigh more may be more dominant, more successful foragers, healthier, and also have higher tolerance against glochidia than their underweight conspecifics.

Here one could argue that some sort of condition index taking fish length, weight, and total gill area into account is preferable while standardizing the influence of parasitic glochidia. Length and weight combined can give a good index over the overall condition of the fish, and gill area can be an interesting factor to implement in the equation to see how large the proportion of the gills covered in glochidia are, which can influence the fish’s respiratory capacity. Here we stumble upon another issue, the fact that the glochidia grow while they are encysted on the host’s gills. It can be hypothesized that infestation has lower effect on the host fish in the beginning of the infestation period than in the end, since the glochidia grow while encysted on the gills and successively covers a larger proportion of the host’s gills. Thus, another aspect to take into account while estimating the influence of parasitic glochidia may be to measure the size of the glochidia, to get an even more representative measure on how large proportion of the gills are covered in glochidia.

**Implications for mussel-host fish ecology and management**

An important question to ask is the causation between glochidia encystment and the effect seen on brown trout. It seems reasonable that salmonids with lots of parasites on their gills would be in poor health condition, and thus be less active and have poorer fighting capabilities than uninfested fish because of the negative effect of the parasites. However, this study only investigates the relationship between glochidia parasitism and brown trout behavior. Less active fish may be more susceptible to glochidia infestation than active dominant fish, and that may be the reason for why infested fish are observed to be less active than uninfested fish. To investigate this further it can be of interest to perform a study where the initial activity (or another behavioral parameter) of the uninfested fish is estimated, and thereafter to infest the fish with glochidia to examine behavioral changes as the trout becomes infested. It may also be of interest to investigate these parameters both during the beginning and the end of the encystment period as the glochidia grow, to see if behavioral changes occur from when the glochidia are newly encysted and small to the end of the encystment period when the glochidia have grown larger. When the glochidia have excysted, it can be of interest to note the behavior of the fish again to see if any changes occur.
From this study knowledge has been furthered on the relationship between the freshwater pearl mussel *M. margaritifera* and its host brown trout. It was observed that the glochidia of the mussels can negatively influence the fish, consistent to what have been seen in previous studies. It seems that glochidia decrease activity of the trout and may also affect dominance traits, which often are traits linked to fitness. Regarding the severe conservation status of *M. margaritifera* it is interesting to know how the species affects its host, and if the influence of parasitic glochidia affects the trout in ways which in turn affect mussel recruitment. The results presented in this thesis may have several implications for the conservation of the critically endangered freshwater pearl mussel. One interesting implication is how the glochidia may disperse while encysted on the gills of juvenile salmonids. Conservational efforts for *M. margaritifera* have in many cases been targeting this certain life stage of the species, where artificially infested fish have been released into streams inhabited by adult mussels to restore mussel populations (Hastie & Young 2003; Thomas *et al.* 2010; Taubert & Geist 2013). In these conservational programs heavily infested fish have been released in order to promote artificial propagation. In the study presented in this thesis it was observed that glochidia abundance was greatest on the largest fish, suggesting that glochidia attachment is a function of gill surface area. This relationship has been reported earlier, both regarding *M. margaritifera* and brown trout (Thomas *et al.* 2014), and other species (Poulin 2000). Captive breeding programs for freshwater pearl mussels usually aim for high infestation rates (Thomas *et al.* 2010). This study, consistent with previous work, implies that it may be beneficial to select the largest fish as hosts since they have more glochidia on their gills. However, previous studies have shown that no relationship exists between body size and glochidia loads at 160 to 167 days post exposure, and this may indicate that larger fish are more efficient at shedding glochidia than smaller conspecifics (Thomas *et al.* 2014). A potential tradeoff may therefore exist between encystment rates and transformation success, and thus not make larger fish more suitable for glochidia propagation. Artificial encystment typically results in glochidia loads many times higher than those commonly found in the wild (Karna & Millemann 1978; Hruska 2001). Although glochidia loads up to 800 per gram fish weight result in little (approximately 1 %) or no mortality (Hastie & Young 2001, 2003; Preston *et al.* 2007) the glochidia may affect host fish in other ways, both behaviorally and physiological. If this is the case, aiming for high glochidia loads may not be the best option for mussel propagation programs, if high glochidia loads compromises host fitness and thus the probability that the glochidia will survive until they excyst.

In these cases it can be important to take into account that heavily infested fish are less active than fish with lower glochidia loads. It may be beneficial to release fish with lower infestation rates if the aim is for the fish to disperse. However, it is not known if highly infested trout are more stationary to a certain area simply because they are less active. One potential way that infested fish may disperse to new areas, even though they are not as active as uninfested fish, may be that they have poorer fighting capabilities, and therefore lose in disputes over good areas for foraging or shelter. Although the infested fish are less active, they may still have to disperse to other areas as they cannot sustain themselves in areas where there is lot of competition from conspecifics. Thus, this is a potential way for the encysted glochidia to disperse on less active fish.

The recruitment of glochidia is linked to the survival of the host. Therefore it may be of interest to investigate which glochidia abundances on the host fish that result in adequate dispersal of juvenile mussels and good mussel recruitment over all. Since infested fish should be able to withstand high velocities in streams where *M. margaritifera* is present, glochidia loads should preferably not impair the fish’s swimming performance. Slower sustained
swimming speeds may have a number of implications for infested fish. Factors that reduce swimming performance can be considered since they may result in higher risks of predation, reduced ability to obtain food, as well as a higher risk for the host fish to be carried downstream during peak flow events. However, *M. margaritifera* may benefit from these effects if highly infested trout prefer habitats with lower velocities where the chance of settlement for newly encysted juvenile mussels can be higher compared to areas with higher velocities. Thus, further studies on how encysted glochidia affect dispersal and habitat choice of juvenile salmonids may be of interest to increase knowledge on *M. margaritifera* ecology and applied conservation work. This knowledge can be of importance to obtain both when investigating the behavior of naturally infested fish, and for implications on the release of artificially infested fish in streams inhabited by *M. margaritifera*.

**Acknowledgements**

Many persons who I wish to acknowledge have been involved in this thesis. First of all I would like to thank my supervisors Johan Höjesjö and Niklas Wengström for your never ending enthusiasm about this project and giving me the opportunity to go through with this thesis. I also want to thank my co-master students Tina Petersson and Fredrik Wahlqvist, without you the work presented in this thesis would never have been done. Thanks Tina for wanting to participate in this study and to further it to what it became. Thanks Fredrik for the help with collecting the fish and for transporting it back and forth to Karlstad with me. I also want to thank the PhD students Jeroen Brijs, Joacim Näslund, David Aldvén and Libor Závorka at the department of biological and environmental sciences in Gothenburg for taking interest in my thesis and for all the effort you put into this project. Thanks Anna-Marie Kellermann and Marie Adamsson for your help with the respirometry trials and blood sampling. I also want to show my gratitude to the people at Karlstad University. Thanks Martin Österling and John Piccolo for being our supervisors in Karlstad, and for providing us heaps with help and sharing your knowledge about freshwater pearl mussels and salmonid foraging behavior. Thanks Johan Watz for answering our never ending questions on how to set the thermostats and timers among the stream tanks. Thanks Bror Jonsson for taking several looks at our experimental setup and giving useful comments. I would also like to thank my examiner Professor Lotta Kvarnemo for taking her time reading and grading my paper.

**References**


