Author(s): Chowdhury, Motiur; Salonen, Jouni; Marjomäki, Timo; Taskinen, Jouni

Title: Interaction between the endangered freshwater pearl mussel Margaritifera margaritifera, the duck mussel Anodonta anatina and the fish host (Salmo) : acquired and cross-immunity

Year: 2018

Version:

Please cite the original version:

Chowdhury, M., Salonen, J., Marjomäki, T., & Taskinen, J. (2018). Interaction between the endangered freshwater pearl mussel Margaritifera margaritifera, the duck mussel Anodonta anatina and the fish host (Salmo) : acquired and cross-immunity. Hydrobiologia, 810(1), 273-281. https://doi.org/10.1007/s10750-017-3114-6

Interaction between the endangered freshwater pearl mussel *Margaritifera margaritifera*, the duck mussel *Anodonta anatina* and the fish host (*Salmo*): acquired and cross immunity

M. Motiur R. Chowdhury*[1], Jouni K. Salonen[1], Timo J. Marjomäki[1], and Jouni Taskinen[1]

[1]Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014 University of Jyväskylä, Finland.

*Correspondence to: Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014 University of Jyväskylä, Finland. E-mail: motiur.chowdhury@jyu.fi. Tel. +358-40-7041004

Abstract

The common duck mussel *Anodonta anatina* can live in sympatry with– and use the same host, brown trout (*Salmo trutta*) – as the endangered freshwater pearl mussel *Margaritifera margaritifera*. Since the glochidia release of *A. anatina* takes place seasonally earlier than that of *M. margaritifera*, brown trout can be sequentially exposed first to *A. anatina* and then to *M. margaritifera*. Cross immunity, an immune reaction induced in fish host against glochidia after the infection with glochidia of another mussel species, is possible. Thus, it was studied experimentally if brown trout can be cross immunized against *M. margaritifera* by earlier infection with *A. anatina*. In addition, the hypothesis that consecutive exposures of same glochidial species in different years in the same host may create acquired immunity was tested in brown trout against *M. margaritifera*. Furthermore, the dose dependence of acquired immunity against *M. margaritifera* glochidia in the Atlantic salmon (*S. salar*) was also studied. Cross immunity was not found; suggesting that occurrence of *A. anatina* does not pose a threat to *M. margaritifera*. Instead, acquired immunity and its dose dependence were evident, emphasizing the significance of availability of 0+ age group, immunologically naïve Atlantic salmon/brown trout for efficient conservation of *M. margaritifera*.

Keywords: Bivalves, Brown trout, Atlantic salmon, Conservation, Unionoida

Introduction

The freshwater pearl mussel (*Margaritifera margaritifera*) is a long-lived (Helama & Valovirta 2008), river dwelling bivalve mollusc, which occurs in Europe and North-East North America, but is now critically endangered throughout its range of occurrence (Geist 2010, Lopes-Lima et al. 2016). *M. margaritifera* has a larval stage, glochidium, which is parasitic on the Atlantic salmon (*Salmo salar*) and/or the brown trout (*S. trutta*) (e.g., Young & Williams 1984, Salonen et al. 2016). Some pearl mussel populations exclusively develop on Atlantic salmon (e.g., Ieshko et al. 2016) and others exclusively on brown trout (e.g. Geist et al. 2006). *M. margaritifera* fulfils the criteria of indicator, flagship, key stone and umbrella species, and can thus be considered an ideal target species for the conservation of aquatic ecosystem functioning (Geist 2010).

The duck mussel, *Anodonta anatina*, occurs commonly in lakes and rivers of Europe (Lopes-Lima et al. 2016). The glochidia of *A. anatina* are known to be able to complete their development on 15 fish species, including the brown trout (Bauer et al. 1991). Although the results by Bauer et al. (1991) suggest that the co-occurrence of both *M. margaritifera* and *A. anatina* in a river is not frequent, it is still possible. For example, the River Mustionjoki/Svartå (Finland) and the River Wye (UK) inhabit both mussel species (Lopes-Lima et al. 2016). As the glochidia shedding of *A. anatina* takes place seasonally earlier (winter-spring, as late as May–June, Taskinen et al. 1997) than that of *M. margaritifera* (summer–autumn, e.g., Salonen & Taskinen 2016), it is likely that brown trout can be sequentially exposed to the glochidia of *A. anatina* and *M. margaritifera*.

The immune defense system in vertebrates includes innate and acquired (adaptive, specific) components so that the acquired immunity is based on antibodies that bind to a specific antigen. In a repeated contact with the same parasite or pathogen, the immune reaction is quicker and stronger (memory) due to a faster antibody production and the antibody reserves left from the previous infection (Mutoloki et al. 2014). For example, fish hosts can develop an acquired immunity against the glochidia of unionoid mussels (Bauer and Vogel 1987, Rogers and Dimock 2003, Dodd et al. 2006, Treasurer et al. 2006). Sequential infection with parasites belonging to different (often closely related) species can also provide protection, which is called cross-immunity or cross-resistance. Thus, cross-immunity is a special type of acquired immunity. It has been shown, for instance, that infection with glochidia of one unionid mussel can result in immunity against glochidia of another unionid species (Dodd et al. 2005). As the parasitic stage can largely contribute to the reproduction success of the endangered *M.*

margaritifera, the role of acquired immunity and cross immunity in conservation of *M. margaritifera* requires attention.

Bauer & Vogel (1987) showed that brown trout can eliminate *M. margaritifera* glochidia by both tissue and humoral reaction so that in repeated exposures to *M. margaritifera* glochidia the immunologic responses by brown trout against glochidia are strengthened, indicating an acquired immunity against *M. margaritifera*. In the case of *M. margaritifera*, lower infection rates have been found in older brown trout in the field or after second infection in the laboratory, suggesting acquired immunity (e.g. Bauer 1987, Ziuganov et al. 1994, Hastie & Young 2001). Nevertheless, even a higher infection success in the second infection has been recorded (see Wächtler et al. 2001). In spite of these contradictory results, the acquired immunity in salmonid hosts against *M. margaritifera* glochidia has not received much attention by experimental studies. For example, an acquired immune response would mean that the success of *M. margaritifera* would largely depend on immunologically naïve 0+ host fish in the environment – with important consequences for the conservation of the species.

The question remains whether the infection by *A. anatina* can cross-immunize brown trout against *M. margaritifera* glochidia such a phenomenon could pose a threat to declined *M. margaritifera* populations in rivers where these unionids live in sympatry. Nevertheless, this is still a widely under researched issue. Bauer et al. (1991) conducted an experiment by infecting brown trout first with *A. piscinalis* (= *A. anatina*) glochidia, and subsequently with *M. margaritifera* glochidia. No evidence for cross-immunity was achieved in this short-term (35 days) experiment. However, as the length of parasitic period of *M. margaritifera* can be even more than 300 days (Young & Williams 1984), a thorough evaluation of the likelihood and strength of this phenomenon should cover the whole parasitic period.

The aim of the present study is to investigate cross-immunity, i.e. whether the infection with glochidia of the duck mussel, *A. anatina*, will induce immunity against *M. margaritifera* in an experiment covering the whole parasitic period of *M. margaritifera*, as well as acquired immunity in salmonids hosts by *M. margaritifera*. The aim of the acquired immunity experiment is to examine the magnitude of acquired immunity in brown trout and the dose-dependent acquired immunity in Atlantic salmon against the glochidia of *M. margaritifera*, i.e. whether the intensity of immunity depends on the number of glochidia to which fish are exposed. Our hypotheses are that (i) *Anodonta* infection induces cross immunity against *M. margaritifera* glochidia, (ii) Atlantic salmon and

brown trout develop acquired immunity against *M. margaritifera* and that (iii) the acquired immunity is dose dependent.

Materials and Methods

In all the experiments, an effort was made to fulfil the key requirements for unbiased procedures for priming and challenge infections of fish by mussel glochidia (see Taeubert et al. 2013). These included e.g., maintenance of experimental fish groups in identical conditions throughout the experiments, identical exposure of fish to glochidia, randomization and sufficient number of replicate fish individuals.

Cross immunity experiment

A total of 300 brown trout fry (age group 1+, River Iijoki stock) were transported from the Taivalkoski fish farm of the Natural Resources Institute Finland (Luke) to Konnevesi research station, University of Jyväskylä, on May 23, 2012. Fish had not been exposed (hereafter 'exposed' and 'infected' are synonymous) to *M. margaritifera* glochidia in the fish farm. Dissection and examination of the gills of five individuals verified that the trout were not previously infected by glochidia. Fish were randomly allocated into four 163 L flow-through tanks with 100, 100, 50 and 50 individuals per tank. Two-hour exposure of trout with glochidia dissected from *A. anatina* (collected from Lake Koijärvi, eastern Finland), was performed on May 24, 2012, by decreasing water volume to 70 L and adding 12.3×10^4 and 7.1×10^4 glochidia to the two tanks with 100 and 50 trout, respectively. The two control tanks holding another 100 and 50 fish per tank received *A. anatina* gill extract suspension without glochidia. Water temperature during the priming infection with *A. anatina* was 7.7 °C.

Five brown trout from the 100-fish-tanks and three from the 50-fish-tanks from both primed and control groups were examined for glochidia five days post infection. All primed fish were infected and the number of *A. anatina* in primed fish varied from 90 to 232 glochidia $fish^{-1}$, indicating a successful priming infection. No glochidia were found from the control fish. On 15 August 2012, when the water temperature had increased to 15.7 $^{\circ}$C (2.5 months post infection), one brown trout from each tank was examined and found uninfected, indicating that *A. anatina* glochidia had already excysted (see also Douda et al. 2013).

On 15 August 2012, fish were marked using fin clipping, and randomly re-allocated into four new 163 L flow-through tanks so that all tanks received both primed and control fish. In every other tank the primed fish were

fin-clipped and control fish unclipped while in every other tank the primed fish were unclipped and control fish fin-clipped. The number of primed fish per tank varied from 21 to 36 whereas the number of control fish per tank varied from 21 to 62. Both the fin-clipped and unclipped fish were anesthetized using MS-222 before marking and handled similarly, except for the clipping.

Challenge infection with *M. margaritifera* glochidia was done two weeks after marking, on 28 August, 2012, with glochidia from the River Jukuanoja (the River Iijoki catchment), northern Finland. The 2-hour exposure was performed technically as in the priming infection above, by adding 3.0×10^5 glochidia to all the four tanks. Water temperature was 16.7 °C. Glochidia collection was performed by placing 30 adult *M. margaritifera* in plastic buckets in 5 L of river water for 30 min on the day of infection. The mussels were returned to the river after incubation. Timing of challenge infection was based on the previous knowledge that the River Jukuanoja *Margaritifera* release glochidia in the end of August (Salonen & Taskinen 2016).

Data were collected at four time points; September 2012 (3 weeks post infection), December 2012 (3 months), May 2013 (9 months) and June 2013 (10 months) (Table 1). Primed and control fish were randomly collected, killed with a sharp blow on the head, and measured for the total length and fresh mass. The gills were cut off and glochidia were examined microscopically for the number and size (length from a subsample of 10 random larvae), except for September sampling when only the right side gills were examined. Therefore, only the data for the right side gills were used in statistical analyses. Throughout the experiment, fish were daily fed with commercial food pellets. During this phase of the experiment the minimum and maximum temperatures were 1.1 °C and 16.8 °C, being the highest in September 2012 and June 2013.

Acquired immunity experiment

Testing of acquired immunity was performed for both of the salmonid host species of *M. margaritifera*, Atlantic salmon and brown trout. Both host species individuals (age group 0+) originated from the River Iijoki stock reared at Taivalkoski fish farm of the Natural Resources Institute Finland (Luke), from where they were moved to Konnevesi research station on August 21, 2012.

In the brown trout experiment, fish were first randomly allocated to primed vs. control groups in two separate 163 L flow-through tanks with 50 fish per tank. Priming infection of trout was performed on August 28, 2012,

using similar methods and origin of *M. margaritifera* glochidia as in the cross immunity experiment, with 2.9 x 10^5 glochidia $tank^{-1}$. Control fish were not exposed to glochidia, but experienced otherwise the same treatment as the primed fish. Next year, in August 26, 2013, when age of the fish was 1+, adipose fins of the primed fish were cut, after which both the primed and control fish were put in one tank. On August 28, 2013, the fish were infected with *M. margaritifera* glochidia (2.3 x 10^5 glochidia $tank^{-1}$) collected from the River Koivuoja (the River Iijoki catchment), northern Finland. In November 25 (3 months post infection), all the fish were examined with the methods described above (Table 1).

Atlantic salmon were primed at the same time (August 28, 2012), with same origin of glochidia and with the same methods as brown trout mentioned above. Salmon were allocated to two tanks with three treatment groups in each, (1) primed with a high dose (8.8 x 10^5 *M. margaritifera* glochidia, tip of the right pectoral fin clipped), (2) primed with a low dose (1.7 x 10^5 *M. margaritifera* glochidia, tip of the left pectoral fin clipped), and (3) control group (not prime infected, adipose fin clipped) with 17, 14 and 13 fish per tank, respectively. After one year, in August 28, 2013, all salmon were infected with *M. margaritifera* glochidia collected from the River Luttojoki (the River Tuuloma catchment), northern Finland. The challenge infection was performed with 6.0 x 10^5 glochidia $tank^{-1}$. As brown trout above, all salmon were examined on November 25, 2013 (3 months post infection) (Table 1). Throughout the experiments, fish were daily fed with commercial food pellets. Due to lack of logistic supports, the dose-dependence experiment was not performed for brown trout.

Statistical analyses

The effect of the previous infection with either *A. anatina* (cross immunity) or *M. margaritifera* (acquired immunity) and other factors (month, dose, tank) on glochidia number in gills and the size of glochidia was analysed by ANOVA. If the tank effect was not significant, the analysis was reduced to the effect of other variables. If necessary, the response variables studied were transferred by Box–Cox-transformation {BCSN = $(N^{\lambda}-1)/\lambda$} to yield as normally distributed variable as possible within each treatment cell. In some cases the distribution within a treatment cell still deviated significantly from normal, which induced a tendency for incorrect rejection of H_0-hypothesis (bias for too low p-value). Therefore, if the H_0 was rejected ($p < 0.05$) the hypothesis was also tested using more conservative non-parametric tests (e.g. Kruskall–Wallis).Fish were not measured for length and weight at the time of exposure but when examined. Therefore, the number of *M.*

margaritifera in the gills was not standardised based on the size of the fish as the individual growth rate after exposure, and consequently the size during exposure, was not known.

Results

Cross immunity experiment

Previous infection with *A. anatina* glochidia had no statistically significant effect (ANOVA) on the number of *M. margaritifera* glochidia (Fig. 1) or glochidium size (Fig. 2). Thus, brown trout does not develop any non-specific immunity that would decrease the success of *M. margaritifera* to parasitize them. Month had a significant effect (ANOVA and Kruskall–Wallis $p < 0.001$) on the response variables: the number of glochidia declined during the incubation period (Fig. 1) and their size (Fig. 2) increased.

Acquired immunity experiment

The previous infection of brown trout with *M. margaritifera* had a significant effect on the number of glochidia (Mann-Whitney U, $p < 0.001$) when re-infected with *M. margaritifera* glochidia (Fig. 3). Brown trout individuals earlier exposed to glochidia had significantly (Tukey and non-parametric pairwise test $p < 0.01$) less glochidia than the control (no exposure) group. No significant difference ($p > 0.05$) in the size of glochidia between the control and exposed group was found (Fig. 4).

Previous infection of Atlantic salmon with *M. margaritifera* had a significant dose-dependent effect on the number of glochidia (ANOVA and Kruskall-Wallis $p < 0.001$) when re-infected with *M. margaritifera* glochidia (Fig. 5). Salmon individuals exposed to high dose of glochidia had significantly (ANOVA with Tukey and Kruskall-Wallis with non-parametric pairwise test $p < 0.01$) less glochidia than the low dose group or the control group. The difference in the number of glochidia between the control and low dose treatment groups was not significant.

In addition, previous infection with *M. margaritifera* had a significant effect ($p < 0.05$) on the size of *M. margaritifera* glochidia when re-infected (Fig. 6). Glochidium size was significantly smaller in the high dose treatment than in the control group ($p < 0.05$). The tank effect was also significant with one tank having larger glochidia than the other of the two replicate tanks, presumably due to higher water temperature. Thus, previous

infection with *M. margaritifera* led to both lower intensity of infection and poorer growth of *M. margaritifera* larvae when re-infected.

Discussion

In natural populations, individuals are usually infected not only by one but also with multiple parasitic species. Interaction between the co-infecting parasitic species within a host individual can be negative (antagonistic), leading – in extreme cases – to competitive exclusion (Holmes 1961). However, species can also be independent of each other or the interaction can be even positive (co-operation, facilitation) (Poulin 2001, Lello et al. 2004). Thus, the interaction between *A. anatina* and *M. margaritifera* could also lead to one of these three possible outcomes. Because the earlier study by Dodd et al. (2005) showed that the previous infection of the host fish with the glochidia of *Lampsilis reeveiana* lowered the infection success of other unionid mussels, *L. abrupta*, *Villosa iris* and *Utterbackia imbecillis*, we hypothesised that the effect of *A. anatina* infection on *M. margaritifera* would be negative, or at most insignificant. We also hypothesised that if the effect of *A. anatina* infection on *M. margaritifer*a would be negative, it would be due to cross-immunity – representing so-called immune-mediated 'apparent competition' where one parasite species elicits an immune response which harms its competitors (see Read & Taylor 2001). In natural conditions, the possible negative impact of *A. anatina* on *M. margaritifera* could be also due to direct interference competition. *M. margaritifera* glochidia that occupy brown trout gills in autumn could be interfered by the glochidia of *A. anatina* in the spring when the glochidia shedding of the latter species takes place. However, in the present study, the exposure of brown trout was sequential so that *A. anatin*a infection occurred earlier (in spring/early summer) and that of *M. margaritifera* started in autumn; glochidia of only one species was present in brown trout at a time.

The acquired (adaptive) immune system of vertebrates activates slowly, but brings a specific and long-lasting immunity against subsequent infections. However, the acquired immune response developed against one parasite genotype may be cross-reactive and provide protection against other genotypes of the same species (e.g., Rellstab et al. 2013), or even to those of different species (e.g., Dodd et al. 2005, Karvonen et al. 2009). From our point of view, in the parasite, two factors determine the importance of cross immunity in the case of multiple infections. First, probability of cross immunity decreases with the genetic distance between the infecting parasites strains/species (Read & Taylor 2001). Second, the sequence of infections influences on the relative benefits and costs of cross immunity. In sequential exposure, only the first parasite enjoys the slow

236 activation of the adaptive immune system whereas the later arrival bears the full costs of the acquired immunity
237 (see Jackson et al. 2006, Hoverman et al. 2013, Klemme et al. 2016).
238
239 The previous experimental study on the acquired immunity in brown trout fish host against the glochidia of *M.*
240 *margaritifera* (Bauer & Vogel 1987) suggested acquired resistance; brown trout developed humoral
241 immunological response and the infection success decreased in repeated infection. Our results verified this
242 finding. Three months after the challenge infection the number of *M. margaritifera* glochidia was lower in
243 individuals infected 1 y earlier with *M. margaritifera* glochidia than in the control group that were not
244 previously exposed to *M. margaritifera*. Furthermore, evidence for acquired immunity was obtained not only in
245 brown trout but also in the Atlantic salmon, indicating that both of the two suitable host fishes of *M.*
246 *margaritifera* (see e.g., Salonen et al. 2016) are able to mount an acquired immune reaction against *M.*
247 *margaritifera*.
248
249 In addition to the number of glochidia, the negative impact of previous infection on success of *M. margaritifera*
250 was seen also in the growth rate of glochidia. When measured 3 months after the challenge infection, glochidia
251 in previously infected fish were smaller than in control fish in the Atlantic salmon. The size of glochidium at the
252 time of excystment from the fish host correlates with the survival rate of the juvenile *M. margaritifera* (Eybe et
253 al. 2015). Thus, previously infected hosts produce less and lower quality juveniles than immunologically naïve
254 hosts.
255
256 A novel finding in the present study was the dose dependence of acquired immunity in *M. margaritifera*-fish
257 host relationship. The higher the number of *M. margaritifera* glochidia that the fish were exposed to in priming
258 the lower the number of glochidia, and the smaller their size, after re-infection with *M. margaritifera* glochidia.
259 Dose dependence was evident in Atlantic salmon but since the dose dependence has been earlier observed in
260 immunization and vaccination of salmonids (Munag'andu et al. 2013, Ballesteros et al. 2015) it is reasonable to
261 assume that the result can be extrapolated also to brown trout. Thus, the negative effect of the previous infection
262 on both the number and the quality of *M. margaritifera* glochidia (and juveniles) depends on the density of
263 glochidia in the previous exposure.
264

The acquired immunity could explain the previous contrasting findings of *M. margaritifera* infection rate with respect to host fish age. In some studies the infection rate has been lower in older host fish (Bauer 1987, Hastie & Young 2001) while in some studies the opposite was found (see Wächtler et al. 2001). For example, if production of glochidia does not take place every year in a particular *M. margaritifera* population, there can be years in which both the 0+ and 1+ age group fish are immunologically naïve with respect to *M. margaritifera* glochidia. In such a condition, the larger sized 1+ fish, due to their large gill area, are probably more intensively parasitized by *M. margaritifera* glochidia than the 0+ fish that is also supported by Geist et al. (2006). During the year that follows production of *M. margaritifera* glochidia, the negative effect of acquired immunity may override the positive effect of larger size among the 1+ age group fish, resulting in situation where the younger and smaller but immunologically naïve 0+ individuals are more heavily infected by *M. margaritifera* than the 1+ fish. The dose dependence of acquired immunity can strengthen this process.

Importantly, the acquired immunity emphasizes the importance of the availability of 0+ age group fish for *M. margaritifera* – and explains the association between the density of 0+ fish hosts and density of young *M. margaritifera* in the population (Bauer 1987). For the conservation of *M. margaritifera*, therefore, the availability of 0+ aged (immunologically naïve) hosts is essential. In other words, the acquired immunity would mean that the recruitment success to post-parasitic life stage of *M. margaritifera* could strongly depend on the abundance of immunologically naïve 0+ host fish in the environment.

Our results suggest that the cross immunity between *M. margaritifera* and *A. anatina* is not as important an impediment for the success and conservation of *M. margaritifera* as the acquired immunity. Brown trout primed with glochidia of the unionid mussel *A. anatina* did not harbor significantly lower number of glochidia when challenged with *M. margaritifera*. The pattern was consistent throughout the 9-month parasitic period of *M. margaritifera*. This is in line with the results of the short term experiment performed earlier: previous infection with *A. anatina* had no influence on the survival of *M. margaritifera* glochidia in brown trout within 35 d (Bauer et al. 1991). That study also showed that previous infection with another unionoid species, *Unio crassus*, had no influence on the survival of *M. margaritifera* glochidia in brown trout (Bauer et al. 1991). Together these results indicate that the exposure of host fish to glochidia of other mussel species would not pose a threat to the endangered freshwater pearl mussel, *M. margaritifera*. However, the present study shows that the immunity is

related to the dose of exposure. Thus, possibility of such a cross immunity cannot be ruled completely out, for example, if brown trout is heavily exposed to *A. anatina* glochidia.

As hypothesized, acquired immunity and its dose dependence existed in *M. margaritifera*-host fish relationship. However, our experiment, as the previous study by Bauer et al. (1991) did not find evidence for cross immunity between *M. margaritifera* and *A. anatina* – contrasting the earlier study by Dodd et al. (2005) conducted between two Unionidae species. It is possible that *M. margaritifera* (family Margaritiferidae) and *A. anatina* (family Unionidae) are immunologically so distant that the antibodies produced for one species do not protect against the other species.

Acknowledgements

We are thankful to laboratory technicians of the Konnevesi research station and Tapani Säkkinen for their help in conducting the experiments. We are also grateful to Shariful Islam for proofreading. This research was funded by Maj and Tor Nessling Foundation, Raija and Ossi Tuuliainen Foundation and the EU Interreg IV A Nord Programme.

References

Ballesteros, N. A., M. Alonso, S. R. Saint-Jean & S. I. Perez-Prieto, 2015. An oral DNA vaccine against infectious haematopoietic necrosis virus (IHNV) encapsulated in alginate microspheres induces dose-dependent immune responses and significant protection in rainbow trout (*Oncorrhynchus mykiss*). Fish & Shellfish Immunology 45: 877–888.

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

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

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

Dodd, B. J., M. C. Barnhart, C. L. Rogers-Lowery, T. B. Fobian & R. V. Ronald Jr, 2005. Cross-resistance of largemouth bass to glochidia of unionid mussels. Journal of Parasitology 91: 1064–1072.

323 Dodd, B. J., M. C. Barnhart, C. L. Rogers-Lowery, T. B. Fobian & R. V. Ronald Jr, 2006. Persistence of host
324 response against glochidia larvae in *Micropterus salmoides*. Fish & Shellfish Immunology 21: 473–484.
325 Douda, K., M. Lopes-Lima, M. Hinzmann, J. Machado, S. Varandas, A. Teixeira & R. Sousa, 2013. Biotic
326 homogenization as a threat to native affiliate species: fish introductions dilute freshwater mussel's host
327 resources. Diversity and Distributions 19: 933–942. doi:10.1111/ddi.12044.
328 Eybe, T., F. Thielen, T. Bohn & B. Sures, 2015. Influence of the excystment time on the breeding success of
329 juvenile freshwater pearl mussels (*Margaritifera margaritifera*). Aquatic Conservation: Marine and
330 Freshwater Ecosystems 25: 21–30. doi: 10.1002/aqc.2471.
331 Geist, J., 2010. Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera*
332 *margaritifera* L.): a synthesis of conservation genetics and ecology. Hydrobiologia 644: 69–88.
333 Geist, J., M. Porkka, R. Kuehn, 2006. The status of host fish populations and fish species richness in European
334 freshwater pearl mussel (*Margaritifera margaritifera* L.) streams. Aquatic Conservation: Marine and
335 Freshwater Ecosystems 16: 251-266.
336 Hastie, L. C. & M. R. Young, 2001. Freshwater pearl mussel (*Margaritifera margaritifera*) glochidiosis in wild
337 and farmed salmonid stocks in Scotland. Hydrobiologia 445: 109–119.
338 Helama, S. & I. Valovirta, 2008. The oldest recorded animal in Finland: ontogenetic age and growth in
339 *Margaritifera margaritifera* (L. 1758) based on internal shell increments. Memoranda Societatis pro
340 Fauna et Flora Fennica 84: 20–30.
341 Holmes, J. C., 1961. Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis*
342 *dubius* (Acanthocephala). I. General effects and comparison with crowding. Journal of Parasitology 47:
343 209–216.
344 Ieshko, E. P., J. Geist, S. A. Murzina, A. E. Veselov, D. I. Lebedeva & V. V. Ziuganov, 2016. The
345 characteristics of the infection of juvenile Atlantic salmon with glochidia of the freshwater pearl mussel
346 in rivers of Northwest Russia. Knowledge & Management of Aquatic Ecosystems 417: 6.
347 doi:http://dx.doi.org/10.1051/kmae/2015039
348 Jackson, J. A., R. J. Pleass, J. Cable, J. E. Bradley & R. C. Tinsley, 2006. Heterogenous interspecific
349 interactions in a host-parasite system. International Journal for Parasitology 36: 1341–1349.
350 Karvonen, A., O. Seppälä & E. T. Valtonen, 2009. Host immunization shapes interspecific associations in
351 trematode parasites. Journal of Animal Ecology 78: 945–952.

352 Klemme, I., K. R. Louhi & A. Karvonen, 2016. Host infection history modifies co-infection success of multiple
353 parasite genotypes. Journal of Animal Ecology 85: 591–597.
354 Hoverman, J.T., B. J. Hoye & P. T. J. Johnson, 2013. Does timing matter? How priority effects influence the
355 outcome of parasite interactions within hosts. Oecologia 173: 1471–1480.
356 Lello, J., B. Boag, A. Fenton, I. R. Stevenson & P. J. Hudson, 2004. Competition and mutualism among the gut
357 helminths of a mammalian host. Nature 428: 840–844.
358 Lopes-Lima, M., R. Sousa, J. Geist, D. C. Aldridge, R. Araujo, J. Bergengren, Y. Bespalaya, E. Bódis, L.
359 Burlakova, D. Van Damme, K. Douda, E. Froufe, D. Georgiev, C. Gumpinger, A. Karatayev, Ü.
360 Kebapçi, I. Killeen, J. Lajtner, B. M. Larsen, R. Lauceri, A. Legakis, S. Lois, S. Lundberg, E. Moorkens,
361 G. Motte, K.-O. Nagel, P. Ondina, A. Outeiro, M. Paunovic, V. Prié, T. von Proschwitz, N. Riccardi, M.
362 Rudzīte, M. Rudzītis, C. Scheder, M. Seddon, H. Şereflişan, V. Simić, S. Sokolova, K. Stoeckl, J.
363 Taskinen, A. Teixeira, F. Thielen, T. Trichkova, S. Varandas, H. Vicentini, K. Zajac, T. Zajac, & S.
364 Zogaris, 2016. Conservation status of freshwater mussels in Europe: state of the art and future
365 challenges. Biological Reviews. doi:10.1111/brv.12244.
366 Munang'andu, H. M., B. N. Fredriksen, S. Mutoloki, R. A. Dalmo & Ø. Evensen, 2013. Antigen dose and
367 humoral immune response correspond with protection for inactivated infectious pancreatic necrosis virus
368 vaccines in Atlantic salmon (*Salmo salar* L). Veterinary Research 47: 7.
369 Mutoloki, S., J. B. Jørgensen & Ø. Evensen, 2014. The adaptive immune response in fish. In Gudding, R., A.
370 Lillehaug & Ø. Evensen, Fish vaccination. Wiley Blackwell, West Sussex, UK: 104–115.
371 Poulin, R., 2001. Interactions between species and the structure of helminth communities. Parasitology 122: S3–
372 S11.
373 Read, A. F. & L. H. Taylor, 2001. The ecology of genetically diverse infections. Science 292: 1099–1102.
374 Rellstab, C., A. Karvonen, K. R. Louhi & J. Jokela, 2013. Genotype-specific vs. cross-reactive host immunity
375 against a macroparasite. PLoS ONE, 8, e78427.
376 Rogers, C. L. & R. V. Dimock Jr, 2003. Acquired resistance of bluegill sunfish *Lepomis macrochirus* to
377 glochidia larvae of the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) after multiple
378 infections. The Journal of Parasitology 89: 51–56.
379 Salonen, J. K., T. J. Marjomäki & J. Taskinen, 2016. An alien fish threatens an endangered parasitic bivalve: the
380 relationship between brook trout (*Salvelinus fontinalis*) and freshwater pearl mussel (*Margaritifera*

margaritifera) in northern Europe. Aquatic Conservation: Marine and Freshwater Ecosystems. doi: 10.1002/aqc.2614.

Salonen, J. K. & J. Taskinen, 2016. Electrofishing as a new method to search for unknown populations of the endangered freshwater pearl mussel *Margaritifera margaritifera*. Aquatic Conservation: Marine and Freshwater Ecosystems. doi: 10.1002/aqc.2667.

Taeubert, J. E., B. Gum & J. Geist, 2013. Towards standardization of studies into host relationships of freshwater mussels. Biological Conservation 159: 550-551.

Taskinen, J., T. Mäkelä & E. T. Valtonen, 1997. Exploitation of *Anodonta piscinalis* (Bivalvia) by trematodes: parasite tactics and host longevity. Annales Zoologici Fennici 34: 37–46.

Treasurer, J. W., L. C. Hastie, D. Hunter, F. Duncan & C. M. Treasurer, 2006. Effects of (*Margaritifera margaritifera*) glochidial infection on performance of tank-reared Atlantic salmon (*Salmo salar*). Aquaculture 256: 74–79.

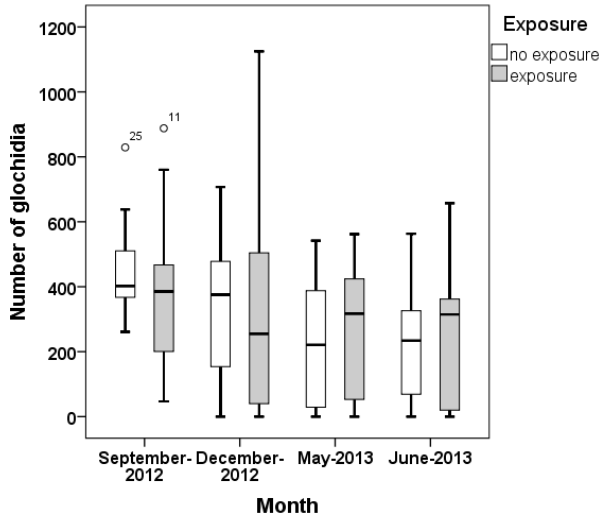
Wächtler, K, M. C. Dreher-Mansur & T. Richter, 2001. Larval types and early postlarval biology in naiads (Unionoida). In Bauer, G. & K. Wächtler (eds), Ecology and Evolution of the Freshwater Mussels Unionoida. Springer-Verlag, Berlin: 93–125.

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

Ziuganov, V., A. Zotin, L. Nezlin & V. Tretiakov, 1994. The freshwater pearl mussels and their relationships with salmonid fish. Russian Federal Research Institute of Fisheries & Oceanography, Moscow.

401 **Figures**

402

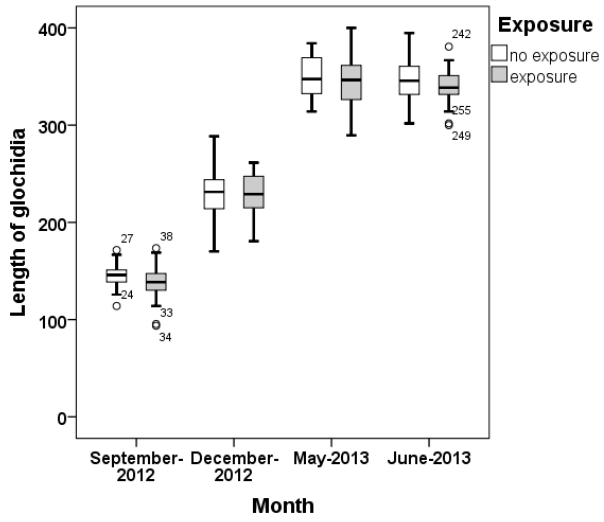


403

404 Figure 1. Box-plot showing the number of *M. margaritifera* in the gills of brown trout at different times after
405 challenging with *M. margaritifera* on 28 August, 2012. No exposure = the fish were not exposed to *A. anatina*
406 before challenging with *M. margaritifera*, exposure = the fish were exposed to *A. anatina* before challenging.
407 The box indicates range between lower and higher quartile, the vertical line in the box is median and the
408 whiskers indicate minimum and maximum values, excluding outliers (values deviating more than 1.5
409 interquartile ranges from the closest quartile).

410

411



412

Figure 2. Box-plot showing the length (μm) of *M. margaritifera* glochidia at different times. No exposure = the fish were not exposed to *A. anatina* before challenging with *M. margaritifera*, exposure = the fish were exposed to *A. anatina* before challenging.

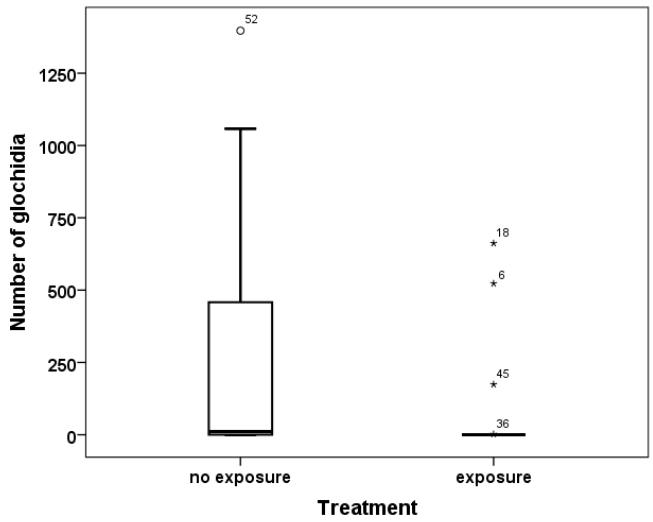


Figure 3. Box-plot showing the number (N) of *M. margaritifera* glochidia in the gills of brown trout in individuals previously not infected with *M. margaritifera* (no exposure) and infected with *M. margaritifera* glochidia (exposure).

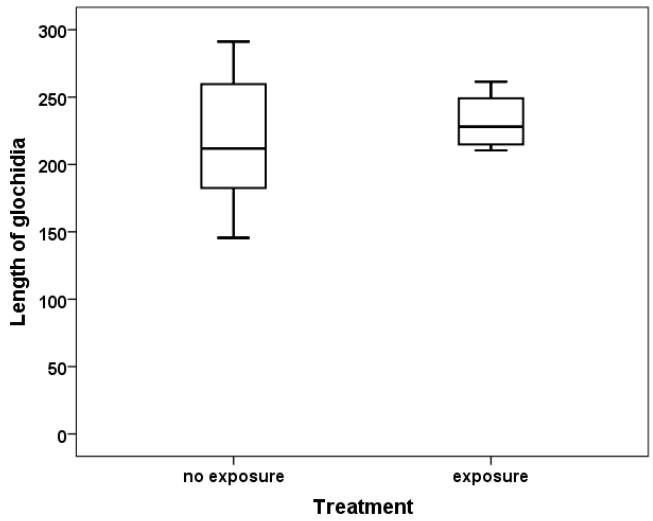


Figure 4. Box-plot showing the length (μm) of *M. margaritifera* glochidia in the gills of brown trout in individuals previously not infected with *M. margaritifera* (no exposure) and infected with *M. margaritifera* glochidia (exposure).

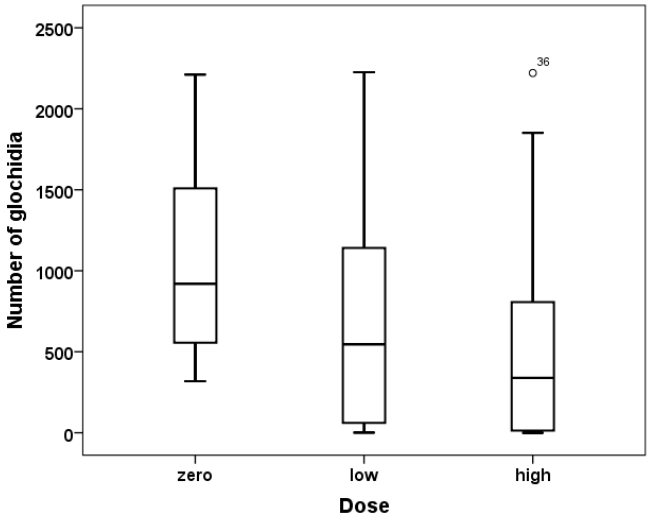


Figure 5. Box-plot showing the number (N) of *M. margaritifera* glochidia in the gills of Atlantic salmon in individuals previously not exposed to *M. margaritifera* (zero dose), exposed to a low number of *M. margaritifera* glochidia (low dose) and to a high number of *M. margaritifera* glochidia (high dose).

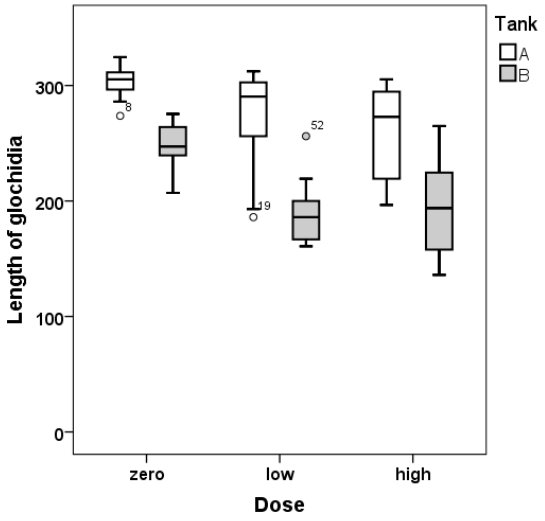


Figure 6. Box-plot showing the length (μm) of *M. margaritifera* glochidia in the gills of Atlantic salmon in individuals previously not infected with *M. margaritifera* (zero dose), infected with a low number of *M. margaritifera* glochidia (low dose) and with a high number of *M. margaritifera* glochidia (high dose).

Table 1. Different time points of infection and fish examination along with fish mortality throughout the experiments.

Experiment	Infection	Challenge Infection	Examination					Mortality
			Sep. 2012	Dec. 2012	May 2013	Jun. 2013	Nov. 2013	
Cross immunity in trout	May 2012	August 2012	20 Control + 20Infected	30 Control + 20Infected	29Control + 45Infected	61Control + 30Infected	--	20 fish in 13 month
Acquired immunity in trout	August 2012	August 2013	--	--	--	--	34 Control + 21Infected	25 fish in 16 month
Acquired immunity in salmon	August 2012	August 2013	--	--	--	--	33 high& 21low dose Infected + 22 control	6 fish in 16 month