DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

A PARASITIC BIVALVE AFFECTS THE INTERACTION BETWEEN A NATIVE AND AN INVASIVE SALMONID

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Frontpage photo depicting a juvenile brown trout. From: Persson, J. (2014). Öringen leker i Tämnarån. The Uppland Foundation*. Available:* *https://www.upplandsstiftelsen.se/Aktuellt/oringen-leker-i-tamnaran__5250. Downloaded 2022-08-21.*

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Abstract

The glochidia larvae of the freshwater pear mussel parasitize on juvenile brown trout during their maturation into tiny mussels. The parasitation has several negative impacts on the trout, one of the less studied being behavioral effects. As such, this study aimed to determine if the mussel infestation had any negative impacts on the brown trout's dominance performance when encountering larvae free brown trout and invasive brook trout. I hypothesized that the general dominance behavior, the number of strikes per individual fish and the proportion of strikes per trout in pairwise interactions would decrease with increasing infestation. It was also hypothesized that infested brown trout would perform differently when encountering either non-infested brown trout or brook trout, that infested fish in an isolated group would have a lower number of initiated aggressions and that infested brook trout would eject their glochidia larvae before the latter complete their metamorphosis. Most of the hypotheses were tested in two experiments where the behavior was recorded with cameras. The brook trout's infestation was tested separately. No glochidia larva completed their metamorphosis on these fish. The isolated group consisting of infested brown trout had a lower average number of initiated aggressions. In the pairwise interactions, the larvae infestation only significantly decreased the general dominance behavior and the proportion of strikes. The number of strikes varied greatly between the fish. The infested brown trout's performance did not differ between interactions with either non-infested brown trout or brook trout. These results portray a problematic scenario for the freshwater pearl mussel. As their hosts are more prone to take subordinate roles and since invasive brook trout does not act as a functional host, the latter will have a greater opportunity to dominate streams inhabited by the bivalve, increasing the likelihood of the mussel's local extinction.

Key words

- Glochidia larvae
- Infestation class
- General dominance behavior
- Dominant/subordinate
- Strikes

Sammanfattning

Flodpärlmusslans glochidialarver lever som parasiter på gälarna hos juvenila öringar under deras utvecklingsfas till små musslor. Parasiteringen har flera negativa effekter på öringens hälsa och dess beteende, det senare är något mindre forskat. Just därför så ämnade denna studie att undersöka om musslans infestering hade några negativa effekter på öringens dominanta beteende under möten med larvfria öringar och invasiva bäckrödingar. Min hypotes var att det generella dominansbeteendet, antalet utfall per individuell fisk och andelen utfall per fisk under parvisa interaktioner skulle minska med ökad mängd larver. Det gjordes också hypoteser om att den infekterade öringens prestanda skulle skilja sig mellan möten med icke infekterade öringar och bäckrödingar, att infekterad fisk i en isolerad grupp skulle ha ett lägre antal initierade aggressioner och att infekterade bäckrödingar skulle förlora sina larver innan den senare fullbordade sin metamorfos. De flesta hypoteserna testades i två experiment där beteendet spelades in med kameror. Bäckrödingarnas infestering testades separat. Ingen glochidialarv fullbordade sin metamorfos på dessa fiskar. Den isolerade gruppen bestående av infekterade öringar hade färre initierade aggressioner. I de parvisa interaktionerna minskade parasiteringen signifikant endast det generella dominansbeteendet och andelen utfall. Antalet utfall varierade mycket mellan fiskarna. Den larvinfekterade öringens prestanda skiljde sig inte mellan möten med larvfria öringar eller bäckrödingar. Dessa resultat porträtterar ett problematiskt scenario för flodpärlmusslan. Då deras värdar är mer benägna till att ta undergivna roller och då bäckröding är en icke fungerande värd, så kommer den invasiva fisken att ha en större möjlighet att dominera musselbebodda bäckar och floder, vilket ökar risken för molluskens lokala utrotning.

Nyckelord

- Glochidialarver
- Infekteringssklass
- Generellt dominansbeteende
- Dominant/undergiven
- Utfall

1 Introduction

This study aimed to determine how the glochidia infestation of the freshwater pearl mussel (*Margaritifera margaritifera*) affects the behavior of juvenile brown trout (*Salmo trutta*) and their interactions with invasive brook trout (*Salvelinus fontinalis*).

1.1 The freshwater pearl mussel

The freshwater pearl mussel is a highly endangered bivalve. The life cycle of the freshwater pearl mussel is complex and includes several stages (Figure 1). The genders are usually split between different individuals, but females can become hermaphrodites during critical situations. This most often occurs at periods of decreasing population density. Mating takes place from June to August and begins with the males releasing their sperm into the open water. The females inhale the sperm and fertilize their eggs. The newly hatched glochidia larvae spend the first part of their lives on their mother's gills, which in Sweden lasts about 4-6 weeks, whereafter the females pump out their offspring into the open water mass. The larvae then attach themselves to the gills of certain species of salmonid fish, which they immediately start to parasitize on. The choice of host species depends on where the mussel lives, but used host hosts are always juveniles. In Europe, the larvae attach to juveniles of brown trout and Atlantic salmon (*Salmo salar*). Only 1 of 100 000 larvae succeeds in finding a host. The surviving larvae start to metamorphose into small mussels, a process which takes 9-11 months for Swedish populations. After they have left the safe haven of the fish gills, the young mussels burrow themselves down into the river bottom substrate, to a depth of 35 cm. Here, the mussels start to slowly grow. When reaching the length of 1 cm, the mussels travel up to the surface layer of the bottom substrate. This process can be very long. In nutrient poor environments, it lasts 5-8 years. The Swedish freshwater pearl mussels have an average life span of 70-80 years. The growth and life span of the species vary greatly with the climate, individuals in colder regions grow slower and live longer. The freshwater pearl mussel is an effective filtrator and cleanse their rivers of over-abundant nutrients and particulate matter ([2]SLU Artdatabanken, 2022; Taubert & Geist, 2017).

Since the beginning of the 20th century, the species has lost one third of its natural habitats, due to local extinctions caused by environmental changes and pollutions. Today, it is found in running waters of Northern Europe, Eastern U.S. and Canada. Many local populations are eradicated every year, while some of the remaining sites completely lack any recruitment. About one third of the current habitats has undergone rejuvenation since the 1980s. The mussel is very sensitive to environmental changes, which has led to its poor population status. In order for the species to thrive, the water needs to be well oxygenated and the river bottom covered with gravel or rocks. The freshwater pearl mussel demands relatively pristine water, as sludge and mud halt its filtering mechanism. Muddy river systems have become more common due to climate change, as certain extreme events (e.g., heavy loads of rain) often cause loose sediment at the river banks. The species is also sensitive to low pH levels and several types of chemical pollution. pH levels below 5, in combination with high concentration of aluminum ions (Al^{3+}), are lethal to the mussel Baldan et al., 2021; Bauer, 1988; [2]SLU Artdatabanken, 2022; Swedish EPA, 2011). However, the last-mentioned threat is quite uncommon in Sweden (Höjesjö, 2023).

As mentioned, the freshwater pearl mussel is an effective filtrator that keeps their streams relatively clean. Through this feeding method, the mussel can have a positive effect on their hosts that inhabit said waters. However, the infestation period seems to be detrimental to the health of the infected salmonids (Chowdhury et al., 2019; [2]SLU Artdatabanken, 2022; Taubert & Geist, 2017; Österling et al., 2014).

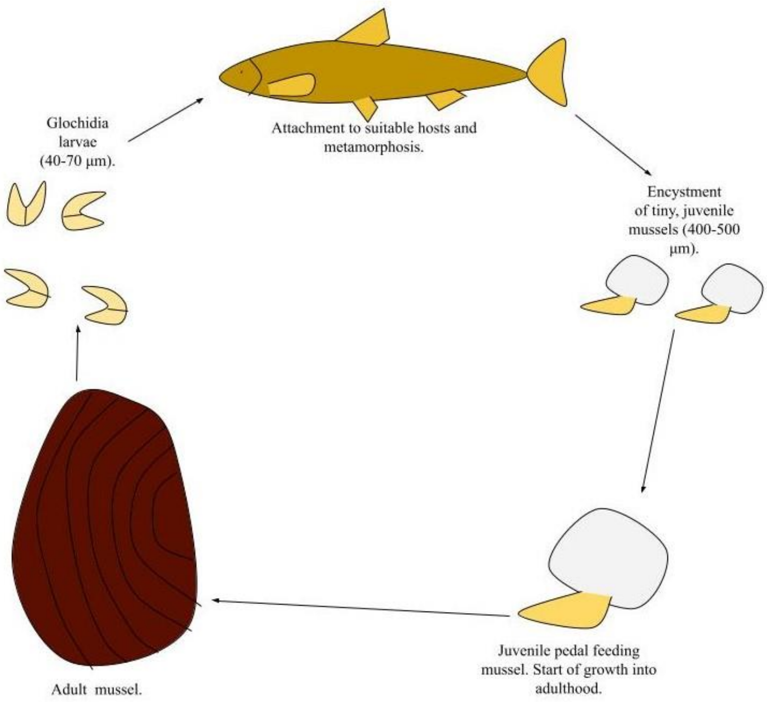

Figure 1. The life cycle of the freshwater pearl mussel (illustration made in Google Drawings).

1.2 The brown trout

One of the essential hosts of the freshwater pearl mussel is the brown trout (Figure 2). The species often lives in local populations that consists of many individuals. Brown trout can be anadromous (marine individuals are called sea trout), but many individuals spend their entire lives in freshwater systems. Unlike some other salmonid species, the brown trout is iteroparous and mates several times during its life. Individual fish become sexually mature at an age of 2-5 years and local populations spawn yearly. However, individual brown trout will not mate again for another two years after spawning. The mating period takes place between August and December during 2-3 weeks. Large females can release as many as 10 000 eggs, which will hatch the following spring. Some of the juvenile trout will after a period of growth (1-5 years) start to migrate to the sea or to freshwater lakes in a form known as smolt. These individuals tend to grow faster and become bigger than those staying in the stream, although brown trout in smaller lakes has the same growth rate as stream-dwelling fish. Despite their different habitats, all forms of brown trout spawn in freshwater streams. This species often lives for 7-10 years, but it can become as old as 21 years. (Degerman, 2015; Sportfiskarna, 2022; [3]SLU Artdatabanken, 2022).

Figure 2. A juvenile brown trout (Bergdahl, 2020).

The brown trout is both a beloved and a controversial species. The former viewpoint is dominating in Sweden, as it is one of our native salmonids. However, due to human actions, the brown trout has been introduced to several new environments. In many habitats, it has started to outcompete fish native to the area and is thus classified as an invasive species (Wild Trout Trust, 2022). Originally from streams and rivers of Northern and Western Europe, it can today also be found in Southern Europe, the Caspian Sea, the Black Sea, North and South America, Africa, Asia, Australia and New Zealand. As mentioned, Swedish waters are some of the species' native habitats and many studies are made yearly to determine its status in this country ([3]SLU Artdatabanken, 2022).

One of the aspects of the species that has been closely examined is the infestation of freshwater pearl mussel larvae, attaching themselves to the gills of the brown trout (Figure 3). The glochidia larvae rely on their host for growth and their transformation into small mussels. It is one of the pivotal stages of the endangered mollusc's life cycle, which likely has contributed to the increasing amount of research on the relationship between the bivalve and the fish. Due to the parasitic nature of the interaction, several negative-impacting changes to the trout occur during the glochidia infestation. Reduced drift feeding rate, growth, dominance performance and critical swimming speed have all been observed in studies (Chowdhury et al., 2019; Filipsson et al., 2016; Taeubert & Geist, 2013; Wengström et al., 2022; Österling et al., 2014). Other effects include increased metabolic rate, haematocrit and enlargement of the spleen (Filipsson et al., 2017; Thomas et al., 2013).

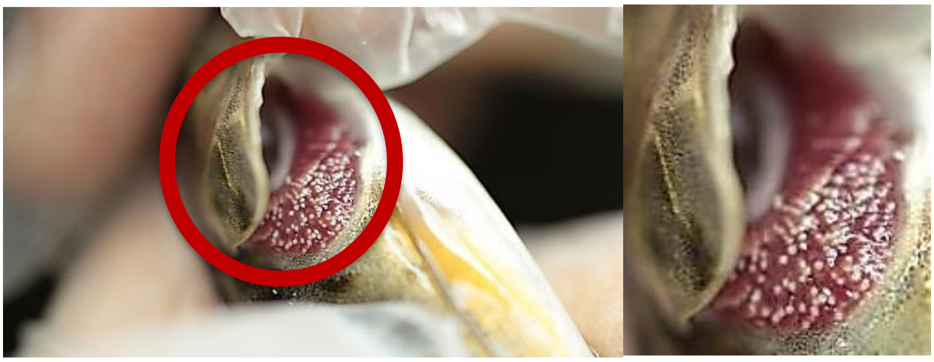

Figure 3. The gill lamellae of a brown trout (marked with the red ring) infected with glochidia larvae of the freshwater pearl mussel, seen as light-colored dots. The image to the right is zoomed in on the gills and the glochidia larvae (Kvarnliden, 2022).

A less studied subject in this area is the effect of glochidia infestation on the behavior of the brown trout. One of the observed changes is the aforementioned reduction in dominance performance, but foraging activity is also seemingly impacted in a negative way (Filipsson, et al., 2016). The glochidia infection likely changes the interaction between host individuals within and between species. It is not unreasonable to assume that the change in behavior could ultimately prove to be lethal for the host, as subordinate trout often have less access to food and valuable territories. As such, this parasite-host relationship should be a major concern for conservationists, as it could potentially have a negative impact on the status of both the brown trout and the freshwater pearl mussel.

1.3 The brook trout

Originating from North America, brook trout (Figure 4) was introduced Swedish waters first in 1892. It was continuously placed in rivers and streams for decades (primarily during the 1950s and -60s) and now constitutes more than 300 reproductive populations. The brook trout generally resides in small running waters, some populations are anadromous. A number of trout is also found in lakes, ponds and wells. Mating primarily takes place in streams, but has been also observed in cold-water lakes. The brook trout becomes sexually mature at an age of 2-4 years, but it usually lives for another 1-3 years. The species is surprisingly resilient in some aspects, as it can handle both pH as low as 5 and a temperature range of 0-25 °C. However, it is sensitive to low oxygen levels and turbid water ([1]SLU Artdatabanken, 2022).

Figure 4. A juvenile brook trout (Hagerty, 2022).

As a consequence of its arrival in Sweden, the species has become invasive in several rivers, streams and other bodies of water. It can be found in habitats used by native brown trout, which is slowly replaced by the invader. The brook trout usually tends to occupy the upper portions of the streams, close to the water source. Brown trout avoids this area and is found further downstream. However, the two species meet and coexist in an overlap zone between these areas, where they start to compete against each other. A higher growth rate and generally earlier reproductive age have given the brook trout major advantages against the native brown trout (Degerman et al, 2005; HaV, 2016; [1]SLU Artdatabanken, 2022; Spens et al., 2007). Some studies suggest that juveniles of the invading species are also better at keeping territories and feed a lot faster than the natives. Another important note of interest is that the interaction between the two species become more aggressive the longer the residence time is in the territory, increasing the probability of physical confrontation (Lovén Wallerius et al., 2022). Juvenile brown trout tend to avoid areas inhabited by adult brook trout (Lovén Wallerius, 2021), suggesting that they are unable to compete with grown-up invaders. Brown trout also act more diurnal and aggregate in the presence of brook trout (Larranaga et al., 2018). The composition of phenotypic traits in the parrs of the native is reduced during these circumstances as well; the brown trout starts to consume more terrestrial prey, the body shape becomes stouter and the specific growth rate is reduced (Závorka et al., 2017).

There is a fear among conservation biologists that the spread of brook trout will negatively impact the status of the freshwater pearl mussel. Not only does the brook trout out-compete one of the bivalve's primary hosts in multiple areas, but it serves as a very poor stand-in host for the mollusc. Extremely few of the glochidia larvae manage to complete their development on this salmonid according to a study by Salonen et al. (2016). The mussel also adapts to their local host fish (Taskinen & Salonen, 2022), meaning that entire local populations should be unable to survive on brook trout. Salonen et al. did also observe infected brown trout being more frequently driven away from valuable territories compared with non-infested fishes. However, the question remains of how the larvae infestation affects encounters between the two species on a more mechanical level; e.g., the frequency of "attacks" during food competition.

2 Aim of the study

The main aim of this study was to determine the effects that the glochidia infection of the freshwater pearl mussel have on the brown trout during feeding and interactions both with non-infested individuals of its own species and with the invasive brook trout. More specifically, the study attempted to clarify how the dominance display is affected. Another aim of the study was to test if the number of initiated aggressions differ between individuals in isolated groups of only infested brown trout, non-infested brown trout and non-infested brook trout. Artificial infestation of brook trout was included as a side experiment. The plan was to test how long the glochidia larvae could remain attached to this species. It was hypothesized that this period should be too short for the glochidia larvae to complete their metamorphosis, as this event is very rare in nature (Salonen et al., 2016).

Due to the known negative effects on the brown trout's health (e.g., Chowdhury et al., 2019; Wengström et al., 2022), it was hypothesized that infested fish should take a more submissive role during pairwise interactions. Thus, these fish should also be responsible for a smaller percentage of the total amount of physical strikes (bites and pushes with head, body and tail) during a pairwise meeting. It was also hypothesized that the number of strikes per fish should decrease with increasing infestation. The experiments also aimed to clarify if the infested brown trout will be struggling more against brook trout or other members of its own species during these encounters. It was important to determine whether the infestation could affect the relationship between the native brown trout and the invasive brook trout, as the larvae could be another disadvantage for the endemic species. That is why some tests had non-infested members of both species, to compare how those encounters would change when adding glochidia larvae. The potential outcome of these interactions was unclear before the tests started. There were studies that hinted that the brook trout would have more victorious encounters. For example, experiments on the fry stage showed that brook trout more frequently wins brawls against brown trout (Lovén Wallerius, 2022) and there have been observations of the diel activity of the native being disturbed in the presence of the invader (Larranaga et al., 2018). However, studies from North America suggested the opposite. In this continent, the two species have switched roles, with the brook trout being the native species and the brown trout being invasive (Hoxmeier & Dieterman, 2016; Huntsman et al., 2022). The cause of the role switching is related to environmental factors (Jansson, 2013; Lauterbach, 2006). As this was a laboratory experiment in an artificial environment, the outcome of the pairwise interactions between non-infested brown trout and brook trout remained unknown as important factors from the wild were altered or missing (e.g., varying weather conditions and natural stream flow). In either case, would the infestation turn out to have a significant effect, then it should still mean that infested brown trout would be at a disadvantage when encountering invasive brook trout.

For the group experiments, I hypothesized that the number of initiated aggressions would be much smaller for the infested brown trout compared with the other fish. The non-infested brown trout should have slightly more displacements than the brook trout. The latter species has a tighter group structure during at least their fry stage, hinting at a more sociable behavior (Lovén Wallerius, 2017).

To summarize the hypothesizes:

- *H*1: The glochidia infestation will decrease the number of initiated aggressions in isolated groups of fish.

- *H*2: An isolated group of non-infested brown trout should have a higher number of initiated aggressions than an isolated group of non-infested brook trout.

- *H*3: The general dominance behavior and related parameters will decrease with increasing glochidia infestation, when tested among brown trout.

- *H*4: Species will affect the outcome of the pairwise interactions, although how is unknown due to the artificial environment of the laboratory.

- *H*5: Brook trout cannot remain infested during the entire metamorphosis period of the freshwater pearl mussel larvae.

3 Materials and Methods

This experiment was carried out under the ethical permit number Dnr 5.8.18-06676/2020.

3.1 Field work

The freshwater pearl mussel larvae were retrieved during the second week of August 2022 from Lindåsabäcken, when the local females were ready to release their offspring. This process was artificially initiated by removing the mussels from the stream and placing them in water filled buckets. They soon released the larvae due to stress caused by the sudden change in environment. The glochidia larvae were then moved to water filled vials while their mothers were returned to the stream. Afterwards, the larvae were transported to the Department of Biology and Environmental Sciences, Zoology, the facility where the actual experiment took place. To prevent the larvae from overheating, they were stored in a cooler with a plastic bottle filled with ice during the transport. They were later placed in a cold room with a temperature of 4 °C, where they remained until the artificial infestation. However, this proved to be unsustainable. The water of their native stream had a temperature of ~20 °C during the collection of larvae, which likely caused them to die of temperature shock when placed in the much colder storage room. Another potential cause of death was oxygen depletion. A second batch of larvae was used immediately after arrival to infest the fish, which turned out successfully (procedure described in 3.2.2 Infestation).

The fish were collected with a backpack electrofisher (Smith-Root LR-20B, Vancouver, Washington, USA). As the glochidia larvae only grow on juveniles, the age of collected brown trout and brook trout ranged between under yearlings (0+) and yearlings (1+). All fish were collected from the same catchment area; Viskan, located outside the town of Borås, Sweden.

Lindåsabäcken is located within this area, meaning that the local host fish has very similar-looking genetic make-up to the collected brown trout. The trout were placed in a sturdy casket which was filled with water from the stream. An air pump was connected to the casket to ensure that the fish were supported with oxygen. The casket and its contents were then transported to the Zoology building in Gothenburg app. 50 km from the field. A total of three batches of trouts were collected.

3.2 Laboratory work

3.2.1 Infestation

Two buckets were filled with 5 L water from the recirculation system of the Zoology building. The recirculation system allowed used aquaria to remain relatively clean and keep stable temperatures. The trout were separated between the two buckets depending on age; 0+ in one bucket and 1+ in the other. As the goal was to have a high infestation level on the fish gills, the concentration of glochidia were appr. 100 000 larvae/L. The water was whirled around by hand for 15 min which made the fish to start breathing faster due to the stress, allowing higher quantities of larvae to pass through their gills. The whirling process started before the glochidia larvae were added to the water (via a micro pipette) as the mussels otherwise risked sinking down to the bottom where infestation was less likely to occur. The trout of respective bucket were then moved into separated tanks and left alone for recovery. Not all fish were exposed to the artificial infestation process, as larvae-free fish were needed to test the effects of the glochidia larvae. However, some fish never became infested despite participating in the artificial infestation process, as no larvae successfully attached to their gills.

Two days later, seven fish from each tank were anesthetized (5 g benzocaine/100 mL 90% ethanol, which in turn had ~1.5 mL dissolved in ~1 L water) and controlled for glochidia infestation with the help of a microscope. All of these fish, except one 1^+ brook trout, carried mussel larvae to a varying amount. Three classes of fish were created to define the level of infestation (the classification system is modified version of the one used by Wengström et al., 2016):

- 0: No glochidia larvae on the gills.
- 1: 10-100 glochidia larvae.
- 2: >100 glochidia larvae.

When one check-up was finished, the subjected trout was placed in a recovery bucket. When the fish had retained their mobility and balance, they were quickly returned to their aquariums. A total of seven trouts died within the following week. All of them were 0^+ and six out of seven were brook trout. It is likely that the cause of death was high infestation levels, as four fish (including the brown trout) that were examined post mortem belonged to class 2 and had mucous gills, suggesting that they suffered from severe inflammation. However, the first three deceased brook trout were not controlled for glochidia infestation. None of these trouts were included in any subsequent experiment or analysis.

A side test alongside the main project aimed to determine if intraspecies interactions or interspecies interactions would increase the likelihood of one fish being successfully infested with glochidia larvae. A special treatment group was used for this experiment. The fish of this batch were separated from the others and put pairwise into 18 tanks of 3 L each. Some pairs only consisted of one species while others included both brown trout and brook trout. The trout were exposed to the same concentration of glochidia larvae as the first batch. The gills were checked ~12 h after infestation rather than 2 days. In this side experiment, only three brook trout had been successfully infested, but two of them had lost their glochidia larvae after another three days. The infected individual was placed with the previously infested fish while the others went to the Class 0 tanks.

The fish were initially fed with 1 pinch of dry food per day to see if they would start to eat. When the trout in all tanks started to consume the food, the dose was increased, but not in excess order to prevent growth. Ca $1^1/_2$-2 tsp was used depending on the number of fish and their average size. However, the dry food was eventually changed to frozen *Chironomidae* larvae. These were packed into small cubes, which individually weighed ~3.3 g, with one cube placed in each storage tank during every feeding. The feedings were kept a few days apart.

Despite being infested, it was ultimately decided that neither the 0^+ trout nor the infested 1+ brook trout should be used for the interaction experiments as they were too few in numbers for the statistics. The latter group was therefore moved to a separate aquarium. The glochidia development of the infested brook trout was followed closely to see how long the fish could hold the mussel larvae.

A couple of weeks after the initial animal collecting, another batch of brook trout was brought to the Zoology building. However, these were not artificially infested by glochidia larvae, as it was decided that all tested brook trout should be free from glochidia. Ultimately, the project as a whole included the groups of fish listed in Table 1.

Table 1. Number of trout in every fish type group involved in the study.

Infestation, Age & Species	Number of Fish
Infested 0+ Brown Trout*	10
Non-Infested 0+ Brook Trout*	5
Infested 0+ Brook Trout*	6
Non-Infested 1^+ Brown Trout	21
Infested 1+ Brown Trout	22
Non-Infested 1+ Brook Trout**	31
Infested 1+ Brook Trout***	5

*Not used in the experiments due to their small numbers. Infested 0+ Brook Trout had to be excluded in either case as they soon died after being infested.

**One used individual was infested for at maximum 3 days. Due to the combination of low infestation level (less than 10 larvae), the short infestation period and the 1-month long interval between the infestation and the experiments, it was determined that the fish was healthy enough to participate in the experiments. This trout is not included in the row with Infested 1+ Brook Trout.

***Were only used to test how long the brook trout could remain infested with glochidia larvae. These fish were not used for any of the interaction experiments.

3.2.2 Tagging the fish

To allow individual identification, the fish were tagged with small passive integrated transponders (PIT-tags), after being anaesthetized using benzocaine. In addition, the weight and length of the trout were measured (ID and weight is reported in Appendix III, Table IV & V). A small incision in the fish’s side was made with a scalpel, which served as a gateway when inserting the tag. Before being put into the recovery bucket, the gills of the fish from the infestation tank were controlled for glochidia infection. Two buckets were used to avoid mixing tagged fish with the non-tagged. After regaining most of their balance, the trout were placed into new aquaria. These tanks had recently housed other groups of brown trout and brook trout. The reason behind this was to separate tagged fish from non-tagged individuals. However, after a couple of weeks all fish were separated into three tanks depending on species and if they were carriers of glochidia larvae.

3.2.3 Further preparations

The fish were divided into three identical aquariums in order to separate the brook trout, the non-infested brown trout and the infested brown trout before the actual experiments. The aquariums had a volume of 130 L each (50 cm long×65 cm wide×40 cm high). The bottoms were covered with gravel to create a more natural-looking environment. Rocks and small grey-colored plastic boards were used to make hiding spots and shelters, and the aquaria were covered with black plastic bags along the sides and the back to avoid stress and unnecessary aggressive interactions. The tanks were separately connected to a recirculatory system to keep the water clean and aerated at a stable temperature of 10-11 °C. These aquaria were mainly used to house the fish when they were not experimented on. However, they were also used when recording the fish's daily activity and behavioral traits for the group interaction experiments (more on this in 3.2.4 Recording of general behavior in storage tanks). The laboratory had automatic lights that were turned on every morning. Proximately, the trout were exposed to artificial light ~12 h per day.

3.2.4 Recording of general behavior in storage tanks

The general behavior of the fishes was recorded in their respective groups before the pairwise interaction experiments. These recording sessions were done in 2 days with a total of 5 h being filmed per day, 3 in the morning and 2 in the afternoon. There was also a 1 h break between the filming sessions. The number of initiated interactions was counted when analyzing the recordings (Appendix II, Table I-III).

3.2.5 Pairwise interaction experiment

Every pairwise interaction included two fish of similar size that were tested together. The tests aimed to determine whether the glochidia infestation and the species factor affected the dominance display during the interactions. These experiments were conducted in three, small 15 L (30 cm long×20 cm wide×25 cm high), isolated tanks, filled with ~5 L water. Like the other tanks, the bottom was filled with gravel and a large stone was used as a potential hiding spot. The outside of the sides and the back were covered with dark plastic bags. The fishes were given 30 min to acclimate to their new environment before the cameras started to roll. They were then left alone for the majority of the experiment in order to avoid unnecessary stress and disturbances that could affect the results. A camera for each tank had been previously set up to record the fish during the test. The interactions were constructed in the following way:

- Non-infested brown trout against non-infested brook trout.
- Non-infested brown trout against infested brown trout.
- Non-infested brook trout against infested brown trout.

Every described interaction was repeated a total of ten times, although no trout was used more than once. As such, a total of 60 fish were used; 20 for every interaction which in turn used 10 trout from the included fish groups. Through this design, the eventual results would reveal whether the glochidia infestation and the species factor affected the outcome of the interaction.

The fishes had previously been divided into 6 different interaction classes depending on species, infestation class, weight and species and infestation class of eventual opponent. Only trout within the same class were allowed to face each other, but only if they were of a different species or infestation class. However, trout of Infestation Class 0 were allowed to face each other if they met the other criteria. This was done in order to avoid major differences in size that otherwise risked having a significant effect on the final results. The fishes in each group were divided between smaller and bigger trouts. The individuals in these groups were the ones closest in size to each other. The varying weights were not seen as an obstacle for the experiments, as trouts will seldom be of equal size during natural encounters. Technically speaking, this only made the experiments more realistic, although larger differences in weight was actively avoided. The interaction classes ended up looking as designed in Table 2 below:

Table 2. Description of interaction classes.

Interaction Class	Fish	**Weight (g)**
1a	Small non-infested brown trout and brook trout.	Min. 11.0 Max. 16.4
1b	Big non-infested brown trout and brook trout.	Min 17.3 Max. 24.8
2a	Small non-infested and infested brown trout.	Min. 6.7 Max. 9.3
2b	Big non-infested and infested brown trout.	Min. 9.0 Max. 12.7
3a	Small non-infested brook trout and infested brown trout.	Min. 5.7 Max. 11.4
3b	Big non-infested brook trout and infested brown trout.	Min 8.7 Max. 13.6

Three buckets were used when identifying the fish; one was for the actual identification, another for storing unwanted fish before returning them to the storage tank and the last was for the test fishes. The interaction pair remained in their bucket there for a short amount of time before being moved to the experimental tank. The purpose of this was mainly to prevent any trout from establishing a territory before they were both in the aquarium, but also to make them aware of each other's presence. One experimental session was completed per day; but the actual film recording took only 2 h. When 1 h had passed, roughly a third of a *Chironomidae* cube (~1.1 g) was placed in the tank. To ensure that the trout would eat, feeding was put on a pause a few days before the experiments started. However, they were fed once after the first session to make sure that they remained healthy for the entire experimental period. A total of 30 interactions were filmed, 10 per the interaction designs listed above. No trout was used more than once, as individuals with dominant or submissive personalities could skew the data if they partook in several sessions. After every session, the weight of the fishes was measured and the gills of the infested brown trout was checked once again. Used trout were moved into new aquariums to prevent them from mixing with untested individuals. Every interaction (according to the previously described designs) shifted tank during the subsequent session.

When analyzing the films, every strike per individual fish was counted, with the proportion of strikes per fish during one interaction being calculated from this data. Dominant individuals were defined as the ones with highest number of strikes during the interaction (Appendix III, Table IV & V).

3.3 Statistics

Six ANCOVA-tests (analysis of covariance) with confidence limits of 95% were performed for the pairwise interaction experiment. The ANCOVA test calculates the *F* statistic, a measure of the ratio of the variability between and within groups. Effect size was also calculated for significant variables (Appendix IV, Table VI-XI). Three ANCOVAs tested data before food was added and the other three data after the fishes were fed. The general dominance behavior, the number of strikes and the proportion of strikes per fish were used as the dependent variable. Infestation class and species were kept as fixed factors while difference in weight (g) was used as a covariate. The two competing individuals were of relatively similar weight while it could differ largely between the pairs (the actual weight is reported in Appendix III). As such, the difference in weight was used instead (termed weight advantage in the test), with the smaller fish receiving a negative value of the difference and the larger a positive. Degrees of freedom (df) were also calculated for every tested variable and covariate.

All statistical tests were made in the software SPSS (version 29). All graphs presented in the 4 Results were made in the Excel software (Microsoft Office 2019).

4 Results

4.1 Length of infestation period for brook trout

Five out of six 1+ brook trout became infection-free within the length of a month, but the final brook trout lost its larvae about one and a half month after the infestation (Table 3). All 1+ brook trout initially carried less than 5 glochidia larvae.

Table 3. Length of infestation period for artificially infested 1+ brook trout.

Brook Trout ID	Length of Infestation Period
800071*	3 days
798711	3 days
798724	2 weeks
798786	3 weeks
800086	1 month
798774	$1^1/_2$ months

* Due to the combination of low infestation level (less than 5 larvae), the short infestation period and the 1-month long interval between the infestation and the experiments, it was determined that this fish was healthy enough to participate in the interaction experiments and was thus used while the rest was not.

4.2 Behavior in storage tanks

The non-infested brown trout had a higher average number of initiated aggression than the other two groups. The non-infested brook trout had the second highest number, although it was only a small difference between those fish and the infested brown trout. The results show an obvious trend that hints at a more prevalent aggressive behavior among the glochidia-free brown trout, which is seemingly subdued by the parasites. The brook trout, as predicted, showed less aggression due to their more social behavior (Lovén Wallerius, 2017), even though it was hypothesized that their number would be closer to the non-infested brown trout. Every group had a large standard deviation (Figure 5).

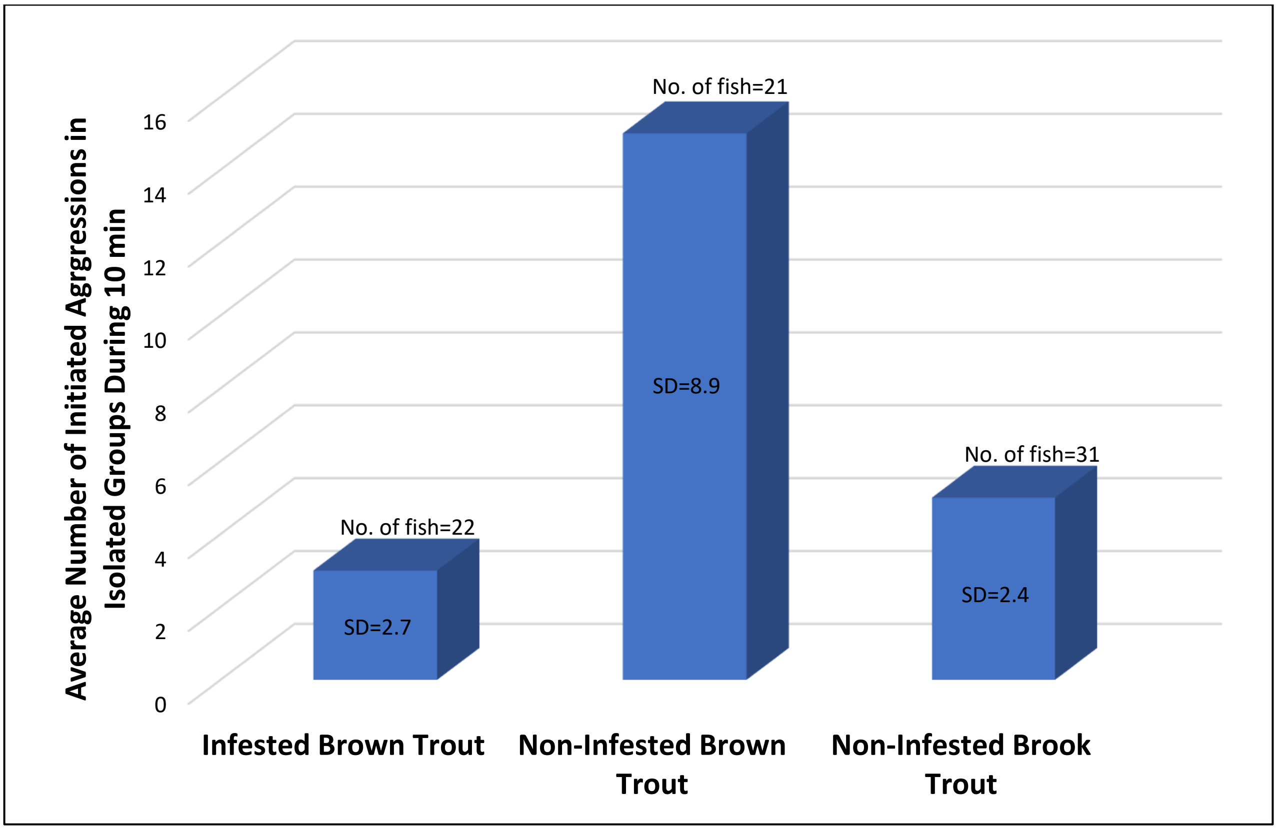

Figure 5. The average number of initiated aggressions in isolated groups of only infested brown trout, non-infested brown trout and non-infested brook trout. The average is calculated from the number of initiated aggressions during random 10 min intervals used in the data analysis. The standard deviation (SD) is included in every column. As every group were held in 1 aquarium, N=1 for every group in this experiment.

4.3 Pairwise interactions

4.3.1 Before feeding

Three ANCOVA-tests with confidence limits of 95% were used to determine whether the general dominance behavior, the number of strikes respectively the proportion of strikes before feeding were significantly affected by infestation class, weight advantage and species. The latter two had 1 df during every test, while infestation class had 2 df.

The infestation class was the only variable that had a significant effect (F=4.578, p=0.014) on the general dominance behavior, with infested fish being less dominant than non-infested fish. However, the actual impact was small, as the effect size was 0.143. Weight difference (F=2.420, p=0.126) and species (F=0.057, p=0.813) were both unsignificant. This means that the larval infestation was of larger importance when determining what role the individual trout would take during the interaction, while species seemingly had very little to no impact. In fact, the infested brown trout had a much lower proportion of dominant individuals than both the non-infested brown trout and the brook trout (Figure 6). While infested fish were less dominant, the general dominance behavior did not decrease linearly with increasing infestation. Closer examination hints that there was only a small difference between Class 1 and Class 2, with the latter even having a larger proportion of dominant trout (Figure 7).

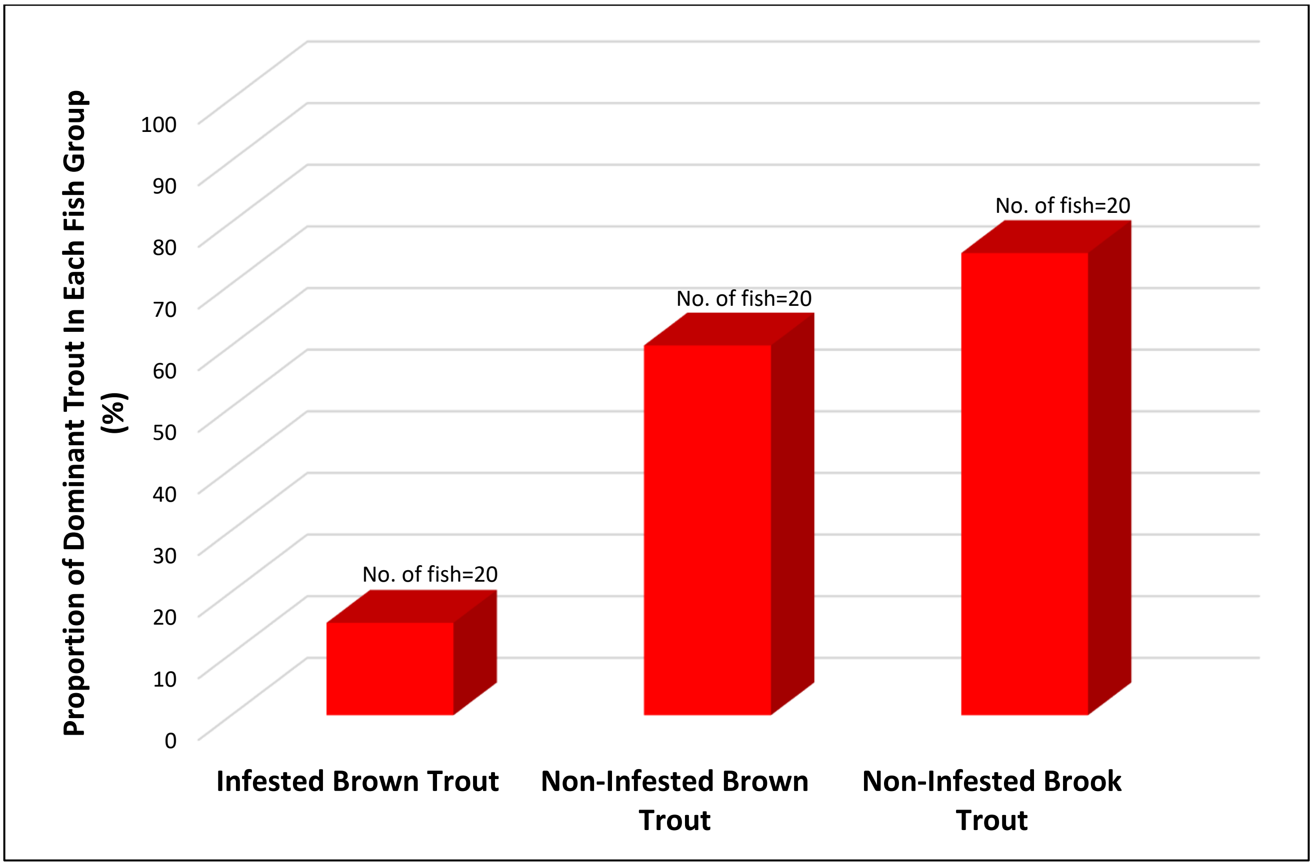

Figure 6. The proportion of dominant individuals in every fish group.

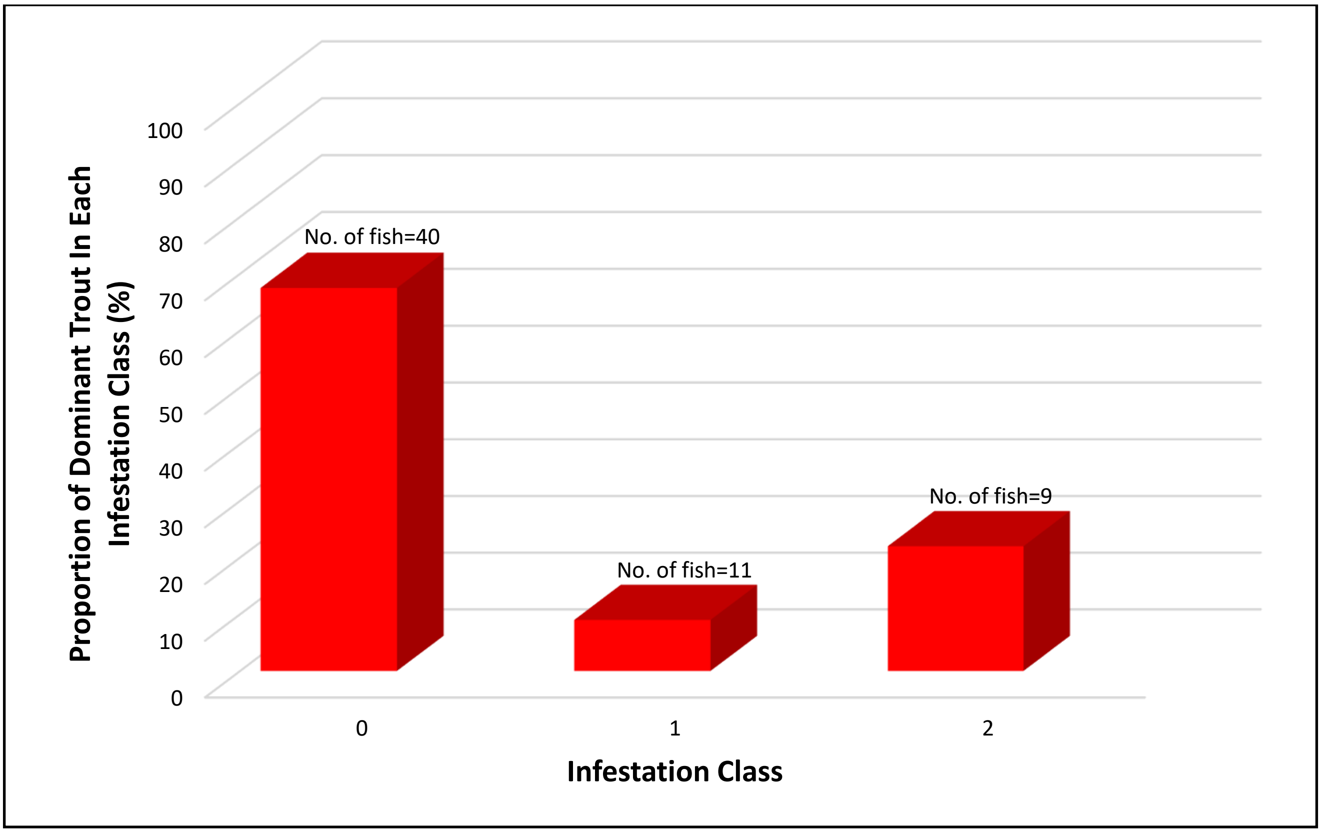

Figure 7. The proportion of dominant trout in every infestation class without the presence of food. Class 0 includes both native brown trout and invasive brown trout, while Class 1 and Class 2 only had brown trout.

Neither species (F=2.462, p=0.122) nor infestation class (F=2.303, p=0.110) had a significant effect on the number of strikes before feeding. Instead, the weight advantage supposedly plays a bigger role as it had a significant effect according to the ANCOVA-test (F=4.826, p=0.032). However, the calculated effect size was small, being only 0.081.

In stark contrast to the no. of strikes-results, the infestation class had a significant effect on the proportion of strikes per fish (F=6.005, p=0.004). Weight advantage did once again play an important role (F=11.223, p=0.001) while species remained unsignificant (F=0.217, p=0.643). None of the significant variables had any large effect size. Infestation class had an effect size of 0.179, while weight advantage had 0.169. While infested fish were generally responsible for a smaller proportion of strikes, Class 2 fish displayed dominant behavior more frequently than Class 1 fish. The results for these classes overlap and the overall difference between them is small. The relationship between infestation class and proportion of strikes is illustrated in Figure 8.

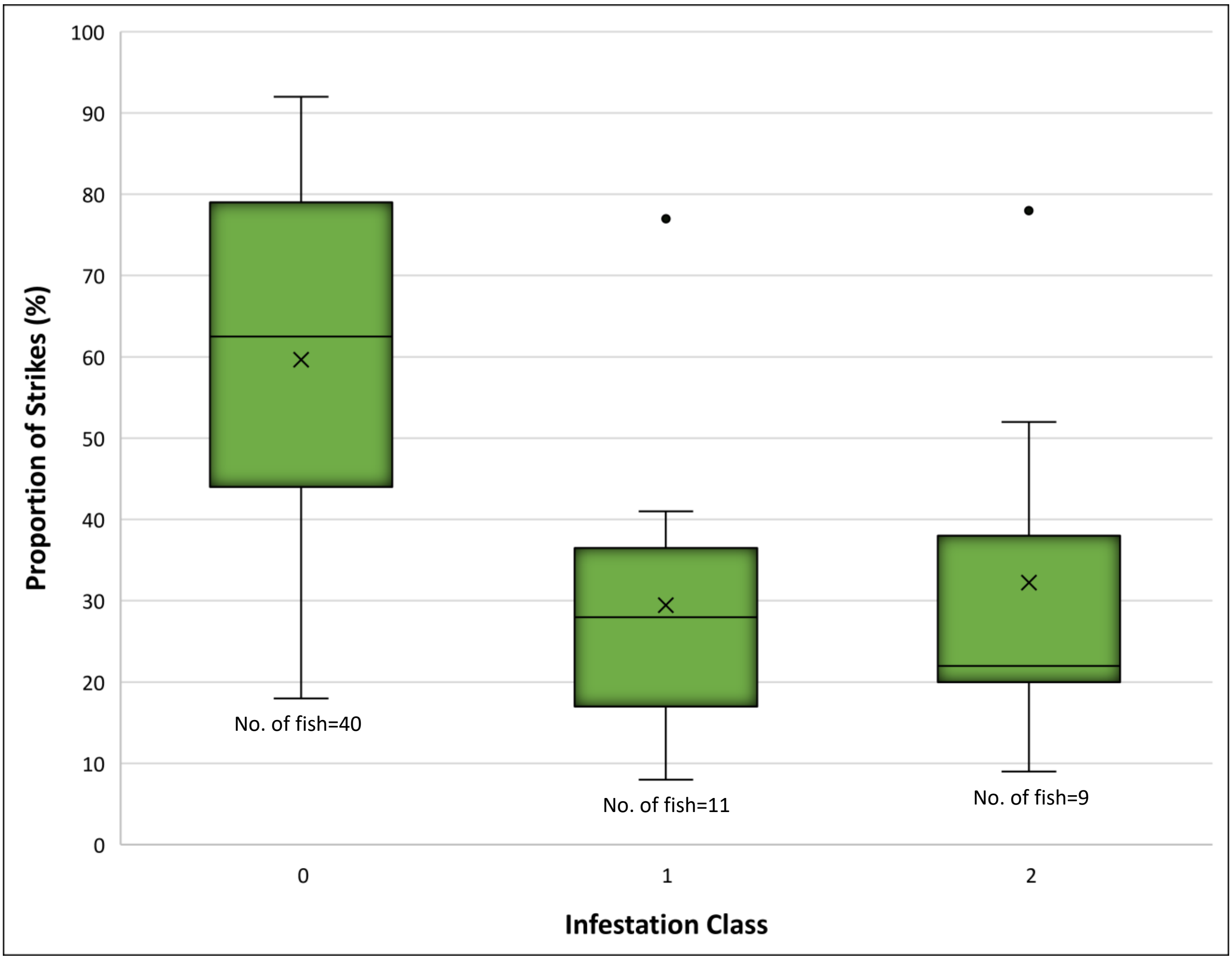

Figure 8. How the proportion of strikes varied between the infestation classes before feeding. × marks the average value, —— is the median and · is an extreme value. The boxes themselves contain 50% of the group's values, with the remaining being divided between the two quartiles (the arrow-looking lines). The lower quartile contains the 25% lowest values, while the upper contain the 25% highest (excluding extreme values).

4.3.2 After feeding

Another three ANCOVA-tests with confidence limits of 95% were used to determine whether general dominance behavior, the number of strikes respectively the proportion of strikes after feeding were significantly affected by infestation class, weight advantage and species. The latter two had 1 df during every test, while infestation class had 2 df.

Infestation class was once again the only variable that had a significant effect (F=11.001, p<0.001) on the general dominance behavior, with a close to moderate effect size of 0.286. Weight advantage (F=3-676, p=0.060) and species (F=2.465, p=0.122) remained unsignificant despite the changed conditions (Figure 9 & 10). While the addition of food triggered some differences in results, the conclusions regarding the importance of the variables remain largely the same. Class 2 had no dominant trout, but as Class 1 has only a very small proportion of dominant individuals, it cannot be certified that there is a linear decrease of general dominance behavior with increasing infestation.

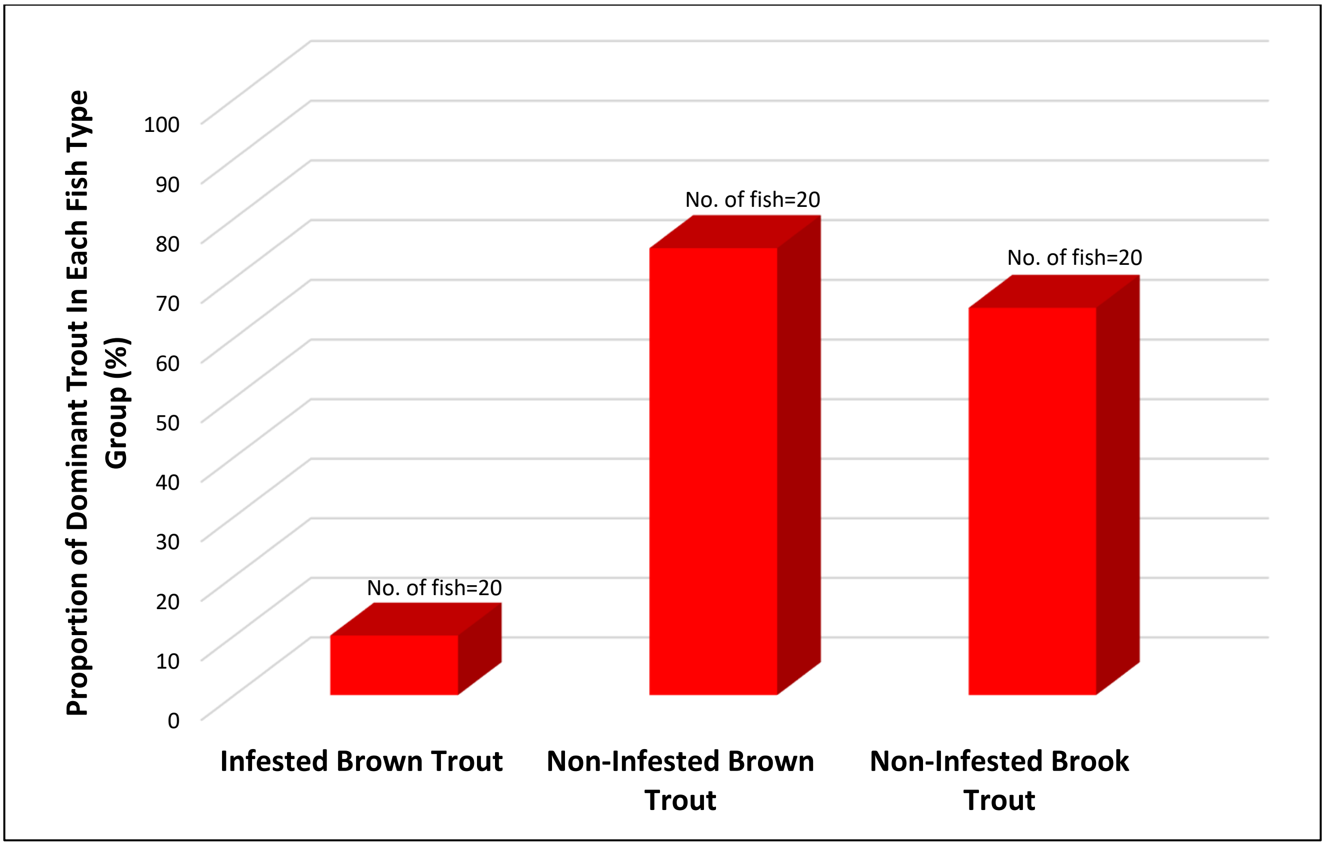

Figure 9. The proportion of dominant individuals in every fish type group.

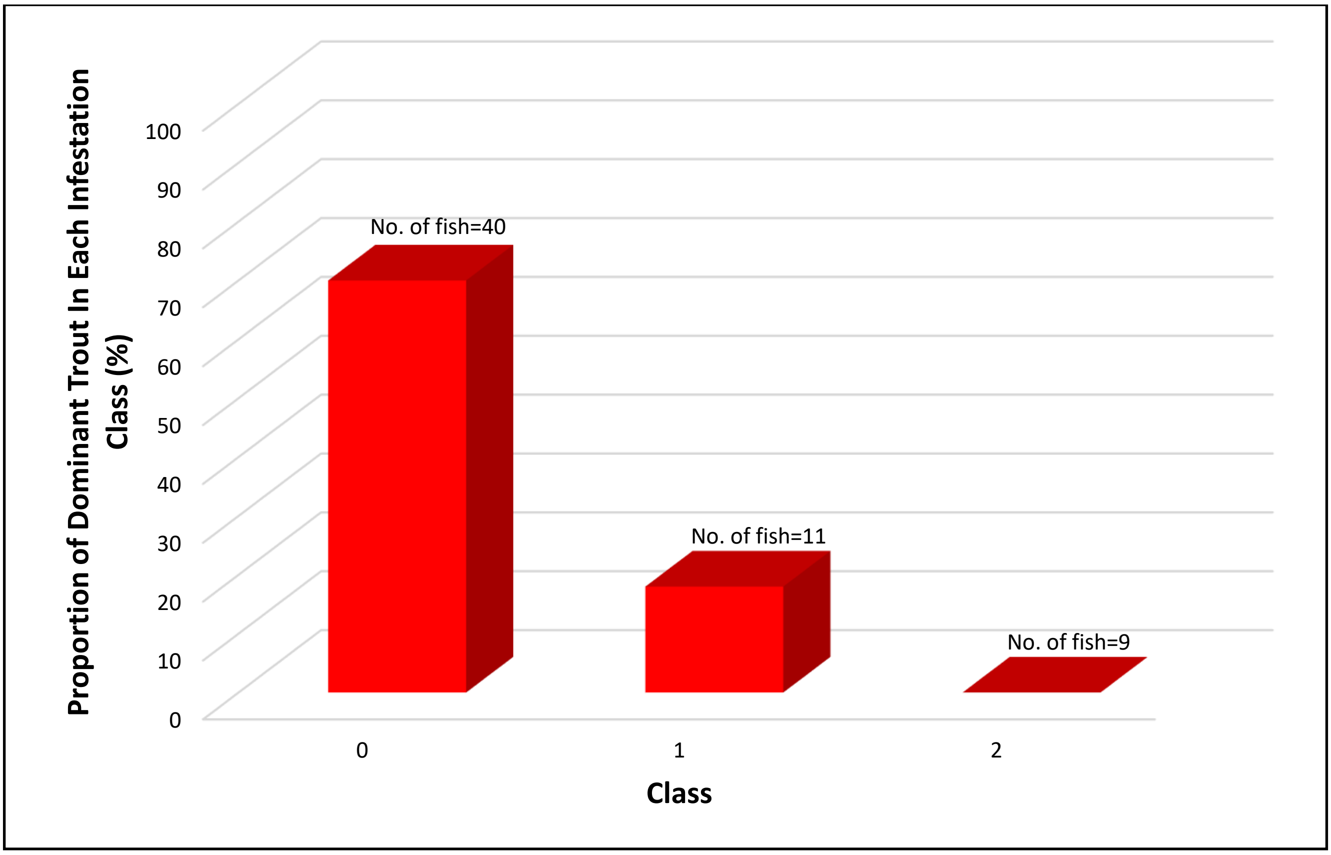

Figure 10. The proportion of subordinate and dominant individuals of each infestation class in the presence of food. Class 0 included both brown trout and brook trout, while Class 1 and Class 2 only had brown trout.

Unlike the interactions without food, weight advantage has no significant effect on the number of strikes after the fish were fed (F=1.710, p=0.196). Likewise, species (F=0.645, p=0.425) was also unsignificant and infestation class (F=3.121, p=0.052) was marginally unsignificant.

Like the previously analyzed interactions, infestation class had a significant effect on the proportion of strikes after feeding as well (F=14.267, p<0.001), with a moderate effect size of 0.342. The weight advantage was also significant (F=8.289, p=0.006), but it had a small effect size of 0.131. Species was once again unsignificant (F=1.876, p=0.176). Figure 11 illustrates these results and shows that on average, Class 1 had slightly more dominant individuals than Class 2. However, Class 1 trout had a larger variance than those of Class 2, having both individuals that were responsible for a larger proportion of strikes and fish with a smaller percentage of strikes. As such, while it looks different than the Before Feeding-interactions, there still seems to exist an overlap between these 2 infestation classes.

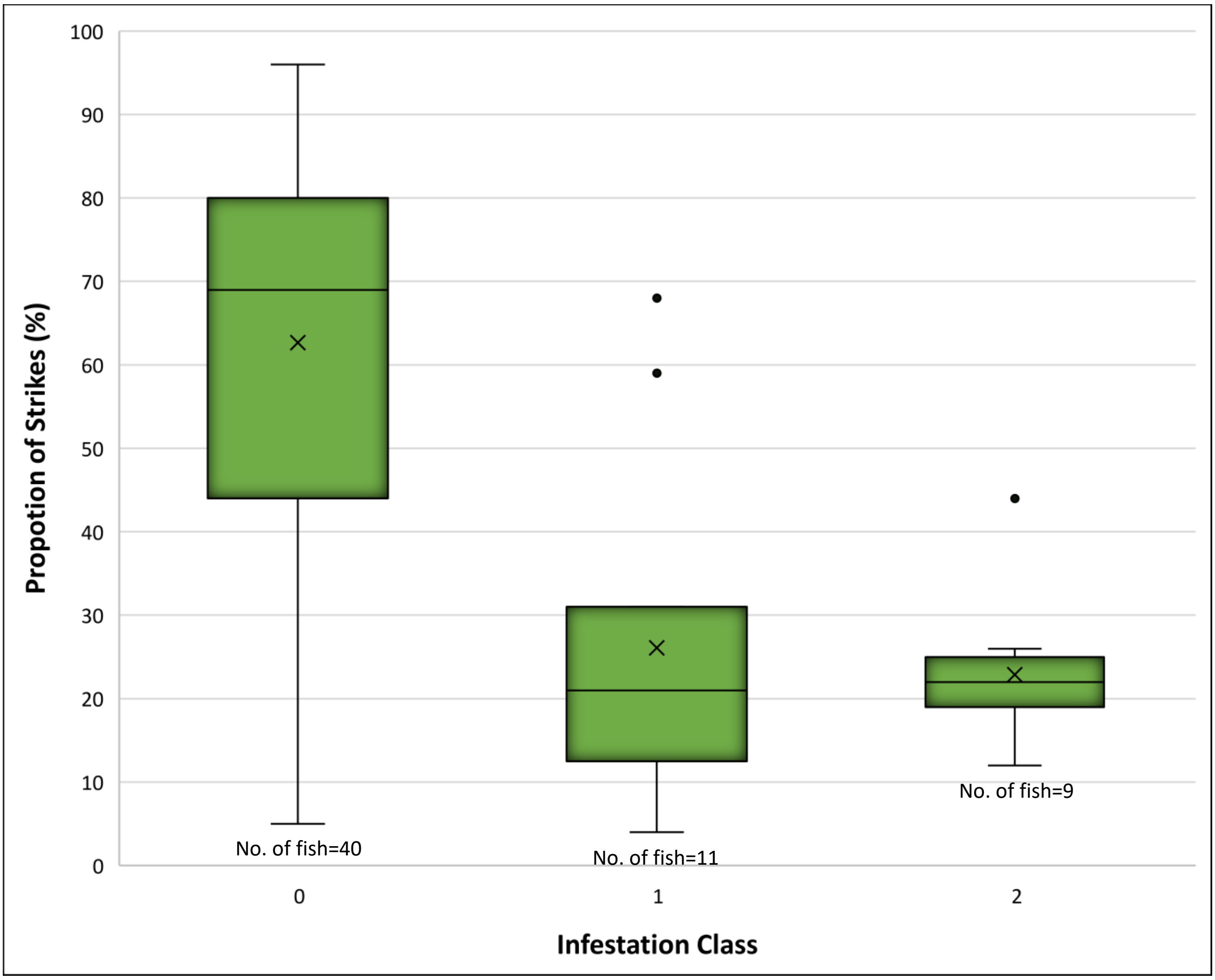

Figure 11. How the proportion of strikes varied between the infestation classes after feeding. × marks the average value, —— is the median and · are extreme values. The boxes themselves contain 50% of the group's values, with the remaining being divided between the two quartiles (the arrow-looking lines). The lower quartile contains the 25% lowest values, while the upper contain the 25% highest (excluding extreme values).

5 Discussion

In agreement with the hypothesis, the glochidia infestation decreased competitive ability of the brown trout, reflected in reduced proportions of dominant individuals and strikes per fish. However, the infestation has no impact on the actual number of strikes. Unfortunately for the freshwater pearl mussel, the brook trout turned out to be a poor substitute for the brown trout. The 0+ brook trout died and the 1+ brook trout lost their larvae in a short span of time (Table 3), confirming that the species is an unsuitable host for the bivalve. All these results offer several interesting implications regarding the behavior of the trouts and the survival of the freshwater pearl mussel.

There is no major surprise over the experiment's outcome regarding the brown trout's behavior. Considering the many physical effects of the glochidia infestation (e.g., Filipsson et al., 2017; Thomas et al., 2013), it would be odd if no behavioral effects were observed at all. Similar results have been reported from projects studying other fish-parasite relationships (Demandt et al., 2020; Horký et al., 2014; Santos & Portes Santos, 2013) and is therefore neither uncommon or exclusive to the brown trout-freshwater pearl mussel relationship. The results also align with the studies by Filipsson et al. (2016), who also noticed reductions in dominance performance.

Unlike this project, their trout were naturally infested, increasing the likelihood of these interactions occurring in the wild as well. One unexpected outcome for these experiments was the small difference between Class 1 and Class 2 trout regarding the dominance display. Before adding food, there was barely any visible difference between their proportions of strikes (Figure 8). Class 2 trout was even slightly more dominant than Class 1 trout during these interactions. One potential explanation is that the Class 2 trout could also have been provoked to a more risk-taking behavior by stress, caused by the heavy glochidia load and their reduced growth rate (Chowdhury et al., 2019). Meanwhile, Class 1 trout would likely try to save energy due to its condition, as full recovery were easier to reach. This could also explain why the proportion of strikes after feeding varied more for this class, despite the larger average. One thing that should be made clear is that the results from the After Feeding-recordings is more relevant for the conclusions of this study. When the trout have valuable resources to compete for, they should be more prone to take the same roles they would otherwise have in the wild.

Some unidentified factors impact the number of strikes per fish of both species. Weight advantage was only significant in interactions without food and it had a low effect size. Something that could have affected the fish is their own individualism. There are reports of observed personality traits in individual brown trout and brook trout that affect their general behavior (Adriaenssens, 2010; Farwell et al., 2014), which has become an increasingly more researched topic (Kortet et al., 2014; White et al., 2017; White et al., 2019; Závorka et al., 2015; Ågren et al., 2019). However, despite the likelihood of potential effects, it is probably not the main cause. There are other possible factors that could have influenced the fish. One with is the fact that the trout were kept in larger storage tanks, where they interacted with several individuals for a few weeks before the experiments. This period certainly gave the trouts the opportunity to establish hierarchies in their respective tank. Even though it is only based on pure speculation, the fish that were more dominant in larger groups should in theory express similar behavior when interacting with a single trout, even if they were strangers to each other. There have been earlier observations of dominant brown trout keeping their social status in subsequent interaction tests (Tiira et al., 2009). It is thus possible that this factor greatly influenced the fish of this study.

While being unable to test this statistically, there is still a strong trend that indicates that isolated groups on non-infested brown trout are more aggressive than groups consisting of the either infested brown trout or non-infested brook trout. While the infested trout had the lowest average number of initiated aggressions, there was barely any difference between them and the brook trout. As such, any meaningful behavioral difference between groups can only be observed between the glochidia free brown trout and the other two groups. However, the infested brown trout were not completely isolated as their tank also were inhabited by three brown trout suffering from swellings in the chest area. Only two of them were infested with glochidia larvae. However, no difference in behavior was observed between these two groups. In fact, the most aggressive brown trout was identified as one bigger individual used in the pairwise interaction experiment.

Of interesting note is the fact that no statistical test showed any meaningful impact of the species variable. This might seem contradictory of the background, as brook trout are outcompeting the brown trout in many streams of Sweden. However, as shortly mentioned in 2 Aim of the study and 3.2.5 Pairwise interaction experiment, the two species can have opposite roles, for example in North America. Many studies have been made on invasive brown trout (e.g., Fost et al., 2016; Hoxmeier & Dieterman, 2016; Huntsman et al., 2022; McKenna Jr. et al., 2013), and the switching roles between the species could explain why the statistics showed no significant results regarding this variable. Why these roles could alter between different environments is not fully known, although the physical environment has been suggested as a key factor. The experiments occurred in an artificial environment, which likely lacked the physical factors that determine the outcome of interspecies conflicts.

Twelve of 20 infested brown trout died shortly after the experiment. In comparison, only three of 20 non-infested brown trout and one of 20 brook trout died. The deaths of the latter two groups were also caused by another fish, while most of the infested trout died or were euthanized due to disease or poor health. Only one of these trout died differently, as it suffocated on gravel that it accidentally swallowed. From these observations alone, there seems to be a trend of generally poorer health of glochidia infested trout, something that was mentioned earlier in this report. However, the high number of casualties is still surprising as this outcome should be something that the parasite wants to avoid. One potential explanation is the simple fact that the brown trout suffered from extra stress as they were outside their natural habitats and exposed to different experiments. However, the main cause was probably the relatively high infestation levels. Low infestation levels consist of 1-10 larvae per gill, a number none of the fish used in the pairwise interaction experiment was even close to (Wengström et al., 2016) (Appendix III).

Although it ultimately only became a small side test beside the main project, it is still interesting to note the brook trout had different immune responses to the mussel infection. While 1+ individuals successfully combated the infestation, younger brook trout soon died due to inflammation on the gills. This hints that the immune responses to this kind of parasitation is not fully developed on such young brook trout, making them less resistant than brown trout of the same age. At least four of six 0+ brook trout that died had a very high infestation level, as they belonged to Class 2. In combination with the glochidia infestation being artificial, it is not strange that the brook trout had so little resilience during these conditions. The scenario is a stark contrast to the older fish's infestation period (Table 1), with the brook trout having a much more effective immune response than the native host. It is those brook trout that will be problematic in the wild, as they have a great advantage over infested brown trout. The invader cannot replace the latter species as a host for the mussel, preventing its offspring from reaching adulthood and sexual maturity. Even if younger brown trout potentially is more durable against the larvae infestation compared with the younger brook trout, they would likely not be the mussel's salvation. Older brook trout are fierce competitors and small brown trout tend to avoid streams inhabited by larger individuals of the invasive species (Lovén Wallerius et al., 2022). Plus, all studied brook trout was artificially infested. It is not certain that they would gain that many glochidia larvae in the wild.

The difference in weight had a significant effect on the proportion of strikes, which indicate that bigger fishes are more dominant and aggressive. This has been observed in other studies for both brown trout (Jacob et al., 2007; Näslund & Johnsson, 2016) and brook trout (Lovén Wallerius, 2021; Macneale et al., 2010). The pairwise interaction experiment aimed to have relatively similar sized trout in every pair. Had the size difference been, for example, more than 10 g, the larger trout would definitely been dominant regardless of infestation class. Lovén Wallerius (2021), reported that juvenile brown trout would avoid territories guarded by adult brook trout, meaning that most individuals are fully aware of how this factor affects their competitive ability.

The gender of the individual fish was not controlled for this experiment due to practical reasons. However, the aim of this study was to only control if any differences in behavior could be observed between the species and infested/non-infested trouts. The sex was never supposed to play a major role in the experiments, although it could serve as basis for potential follow-up studies.

This study provides important information for conservation projects of both brown trout and freshwater pearl mussel. The species' close connection could endanger the preservation work, as infested brown trout is less capable competitors than their non-infested counterparts. The situation is only made more complicated by the growing presence of brook trout. Salonen et al. (2016) came to the same conclusions after their studies, which showed that invasive brook trout were dominating streams that served as habitats for freshwater pearl mussels. The fish met more resistance from brown trout in mussel free streams, meaning that the patterns of this experiment is in effect in the wild. Not only does it give the results more credit, but it could also give additional clues to the biologists. As the brook trout benefits from the brown trout-glochidia larvae relationship to the detriment of the other two species, the goal should be to immediately remove brook trout from all mussel streams. The bivalve is enduring extremely high levels of pressure, removing one obstacle would aid the species' recovery at least a bit. Other measures still need to be made to guarantee the mussel's survival. However, as there already exist removal projects involving the brook trout (Jansson, 2013), this part of the freshwater pearl mussel-conservation could easily be made with only slight rearrangements in the established brook trout programs. Said rearrangements should state that mussel-inhabited water should be of higher priority. Some of these projects are developed and ongoing in Sweden, including the NOBROOK project financed by the Swedish Environmental Protection Agency (SEG, 2023). Similar models could be applied for other endangered parasites, as they may have similar effects as the freshwater pearl mussel during the infestation period. This strategy should also be repeated in streams inhabited by freshwater pearl mussel hosts other than the brown trout, including the Atlantic salmon. Future behavioral studies of glochidia infested salmon could be useful tools in future conservation works. Results of those studies could also be interesting to compare with this and other projects focusing on the brown trout, as there are some reports that state that the survivability and suitability of the host depends on genetic adaptations of the mussel. The most common host in the area usually serves as the main or only host for one mussel population, a relationship that has become very specific through the means of evolution. Freshwater pearl mussel population genetics could differ greatly depending on the dominating host (Karlsson et al., 2014; Marwaha et al., 2021; Salonen et al., 2017; Taskinen & Salonen, 2022).

5.1 Conclusions

To summarize, the results clearly indicate that the glochidia infestation negatively impacts the behavior of the brown trout. Infected fish seldom takes the dominant role during encounters with other fishes and they tend to make a smaller proportion of strikes during the interaction. While this certainly is a problem for the brown trout, especially as it has to compete with the invasive brook trout, there should be a bigger concern for the survival of the freshwater pearl mussel. As it is a highly endangered species, any new threat or obstacle could have dire consequences. The study reveals a major threat as the brook trout have a larger opportunity of replacing brown trout of mussel-inhabited streams. This would prevent the freshwater pearl mussel from gaining new recruits. Therefore, it is highly recommended that rivers and streams containing the bivalve should be prioritized when removing brook trout. Similar strategies could be considered when protecting other endangered parasites.

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Appendices

Appendix I. Popular science summary

AN ENDANGERED MUSSEL ENSURES ITS OWN DOOM

By Isac Brander

Most people likely associate mussels with warm days on the beach. If not observed in the water, you will see their empty shells spread in the sand. However, some species are actually found in freshwater instead of the sea. The fitly named freshwater pearl mussel are one of the more known limnic mussels. It is an important filtrator of its native rivers and streams, but has sadly diminished greatly in numbers due to pollutions and overall worsened conditions of their waters. Another problematic aspect for the survival of the species is their parasitic larvae. These parasites attach themselves to salmonids such as the brown trout for several months of growth. According to my own research, this strategy might potentially backfire as the host fish lose some of their ability to properly compete against rival species.

The mussel and its host

The threatened freshwater pearl mussel (*Margaritifera margaritifera*) is today found in the Northern parts of Europe, Eastern U.S. and Canada. The future of the mussel does not look bright, as several local populations are lost every year. The bivalve has proven to be heavily affected by environmental changes. It demands a stable water system, pristine and non-polluted. Similar environments are sought after by salmonids, a group of fish that plays a very important role in the bivalve's life cycle. Unlike their adult counterparts, the mussel larvae (also known as glochidia larvae) live as parasites that prey on juvenile salmonids. One common host is the brown trout (*Salmo trutta*), whose gills functions as an attachment area for the mussel. The larvae will not let go until 9-11 months later, when they have developed into tiny mussels.

The larva infested gill of a brown trout, marked by a red circle in the left picture. The larvae look like white dots on the pink-coloured gill lamellae (photo by Kvarnliden, 2022).

While the brown trout can benefit from sharing their habitats with the freshwater pearl mussel due to them filtrating and cleaning the water, the fish are still negatively affected by the actual larvae infestation. Several researchers have reported about different ailments related to the infection, including reduced swimming performance, enlargement of the spleen and reduced dominance performance. The last has gathered interest and curiosity from the scientific community, as it could suggest the infested trout are at disadvantage when competing with healthier individuals. The matter has become increasingly complicated with the spread of brook trout (*Salvelinus fontinalis*) in Swedish waters. This fish originates from North America and was brought to Sweden for sport fishing. In several rivers and streams, the brook trout are outcompeting the native brown trout. This is also a threat against the freshwater pearl mussel, as the brook trout is a very poor substitute for the host fish.

The mussel reduces the brown trout's dominant behaviour

Through my study at the University of Gothenburg, more evidence has emerged that the mussel's life style is more of a burden during current circumstances. In this study, infested brown trout and non-infested brown and brook trout met each other pairwise to test if the larvae infestation led to a reduced display of dominance. This was measured by counting the number of pushes and bites (labelled as strikes) the fish would do against each other. While the number of strikes heavily varied between the different tests, the proportion of strikes between the two fishes in each test were clearly in the favour of non-infested trouts. Consequently, these trout were more frequently deemed as the dominant individual. From these results, I drew the conclusion that the glochidia infestation indeed decrease the dominance display.

Sadly, the results of the experiment give dark implications for the future of the freshwater pearl mussel. As the hosts are less resistant against other competitors, they are at a greater risk of suffering from starvation or being killed by territorial trout. Even more problematic is the continuous spread of brook trout, as its increasing presence hinders the mussel populations from gaining new recruits. Luckily enough, there are several projects that attempt to help the brown trout and the freshwater pearl mussel, including frequent removal of invasive brook trout.

Appendix II. Data collected from film recordings of the storage tanks.

Table I. Tank with non-infested brown trout

Day	Time of day	Time interval of analyzed observations	No. of forced displacements
1	Morning	09:00-09:10	4
1	Morning	09:40-09:50	14
1	Morning	09:50-10:00	9
1	Morning	10:10-10:20	19
1	Morning	10:40-10:50	19
1	Morning	11:50-12:00	16
1	Afternoon	13:30-13:40	8
1	Afternoon	14:10-14:20	18
1	Afternoon	14:40-14:50	26
1	Afternoon	14:50-15:00	33
2	Morning	09:00-09:10	7
2	Morning	09:30-09:40	11
2	Morning	10:00-10:10	17
2	Morning	10:20-10:30	24
2	Morning	11:10-11:20	36
2	Morning	11:50-12:00	20
2	Afternoon	13:30-13:40	8
2	Afternoon	13:40-13:50	6
2	Afternoon	14:00-14:10	9
2	Afternoon	14:40-14:50	5

Table II. Tank with infested brown trout.

Day	Time of day	Time interval of analyzed observations	No. of forced displacements
1	Morning	09:30-09:40	4
1	Morning	09:40-09:50	1
1	Morning	10:10-10:20	1
1	Morning	10:30-10:40	5
1	Morning	10:40-10:50	4
1	Morning	11:40-11:50	1
1	Afternoon	13:40-13:50	0
1	Afternoon	13:50-14:00	4
1	Afternoon	14.20-14.30	6
1	Afternoon	14:40-14:50	3
2	Morning	09:40-09:50	2
2	Morning	10:40-10-50	10
2	Morning	11:00-11:10	7
2	Morning	11:30-11:40	3
2	Morning	11:40-11:50	0
2	Morning	11:50-12:00	8
2	Afternoon	13:00-13:10	0
2	Afternoon	13:20-13:30	2
2	Afternoon	14:30-14:40	3
2	Afternoon	14:50-15:00	2

Table III. Tank with non-infested brook trout.

Day	Time of day	Time interval of analyzed observations	No. of forced displacements
1	Morning	09:30-09:40	6
1	Morning	09:40-09:50	9
1	Morning	10:20-10:30	6
1	Morning	11:10-11:20	5
1	Morning	11:40-11:50	6
1	Morning	11:50-12:00	5
1	Afternoon	13:30-13:40	4
1	Afternoon	14:00-14:10	3
1	Afternoon	14:20-14.30	5
1	Afternoon	14:30-14:40	5
2	Morning	10:10-10:20	2
2	Morning	10:20-10:30	5
2	Morning	10:40-10:50	11
2	Morning	11:30-11:40	1
2	Morning	11:40-11:50	6
2	Morning	11:50-12:00	2
2	Afternoon	13:10-13:20	6
2	Afternoon	13:30-13:40	6
2	Afternoon	13:40-13:50	2
2	Afternoon	14:20-14:30	1

Appendix III. Data collected when analyzing the film recordings of the pairwise interaction experiments.

Table IV. Before feeding.

ID	*Species*	*Day*	*Tank*	*Weight (g)*	*Infestation class*	*Interaction class*	*Strikes*	*Strikes (%)*	*Behavior*
800017	Brown trout	1	Left	16.4	0	1a	64	63	Dominant
798762	Brook trout	1	Left	13.5	0	1a	37	37	Subordinate
800055	Brown trout	1	Mid	9.1	0	2a	40	48	Subordinate
798722	Brown trout	1	Mid	7.3	2	2a	43	52	Dominant
798625	Brook trout	1	Right	5.7	0	3a	30	79	Dominant
800040	Brown trout	1	Right	6.3	1	3a	8	21	Subordinate
798661	Brook trout	2	Left	13.6	0	3b	72	68	Dominant
800035	Brown trout	2	Left	10.6	1	3b	34	32	Subordinate
800095	Brown trout	2	Mid	23.3	0	1b	107	22	Subordinate
798624	Brook trout	2	Mid	24.8	0	1b	377	78	Dominant
798791	Brown trout	2	Right	9.3	0	2a	71	59	Dominant
800067	Brown trout	2	Right	9.2	1	2a	49	41	Subordinate
798764	Brown trout	3	Left	10.3	0	2b	262	84	Dominant
798732	Brown trout	3	Left	9.0	1	2b	50	16	Subordinate
798762	Brook trout	3	Mid	11.7	0	3b	190	72	Dominant
798790	Brown trout	3	Mid	9.2	1	3b	73	28	Subordinate
800054	Brown trout	3	Right	19.6	0	1b	180	54	Dominant
798686	Brook trout	3	Right	23.9	0	1b	151	46	Subordinate

800011	Brown trout	4	Left	11.7	0	1a	11	44	Subordinate
798644	Brook trout	4	Left	14.5	0	1a	14	56	Dominant
800076	Brown trout	4	Mid	9.2	0	2b	51	22	Subordinate
800019	Brown trout	4	Mid	11.0	2	2b	180	78	Dominant
800053	Brook trout	4	Right	10.5	0	3a	117	82	Dominant
800087	Brown trout	4	Right	8.8	1	3a	26	18	Subordinate
800039	Brook trout	5	Left	12.9	0	3b	129	79	Dominant
800021	Brown trout	5	Left	10.3	2	3b	35	21	Subordinate
800038	Brown trout	5	Mid	17.5	0	1b	78	44	Subordinate
798701	Brook trout	5	Mid	17.7	0	1b	99	56	Dominant
800093	Brown trout	5	Right	6.7	0	2a	17	23	Subordinate
798783	Brown trout	5	Right	8.4	1	2a	58	77	Dominant
800059	Brown trout	6	Left	10.8	0	2b	113	92	Dominant
798741	Brown trout	6	Left	9.7	1	2b	10	8	Subordinate
798679	Brook trout	6	Mid	11.4	0	3a	25	89	Dominant
798749	Brown trout	6	Mid	7.7	1	3a	3	11	Subordinate
798737	Brown trout	6	Right	17.3	0	1b	28	67	Dominant
798782	Brook trout	6	Right	20.4	0	1b	14	33	Subordinate
800015	Brown trout	7	Left	18.9	0	1b	79	58	Dominant
798676	Brook trout	7	Left	23.5	0	1b	57	42	Subordinate
800057	Brown trout	7	Mid	12.7	0	2b	160	82	Dominant
800062	Brown trout	7	Mid	10.1	2	2b	35	18	Subordinate

800037	Brook trout	7	Right	9.1	0	3a	54	62	Dominant
800018	Brown trout	7	Right	8.4	2	3a	33	38	Subordinate
798757	Brook trout	8	Left	11.8	0	3b	32	91	Dominant
798742	Brown trout	8	Left	8.7	2	3b	3	9	Subordinate
800075	Brown trout	8	Mid	20.6	0	1b	53	80	Dominant
798697	Brook trout	8	Mid	22.2	0	1b	13	20	Subordinate
800090	Brown trout	8	Right	8.4	0	2a	128	80	Dominant
800072	Brown trout	8	Right	7.0	2	2a	33	20	Subordinate
800023	Brown trout	9	Left	9.3	0	2a	157	69	Dominant
800009	Brown trout	9	Left	8.2	1	2a	72	31	Subordinate
800044	Brook trout	9	Mid	10.9	0	3a	26	59	Dominant
800032	Brown trout	9	Mid	10.0	1	3a	18	41	Subordinate
798772	Brown trout	9	Right	11.0	0	1a	61	30	Subordinate
798665	Brook trout	9	Right	13.3	0	1a	140	70	Dominant
800041	Brown trout	10	Left	12.6	0	1a	13	18	Subordinate
800071	Brook trout	10	Left	13.9	0	1a	59	82	Dominant
800082	Brown trout	10	Mid	12.4	0	2b	343	78	Dominant
800058	Brown trout	10	Mid	10.2	2	2b	96	22	Subordinate
798677	Brook trout	10	Right	12.8	0	3b	98	68	Dominant
800060	Brown trout	10	Right	12.8	2	3b	47	32	Subordinate

Table V. After feeding.

ID	*Species*	*Day*	*Tank*	*Weight (g)*	*Infestation Class*	*Interaction class*	*Strikes*	*Strikes (%)*	*Behavior*
800017	Brown trout	1	Left	16.4	0	1a	52	76	Dominant
797862	Brook trout	1	Left	13.5	0	1a	16	24	Subordinate
800055	Brown trout	1	Mid	9.1	0	2a	43	78	Dominant
798722	Brown trout	1	Mid	7.3	2	2a	12	22	Subordinate
798625	Brook trout	1	Right	5.7	0	3a	45	87	Dominant
800040	Brown trout	1	Right	6.3	1	3a	7	13	Subordinate
798661	Brook trout	2	Left	13.6	0	3b	68	69	Dominant
800035	Brown trout	2	Left	10.6	1	3b	30	31	Subordinate
800095	Brown trout	2	Mid	23.3	0	1b	97	20	Subordinate
798624	Brook trout	2	Mid	24.8	0	1b	387	80	Dominant
798791	Brown trout	2	Right	9.3	0	2a	40	69	Dominant
800067	Brown trout	2	Right	9.2	1	2a	18	31	Subordinate
798764	Brown trout	3	Left	10.3	0	2b	297	90	Dominant
798732	Brown trout	3	Left	9.0	1	2b	32	10	Subordinate
798762	Brook trout	3	Mid	11.7	0	3b	198	79	Dominant
798790	Brown trout	3	Mid	9.2	1	3b	54	21	Subordinate
800054	Brown trout	3	Right	19.6	0	1b	92	56	Dominant
798686	Brook trout	3	Right	23.9	0	1b	73	44	Subordinate
800011	Brown trout	4	Left	11.7	0	1a	147	41	Subordinate
798644	Brook trout	4	Left	14.5	0	1a	211	59	Dominant

800076	Brown trout	4	Mid	9.2	0	2b	83	56	Dominant
800019	Brown trout	4	Mid	11.0	2	2b	66	44	Subordinate
800053	Brook trout	4	Right	10.5	0	3a	60	41	Subordinate
800087	Brown trout	4	Right	8.8	1	3a	88	59	Dominant
800039	Brook trout	5	Left	12.9	0	3b	111	74	Dominant
800021	Brown trout	5	Left	10.3	2	3b	40	26	Subordinate
800038	Brown trout	5	Mid	17.5	0	1b	144	56	Dominant
798701	Brook trout	5	Mid	17.7	0	1b	113	44	Subordinate
80093	Brown trout	5	Right	6.7	0	2a	39	32	Subordinate
798783	Brown trout	5	Right	8.4	1	2a	84	68	Dominant
800059	Brown trout	6	Left	10.8	0	2b	153	88	Dominant
798741	Brown trout	6	Left	9.7	1	2b	20	12	Subordinate
798679	Brook trout	6	Mid	11.4	0	3a	22	96	Dominant
798749	Brown trout	6	Mid	7.7	1	3a	1	4	Subordinate
798737	Brown trout	6	Right	17.3	0	1b	51	33	Subordinate
798782	Brook trout	6	Right	20.4	0	1b	103	67	Dominant
800015	Brown trout	7	Left	18.9	0	1b	35	67	Dominant
798678	Brook trout	7	Left	23.5	0	1b	17	33	Subordinate
800057	Brown trout	7	Mid	12.7	0	2b	79	77	Dominant
800062	Brown trout	7	Mid	10.1	2	2b	23	23	Subordinate
800037	Brook trout	7	Right	9.1	0	3a	43	81	Dominant
800018	Brown trout	7	Right	8.4	2	3a	10	19	Subordinate

798757	Brook trout	8	Left	11.8	0	3b	15	88	Dominant
798742	Brown trout	8	Left	8.7	2	3b	2	12	Subordinate
800075	Brown trout	8	Mid	20.6	0	1b	41	95	Dominant
798697	Brook trout	8	Mid	22.2	0	1b	2	5	Subordinate
800090	Brown trout	8	Right	8.4	0	2a	165	85	Dominant
800072	Brown trout	8	Right	7.0	2	2a	28	15	Subordinate
800023	Brown trout	9	Left	9.3	0	2a	96	84	Dominant
800009	Brown trout	9	Left	8.2	1	2a	18	16	Subordinate
800044	Brook trout	9	Mid	10.9	0	3a	73	78	Dominant
800032	Brown trout	9	Mid	10.0	1	3a	20	22	Subordinate
798772	Brown trout	9	Right	11.0	0	1a	5	56	Dominant
798665	Brook trout	9	Right	13.3	0	1a	4	44	Subordinate
800041	Brown trout	10	Left	12.6	0	1a	21	22	Subordinate
800071	Brook trout	10	Left	13.9	0	1a	75	78	Dominant
800082	Brown trout	10	Mid	12.4	0	2b	79	75	Dominant
800058	Brown trout	10	Mid	10.2	2	2b	26	25	Subordinate
798677	Brook trout	10	Right	12.8	0	3b	28	80	Dominant
800060	Brown trout	10	Right	12.8	2	3b	7	20	Subordinate

Appendix IV. Results from the statistics

All ANCOVA tests were done in the SPSS software (version 29). The confidence limits were kept at 95%. The species and the weight advantage variables had 1 degree of freedom, while the infestation class had 2.

Table VI. Results from the ANCOVA test on the general dominance behavior before feeding.

	F	***p***	***Effect size****
Infestation Class	4.578	0.014	0.143
Species	0.057	0.813	"-"
Weight Advantage	2.420	0.126	"-"

*Only reported for significant variables.

Table VII. Results from the ANCOVA test on the number of strikes before feeding.

	F	***p***	***Effect size****
Infestation Class	2.303	0.110	"-"
Species	2.462	0.122	"-"
Weight Advantage	4.826	0.032	0.081

*Only reported for significant variables.

Table VIII. Results from the ANCOVA test on the proportion of strikes before feeding.

	F	***p***	***Effect size****
Infestation Class	6.005	0.004	0.179
Species	0.217	0.643	"-"
Weight Advantage	11.223	0.001	0.169

*Only reported for significant variables.

Table IX. Results from the ANCOVA test on general dominance behavior after feeding.

	F	***p***	***Effect size****
Infestation Class	11.001	<0.001	0.286
Species	2.465	0.122	"-"
Weight Advantage	3.676	0.060	"-"

*Only reported for significant variables.

Table X. Results from the ANCOVA test on the number of strikes after feeding.

	F	***p***	***Effect size****
Infestation Class	3.121	0.052	“-“
Species	0.645	0.425	“-“
Weight Advantage	1.710	0.196	“-“

*Only reported for significant variables.

Table XI. Results from the ANCOVA test on the proportion of strikes after feeding.

	F	***p***	***Effect size****
Infestation Class	14.267	<0.001	0.342
Species	1.876	0.176	“-“
Weight Advantage	8.289	0.006	0.131

*Only reported for significant variables.