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Abstract

In this paper we describe, for the first time, the effects of freshwater pearl mussel (*Margaritifera margaritifera* L.) encystment on the drift-feeding behavior of juvenile brown trout (*Salmo trutta* L.). Because both mussel and salmonid populations are often threatened, this study not only adds knowledge to the understanding of host-parasite systems, but it is also of conservation value. Individual trout, mussel-encysted (25.1±5.7 larvae·g\(^{-1}\) body weight, *n*=5) or non-encysted (*n*=5), were fed with chironomid larvae in a flow-through stream aquarium. Feeding trials were filmed and analyzed by counting the numbers of chironomid larvae each individual ate, and by estimating the prey-capture distance. Non-encysted trout had a significantly higher drift-foraging rate than did encysted trout, and they captured significantly more prey further away from their focal point. The reduced foraging success of encysted trout was mainly due to their failure to catch prey relatively further from their focal point. This suggests that reduced foraging success of encysted trout may be due to poorer energetic status, but the physical effects of mussel larvae on prey handling time cannot be ruled out. Encysted trout caught approximately 20 % fewer prey, which would result in a reduction in growth potential during the period of mussel encystment. Reduced energetic status might also result in reduced competitive ability or in increased exposure to predation risk.

Keywords

Host - Parasite - Unionid mussels - *Margaritifera* - *Salmo trutta* - Drift-feeding - Foraging - Conservation
Parasitic freshwater pearl mussel larvae (*Margaritifera margaritifera* L.) reduce the drift-feeding rate of juvenile brown trout (*Salmo trutta* L.)

Martin E. Österling · Julia Ferm · John J. Piccolo

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**Abstract** In this paper we describe, for the first time, the effects of freshwater pearl mussel (*Margaritifera margaritifera* L.) encystment on the drift-feeding behavior of juvenile brown trout (*Salmo trutta* L.). Because both mussel and salmonid populations are often threatened, this study not only adds knowledge to the understanding of host-parasite systems, but it is also of conservation value. Individual trout, mussel-encysted (25.1 ± 5.7 larvae·g⁻¹ body weight, n=5) or non-encysted (n=5), were fed with chironomid larvae in a flow-through stream aquarium. Feeding trials were filmed and analyzed by counting the numbers of chironomid larvae each individual ate, and by estimating the prey-capture distance. Non-encysted trout had a significantly higher drift-foraging rate then did encysted trout, and they captured significantly more prey further away from their focal point. The reduced foraging success of encysted trout was mainly due to their failure to catch prey relatively further from their focal point. This suggests that reduced foraging success of encysted trout may be due to poorer energetic status, but the physical effects of mussel larvae on prey handling time cannot be ruled out. Encysted trout caught approximately 20 % fewer prey, which would result in a reduction in growth potential during the period of mussel encystment. Reduced energetic status might also result in reduced competitive ability or in increased exposure to predation risk.

**Keywords** Host · Parasite · Unionid mussels · *Margaritifera* · *Salmo trutta* · Drift-feeding · Foraging · Conservation

**Introduction**

Numerous effects of parasites on fish behavior have been described, including effects of both internal and external parasites on foraging and risk-taking behavior of several fish species (Barber et al. 2000). One aspect of parasitism that is just at an early exploration stage is the effect of gill parasites on the drift foraging behavior of stream fish. Crane et al. (2011) measured behavior of two mussels belonging to the order Unionidae. In this paper we describe, for the first time, the effects of a mussel belonging to the family Margaritiferidae, namely the freshwater pearl mussel (*Margaritifera margaritifera* L.) encystment on the drift-feeding behavior of juvenile brown trout (*Salmo trutta* L.). Mussels of this family, as mussels in the family Unionidae, both belong to the order Unionoida, and are examples of organisms that are parasitic on fish during their larval stage, but free-living for most of their lives. From a conservation standpoint this host-parasite system is somewhat unique, because often both unionid mussel species and their host fish species are considered valuable components of ecosystem biodiversity and are threatened (Strayer et al. 2004; Österling and Högberg 2013). Both species are either sensitive or threatened throughout much of their native range (Klemetsen et al. 2003; Geist 2010; Österling et al. 2010; Arvidsson et al. 2013).
The highly threatened unionid mussels live as parasites on the gills of fish during their larval stage, before they drop off the fish to find suitable habitat in the stream substrate (Strayer et al. 2004). The freshwater pearl mussel (classified as endangered in the IUCN classification) is a gill parasite on brown trout or Atlantic salmon (S. salar) for a period of about 10 months (Young and Williams 1983; Young and Williams 1984). The larval parasites may potentially impact growth and reproduction of the host fish, especially since the *M. margaritifera* larvae grow on the host fish during their parasitic stage (Österling and Larsen 2013). Very little is known about the specific interactions between trout and mussels, however, in terms of energetic or physiological effects (Gethin et al. 2013). Because both the mussels and many of the trout populations are considered vulnerable, improving the understanding of the interactions in this unique host-parasite system is of high conservation value.

Stream salmonids, including brown trout, obtain significant portions of their energy intake through drift-feeding, wherein they maintain a position in the stream current and capture invertebrate prey as it drifts past their position, commonly called a focal point (Fausch 1984; Hughes and Dill 1990; Hughes et al. 2003). Drift-feeding salmonids are thought to maximize their net energy intake (NEI) by selecting focal points in slower water velocities which lie nearby areas of faster velocities that have been shown to deliver more prey (Everest and Chapman 1972; Hughes and Dill 1990). There is, in fact, a growing body of literature, both theoretical and applied, that supports the hypothesis that fish select, and compete for, positions that offer relatively high NEI (see Piccolo et al. 2014). Of late, NEI foraging models have served as the foundation for stream production and population-process models (Hayes et al. 2007), and it appears that these models are poised to expand into wider use in stream management.

The ability of fish to capture prey while drift feeding is a major determinant of their NEI (Hill and Grossman 1993; Hughes and Dill 1990; Piccolo et al. 2008a, b). Environmental and biotic factors (e.g. turbidity, velocity and competition) have been shown to impact drift-feeding behavior (Sweka and Hartman 2001; Zamor and Grossman 2007; Hazleton and Grossman 2009).

To our knowledge, however, there have been no investigations of the effects of parasites on drift-feeding efficiency, despite the possible energetic consequences of mussel encystment. Here, we report the results of experiments to test the effects of freshwater pearl mussel glochidia encystment on the drift-foraging behavior of juvenile brown trout. We did this at a period when the encysted mussels have approximately one month until they release from the fish, which is a time when the encysted mussels are at their largest size, and therefore probably has a relatively large impact on the host fish. We hypothesized that brown trout that were encysted with mussel larvae would have reduced drift-foraging abilities compared to non-encysted fish.

**Methods**

In the case of freshwater pearl mussel parasitism on brown trout, glochidia encystments are often greatest on young-of-the-year (YOY) brown trout (Hastie and Young 2001; Österling et al. 2008). Thus, the YOY brown trout we used for our experiments are the age class most likely to be heavily encysted, and therefore negatively influenced by mussel larvae. YOY brown trout originating from the stream Oxsjöä in the Ljungan catchment, Sweden, were collected in September 2010 from a fish farm situated 30 km south of Sundsvall. Gravid mussels were collected in September 2010 in the stream Maljan, a tributary to the Ljungan main stream. The brown trout and the mussels were transported to Karlstad University, and placed in aquaria to acclimatize at a temperature of 16 °C. Inspection for free living, ripe glochidia larvae was performed on a daily basis in the container with mussels, by placing 1 ml of the suspended water under a dissecting microscope.

On 10 September high densities of “snapping” (ripe), glochidia larvae were found suspended in the water. Infestation by mussel larvae on the brown trout gills was performed by placing a concentration of 30 000 larvae·L⁻¹ in containers, whereupon brown trout were put into the containers for 30 min. The larvae in each container were kept in suspension by constant stirring of the water. The infestation was terminated by removing the trout to 100 L aquaria. The water was changed twice a week, and constantly filtered (EHEIM 2215 filter). The light regime was 11 h daylight, 1 h dusk, 11 h darkness and 1 h dawn each day. The fish were fed approximately 2 % of their weight with chironomid larvae 3 times each week. Water temperature was
measured three times a week, and was 18.4±0.67 °C at 10 September, and then slowly decreased to 14.0±0.17 °C when the experiment was started.

The experiment was started at 6 November, when we visually assessed mussel encystment level on trout gills. This was done by sedating the trout (Benzocaine) and gently lifting the gill cover with a probe. We have previously shown this method to accurately describe encystment rate (Österling 2011). Five encysted trout (25.1±5.7 larvae·g⁻¹ body weight) and five non-encysted trout were randomly chosen for the experiment. The mean weight of the trout did not differ between encysted (2.16±0.28 g) and non-encysted (2.62±0.47 g) trout (One-Way ANOVA, F₁,₈=0.88, n=10, p=0.38). The water temperature was measured every hour using loggers (Onset, Hobo pendant temp logger UA-002-64). The number of day degrees between the glochidial attachment and the start of the experiment (769±9.2 day degrees) was similar to the number of day degrees between the infestation and early June approximately 9 months after infestation (727±26.0 day degrees, own unpublished field data from 19 streams in the Ljungan Catchment).

We investigated the effects of mussel encystment on YOY brown trout on drift-foraging rate in a 0.58 m wide, 1.5 m long and 0.16 m deep flow-through aquarium (Piccolo et al. 2007, 2008a). Differences in drift-foraging rate between encysted and non-encysted trout at the two periods (early and late) were analyzed with a mixed effects factorial ANOVA, with Encystment as one factor (between subject effects), and Period as the other factor (within subject effects). Differences in drift-foraging rate between encysted and non-encysted trout at the two distances (close and distant) were analyzed with a mixed effects factorial ANOVA, with Encystment as one factor (between subject effects), and Distance as the other factor (within subject effects). Post hoc tests were performed with Student-Newman-Keuls test (SNK-test) (Tybout and Sterntahl 2001). The data was normally distributed for the encysted and the non-encysted fish at every analysis (Shapiro-Wilks-test, p>0.05). All analyses were performed with the SPSS program (IBM SPSS Statistics, Version 19).

**Results**

The feeding efficiency of non-encysted and encysted trout was 22 % and 16 %, respectively, for the early period. A mixed model analysis for drift-foraging rate showed that there was a significant effect of Encystment (Mixed Effects Factorial Repeated ANOVA, F₁,₈=9.1, n=10, p=0.017), but not of Period (Mixed Effects Factorial Repeated ANOVA, F₁,₈=1.6, n=10, p=0.24) or the interaction of Encystment and Period (Mixed Effects Factorial Repeated ANOVA, F₁,₈=1.6, n=10, p=0.24). Therefore, fish at the early feeding event were not...
considered to be satiated during the early event. The non-encysted trout had a higher foraging rate than did the encysted trout at the early feeding event (SNK-test, $n=10$, $p<0.05$; Fig. 2). This was also true for the late period event (SNK-test, $n=10$, $p<0.05$).

Non-encysted trout caught 65% of their prey at the close feeding distance and 35% of their prey at the distant feeding distance during the early feeding event, whereas encysted trout caught 75% of their prey at the close distance and 25% of their prey at the distant feeding distance at the early feeding event. A mixed model analysis for drift-foraging rate showed that there was a significant effect of Encystment (Mixed Effects Factorial ANOVA, $F_{1,8}=7.6, p=0.025$; see the analyses above and Fig. 2), and of Distance (Mixed Effects Factorial ANOVA, $F_{1,8}=9.8, p=0.014$), but not of the interaction between Distance and Encystment (Mixed Model Analysis, $F_{1,8}=0.31, p=0.60$). The feeding rate was higher at the close than at the distant distance (SNK-test, $p<0.05$). However, the feeding rates between the non-encysted and the encysted trout differed among the two distances. There was no significant difference in the drift-foraging rate between the encysted and non-encysted trout at the close feeding distance (SNK-test, $n=10$, $p>0.05$). At the distant feeding distance, there was a significantly higher drift-foraging rate for the non-encysted trout than for the encysted trout (SNK-test, $n=10$, $p>0.05$; Fig. 3).

**Discussion**

This is the first study to document the effects of unionid mussel encystment on drift-feeding fish. Our study showed that non-encysted fish had a higher drift foraging rate than did encysted fish, primarily due to a...
reduced foraging distance of encysted fish. Ongoing experiments have confirmed that both hatchery and wild trout may have lower foraging rates at different water velocities when encysted by mussel larvae (Österling unpubl. data). Although our study is restricted to a small sample size and a limited portion of the complex host-parasite relationship between brown trout and freshwater pearl mussels, it demonstrates one possible negative effect of the parasite on the host’s energetic status, the trout’s net energy intake (NEI). These findings may have energetic consequences for drift-feeding brown trout during the important growth period in the late spring to the early summer period, even if the impact from the mussel parasite does not reduce the trout fitness to a large extent, given the dependence of the mussel on its host.

Potential energetic consequences of mussel encystment

For drift-feeding fish, the net energy intake (NEI) is considered to be the result of a tradeoff between the costs of swimming to hold position and catch prey, versus the benefits of encountering more prey at faster water velocities (Fausch 1984; Hughes and Dill 1990). Because mussel encystment appears to reduce a fish’s maximum foraging rate, a parasitized fish would experience a reduced NEI, at least at high prey densities. Encysted fish caught approximately 20% fewer prey, which would result in a reduction in growth potential during the period of mussel encystment. Individual-based models of fish distribution and growth based on NEI (Hayes et al. 2007; Railsback and Harvey 2013) have yet to include a parasite effect, but this might be a worthwhile consideration if the models were applied to mussel-trout ecosystems.

The fact that the reduction of foraging rate of encysted fish was largely due to reduced prey capture distance suggests that reduced energetic status, and hence reduced swimming ability (Crane et al. 2011; Taeubert and Geist 2013), may be the underlying mechanism. Fish with encysted larvae have higher respiration rates than do trout without encysted larvae (Crane et al. 2011; Gethin et al. 2013), which may further reduce the energetic status and reduce the prey capture distance. Reduced energetic status due to mussel encystment might also result in reduced competitive ability for encysted fish (Crane et al. 2011), potentially further reducing their NEI or exposing them to increased predation risk. These hypotheses remain to be generally tested, however. Because we did not measure prey handling times, we also cannot rule out the possibility that mussel encystment physically impairs foraging ability by reducing the efficiency of gill raker functioning.

We chose a water velocity, depth, and temperature to simulate approximate conditions that drift-feeding YOY brown trout might encounter around the early summer period in boreal streams (Klemetsen et al. 2003; Eros et al. 2012). Thus our foraging rates ought to approximate natural stream conditions when YOY trout are infected by mussel larvae. We saturated prey density to determine maximum foraging rate – our mean rates for both encysted and non-encysted fish fall roughly within those described for juvenile stream salmonids in the wild under comparable conditions (Hayes et al. 2007). This suggests that during daytime feeding activity periods juvenile salmonids forage at or near their maximum rate. One additional result of our study, therefore, is that we now have a range of maximum foraging rates for this life stage under these conditions, which should be of value in the further development of foraging-based habitat selection models.

Trout-mussel host-parasite interactions

Currently, the mechanisms that allow continued coexistence of the trout-mussel relationship at the physiological, behavioral, and population-level, are largely unknown (Strayer et al. 2004). These include trout physical and immune responses to larvae, encystment mechanisms, and growth and survival of encysted trout. In other fish-mussel systems, for example, mussels have evolved elaborate physical and behavioral mechanisms such as “lures” to entice fish to ingest larvae (Haag and Warren 2000). The freshwater pearl mussel does not seem to have evolved such mechanisms; how it ensures adequate infection rates is unknown. Encystment rates are known to vary within and among streams, and they appear to be higher on both younger trout and on trout strains with a history of within-drainage sympatry with mussels (Taeubert et al. 2012; Österling and Larsen 2013). Thus, trout population structure and migratory behavior probably play an important role for mussel populations. The answers to these broader-perspective questions will need to be based in part on a better understanding on the physiology and behavior of trout and mussels. This should contribute to conservation and restoration of both trout and mussel populations.
Trout have not been observed to benefit in any way from mussels, but we are not aware of any studies that have investigated this. At the ecosystem level, however, other species of freshwater mussels have been shown to both influence organic matter processing and to increase benthic fauna production (Vaughn and Hakenkamp 2001; Vaughn et al. 2004; Vaughn and Spooner 2006; Limm and Power 2011) which could increase invertebrate drift densities. The historic abundances of freshwater pearl mussels and brown trout in streams where they historically co-existed can only be guessed at. In Sweden, mussels were harvested commercially to obtain pearls, and records suggest they could reach great biomass. It is interesting to speculate on the food web effects of extensive mussel beds in trout streams; how might these ecosystem function and how might they influence trout production? Hopefully, efforts to restore mussel populations will allow us the opportunity to find out.

Conclusions

Our experiments were of limited scope in terms of time and space, and were further limited to a narrow set of physical and biological conditions (e.g. temperature, light, prey, fish size, mussel encystment rate, water velocity). Thus, the inferences that we can draw are restricted. These limitations notwithstanding, there is a clear measurable effect of mussel encystment on trout foraging behaviour. The extent to which this effect influences the relationship between trout and mussel populations is unknown, but given the conservation concerns of both species, it offers a fruitful area for further exploration.

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