Freshwater mussels (*Anodonta anatina*) reduce transmission of a common fish trematode (eye fluke, *Diplostomum pseudospathaceum*)

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SUMMARY

Recent results suggest that bivalves can play an important role in restraining the spread of various aquatic infections. However, the ability of mussels to remove free-living stages of macroparasites and reduce their transmission is still understudied, especially for freshwater ecosystems. We investigated the influence of the common freshwater mussel (*Anodonta anatina*) on the transmission of a trematode (eye fluke, *Diplostomum pseudospathaceum*), which frequently infects fish in farms and natural habitats. In our experiments, mussels caused a significant decrease ($P < 0.001$) in the abundance of trematode free-living stages, from 6520 to 1770 cercariae L^{-1} on average (about 4-fold in 2 h). Individual clearance rates of mussels were $0.6–3.7$ L per hour (mean 1.9). These tests were followed by experimental infections of rainbow trout (*Oncorhynchus mykiss*) with different doses of *D. pseudospathaceum* cercariae in the presence or absence of mussels. Exposure of fish to cercariae in the presence of mussels significantly ($P < 0.05$) reduced the infection intensities in fish (by 30–40%) at all exposure doses. Our results indicate that freshwater bivalves can markedly reduce local cercariae densities and could be useful in mitigation of trematodoses harmful to fish farming.

Key words: Cercariae, bivalves, parasite transmission, infection intensity, freshwater ecosystem, clearance rate, diplostomosis, biofilters, Unionidae.

INTRODUCTION

The removal of free-living parasite larvae by aquatic predators has recently been recognized as an important factor limiting parasite transmission and hence their infection success (Thieltges *et al*. 2008*a*; Orlofske *et al*. 2015). It has been shown that various aquatic organisms (e.g. carnivorous plants, cnidarians, bryozoans, oligochaetes, turbellarians, insect larvae, mites, molluscs, fish, newts) consume larval stages of parasites, decreasing their numbers in water (reviewed in Thieltges *et al*. 2008*a*; Johnson *et al*. 2010). Such predation can lead to the significant reduction (up to 99%) of the infection intensity in downstream hosts (Schotthoefer *et al*. 2007; Thieltges *et al*. 2008*a*; Orlofske *et al*. 2012).

Bivalve molluscs are powerful biofilterers (McMahon, 1991), which can effectively cleanse water of a broad spectrum of pathogens, including bacteria (Miller *et al*. 2006; Ismail *et al*. 2015), parasitic protists (Robertson, 2007; Lucy *et al*. 2008; Willis *et al*. 2014; Słodkowicz-Kowalska *et al*. 2015), larval stages of parasitic copepods (Molloy *et al*. 2011; Bartsch *et al*. 2013) and even viruses (Faust *et al*. 2009; Mezzanotte *et al*. 2016, however see Stumpf *et al*. 2010). Thus, the potential of bivalves to reduce various aquatic diseases caused by microparasites (viruses, bacteria, protists) has been demonstrated, while the effect of bivalves on macroparasite transmission is still understudied (Burge *et al*. 2016). We propose that freshwater bivalves can also remove free-living larvae of fish trematodes (i.e. cercariae), which are within the size range (up to 0·25 mm) of particles filtered by bivalves (Gosling, 2003). Trematodes cause various diseases in humans, fish and other animals and have a great epidemiological and economic impact (King and Scholz, 2001; Ross *et al*. 2002; Fenwick, 2012; Shinn *et al*. 2015). Taking into account the high filtration capacities and abundance of bivalves in different ecosystems (Burge *et al*. 2016), they hypothetically could be used to impede trematode transmission in aquaculture and for prevention of human trematodoses (e.g. schistosomiasis, opisthorchiasis).

Data about the influence of bivalves on trematode transmission are still sparse and controversial. Moreover, these data are limited to marine trematodes infecting mussels, while there is no information about

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freshwater parasites (e.g. fish trematodes). For example, some marine mussels (*Crassostrea gigas*, *Mya arenaria*) were reported to reduce parasitic load in host mussel species (*Mytilus edulis*, *Cerastoderma edule*) by filtering trematode cercariae (Thieltges *et al*. 2008a; Goedknegt *et al*. 2015) and by acting as decoys for the trematodes, thus causing a dilution effect (Thieltges *et al*. 2009). However, experiments with other marine (mix of *C. edule*, *M. edulis*, *Ensis americanus*) and freshwater (*Sphaerium* sp.) bivalves indicated that these molluscs did not remove cercariae (Orlofske *et al*. 2012; Welsh *et al*. 2014). Therefore, clarification is needed of the role of bivalves in removal of the trematode larval stages and potential ecosystem effects.

The present study is the first attempt to assess the influence of freshwater mussels on the infection success of common fish trematodes. We experimentally tested (1) the ability of mussels (*Anodonta anatina*) to remove the cercariae of the eye fluke (*Diplostomum pseudospathaceum*) and (2) the influence of mussels on the transmission of this trematode to the host fish (rainbow trout, *Oncorhynchus mykiss*). Our hypothesis was that mussels can effectively decrease cercariae number in water, thus reducing the intensity of trematode infection in fish.

This host–parasite system was chosen because diplostomatids, including *D. pseudospathaceum*, are very common parasites, infecting almost all species of freshwater fishes wherever they occur (Valtonen and Gibson, 1997). They can infect rainbow trout in natural environments (Sokolov, 2010) and are frequently encountered in fish farms (Karvonen *et al*. 2006). *Diplostomum pseudospathaceum* can impair fish physiology and behaviour, including reduced vision and decreased attack distance when catching a prey (Owen *et al*. 1993; Karvonen *et al*. 2004a, b). Moreover, the eye fluke can manipulate host behaviour (Seppälä *et al*. 2004; Mikheev *et al*. 2010a, b; Gopko *et al*. 2015, 2017) often causing a deterioration of anti-predatory traits in their hosts and predisposing them to predation by avian definitive hosts (Crowden and Broom, 1980; Seppälä *et al*. 2004, 2005, 2008; Gopko *et al*. 2017). Although praziquantel decreases the life span of *Diplostomum* cercariae (Voutilainen *et al*. 2009), effective treatment against diplostomosis in fish is not available. Thus, knowledge of the factors controlling the transmission of the eye fluke is important and potentially can be used in sustainable parasite control in the fish farming industry.

MATERIALS AND METHODS

The experiments were carried out in June to September 2016 at the Konnevesi research station (University of Jyväskylä, Finland). To minimize observer bias, blind methods were used in all experiments.

Study organisms

The eye fluke *D. pseudospathaceum* has a three-host life cycle. Infected freshwater snails (e.g. pond snail *Lymnea stagnalis*, the first intermediate host) produce huge numbers (up to 58 000 d^{-1}) of free-living larval cercariae (Lyholt and Buchmann, 1996), which infect freshwater fishes (second intermediate host). In fish, the parasites establish in the eye lenses and develop into metacercariae. Eye flukes complete their life cycle in the intestine of fish-eating birds (definitive host), where they reproduce sexually (Valtonen and Gibson, 1997).

We used *L. stagnalis* snails collected from Lake Konnevesi as a source of the cercariae. Infected snails were allowed to release cercariae for 3 h at 18 °C; therefore, cercariae used for experimental infections were no older than 3 h at the beginning of the experiment. *Anodonta anatina* mussels were collected from the shallow nearshore habitats of Lake Konnevesi. *Anodonta anatina* (Unionidae) is a widespread species (Lopes-Lima *et al*. 2016), which often shares the same microhabitats, shallow littoral areas of lakes (Taskinen and Valtonen, 1995) with *L. stagnalis* (mussels are largely infaunal, partly submerged in the sand, while snails are epibenthic and feed on the aquatic vegetation). Therefore, potential predators of trematode cercariae, *A. anatina* and *D. pseudospathaceum* are sympatric throughout the littoral zone.

Rainbow trout were obtained from a commercial fish farm. Fish were reared in well water untreated with chemicals and therefore were free from macroparasites. Fish and mussels were acclimated to the conditions in the indoor 200 L flow-through tanks for at least a week at 13·1–13·8 °C before the experiments.

Testing cercariae removal by mussels

To study the removal of cercariae by filtering mussels, we measured changes in the cercariae numbers after their incubation in containers with mussels. Similar containers without mussels served as a control. Ten 2 L plastic containers were filled with filtered (100-µm mesh) lake water. An individual mussel was placed in each of the five containers 5 h prior to experiments, to acclimate the mussels to laboratory conditions (water temperature 15–16 °C). Each mussel was observed to filter actively (siphons protruded) before the start of the experiment. The mussel length was from 78 to 93 mm ($n = 5$).

Cercariae produced by eight infected *L. stagnalis* snails were mixed and an equal volume of cercariae suspension (100 mL) was added to the containers to give a final concentration of about 6000 cercariae L^{-1}. The average concentration of cercariae in the initial suspension was counted from three 2-mL samples. The experiment lasted for 2 h and the concentration of cercariae in each container was estimated

at the beginning and at the end of the incubation from three 5-mL samples taken after gentle mixing. Cercariae were counted under a dissecting microscope (28× magnification) in a Bogorov zooplankton counting chamber (Hydro-Bios GmbH, Kiel-Altenholz, Germany) within 2 h of sampling. The average cercariae numbers were calculated for each container and used in the subsequent analysis. The initial number of cercariae in the experiment was 6520 ± 369 cercariae L^{-1} (mean ± s.e.). The effect of mussels on the cercariae numbers was tested using repeated-measures analysis of variance (ANOVA) because it is an appropriate analysis for monitoring temporal changes in mean values. Treatment (mussel present/absent) was a categorical predictor, while cercariae numbers at the beginning and at the end of the experiment were considered to be repeated measurements. Data were checked for normality using the Shapiro–Wilk's test ($W > 0.8$, $P > 0.16$ in all cases). The clearance rates were calculated according to Frost (1972). To calculate the rates of cercariae removal, we used the equations proposed for ingestion rates (Conover, 1978); however, it was not clear from the results of our study, whether cercariae were ingested or only damaged by mussels. After the experiment, the length and mass of each mussel were measured to allow calculation of the correlation between mussel size and reduction of cercariae numbers.

Infection experiment

To determine whether predation on parasite larvae can reduce the infection rate in the next host, we experimentally infected rainbow trout with eye fluke cercariae in the presence (predation treatment) or absence (control) of the mussel *A. anatina*. The three similarly designed experiments were conducted with high, medium and low exposure doses of cercariae (400, 300, 230 cercariae per fish, respectively). Exposure doses were similar to those used in previous studies (Seppälä *et al.* 2004; Karvonen *et al.* 2004*a*, *b*). Metacercariae numbers acquired by the individual rainbow trout in our experiment never exceeded 178 metacercariae/fish and median values were much lower (see Results), and were comparable to the infection intensities in naturally infected fish (Mikheev *et al.* 2014 and references therein).

Rainbow trout were exposed to cercariae individually in 22 semi-transparent plastic containers (30 × 40 × 25 cm) filled with 12 L of lake water. Individual *A. anatina* mussels were placed in 11 randomly chosen containers (*Anodonta* treatment) and 11 containers with empty mussel shells served as controls. However, in the treatment with medium cercariae doses, the number of experimental and control containers was not equal (10 and 12 containers, respectively).

A closed empty *A. anatina* shell was placed in each of the control containers to minimize potential effect of the habitat structure and bottom colouration on the behaviour of rainbow trout. Juvenile rainbow trout may consider dark objects on the bottom (such as a mussel shell) as a shelter (Mikheev *et al.* 2010*a*, *b*) and may ventilate less actively in the presence of a shelter due to decreased stress, which in turn, results in decreased acquisition of the cercariae (Mikheev *et al.* 2014). Besides visual stimuli, chemical signals are also important for fish behaviour. Such signals can carry important information, like a predation threat, that markedly increases ventilation rate (Hawkins *et al.* 2004, 2007). However, there is no evidence that chemical cues produced by mussels could influence ventilation in fish. On the other hand, the presence of fish can induce an increase of the filtration activity of *Anodonta* mussels (Jokela and Palokangas, 1993). We estimated filtration rates of mussels in containers without fish. In experiments with fish and mussels, fish could stimulate filtration of cercariae, thus indirectly decreasing parasite transmission.

Fish and mussels were acclimated in experimental containers (at 15–16 °C) for 2 h before the beginning of experiments. Mussels for each experiment were chosen randomly from the subpopulation of 25 *A. anatina* maintained in the laboratory in a large flow-through tank with water from Lake Konnevesi. The length of mussels ranged from 71 to 119 mm and did not differ from the length of mussel shells in control treatments (one-way ANOVA, $P < 0.14$ in all three experiments). Shells were washed and kept for a week in the same holding tank with living mussels prior to the experiments. Fish were exposed to parasites by adding the mixture of freshly produced *D. pseudospathaceum* cercariae (obtained from five infected *L. stagnalis* snails) to each container to give 400, 300 and 230 cercariae per fish (high, medium and low dose, respectively). Experiments were conducted in an 11-day period and started at the same time of the day (at noon) to exclude potential circadian effects. This time period was chosen for experiments because *Diplostomum* cercariae are mostly released in the day time (Karvonen *et al.* 2004*a*, *b*), when their chances of encountering a fish host are highest.

After 3 h of exposure, fish were caught with a dip net and placed individually in 8 L flow-through tanks for 48 h, which is enough for the parasites to reach the eye lenses of fish (Owen *et al.* 1993; Karvonen *et al.* 2004*a*, *b*). Fish were then killed with an overdose of MS 222 (Sigma Chemical Co., St Louis, Missouri, USA), weighed and dissected.

The number of *D. pseudospathaceum* in the eye lenses of the fish was counted using a dissection microscope (28× magnification). After the experiment, the length and mass of the mussels were also measured to enable correlation between mussel size and the possible reduction of infection intensity in fish. The experiments were conducted with permission from the Centre for

Table 1. Repeated-measures ANOVA summary for the cercariae removal experiment. The number of *Diplostomum pseudospathaceum* cercariae was considered as a response variable, *Anodonta anatina* presence/absence (treatment) as a fixed factor and time (start/end of the experiment) as repeated measurements

Results of the repeated-measures ANOVA

Effect	D.F.	MS	F value	P value
Treatment	1	544·1	21·92	<0·001
Time	1	832·1	43·01	<0·001
Time × treatment	1	586·8	30·34	<0·001
Post hoc comparisons				
Treatment	*Anodonta* (start)	*Anodonta* (end)	Control (start)	
Anodonta (start)				
Anodonta (end)	0·0003[a]			
Control (start)	0·9992	0·0002[a]		
Control (end)	0·8396	0·0002[a]	0·8772	

[a] Significant Tukey HSD *post hoc* comparisons.

Economic Development, Transport and Environment of South Finland (licence number ESAVI/10184/04·10·07/2014). We used the minimum number of fish to produce statistically reproducible results and performed experiments in accordance with the ethical and regulatory guidelines (standards of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and its appendix).

We used negative binomial generalized linear models (GLMs) with log link function to analyse infection intensities, since the negative binomial distribution often provides a good fit to parasite data (Wilson *et al.* 2002; Alexander, 2012). The STATISTICA 10 (StatSoft Inc., 2011) and R Core team (2015) software were used for the statistical analysis. The three exposure doses (high, medium and low) were regarded as separate experiments, and were analysed separately. The 'ggplot' package (Wickham, 2009) was used for graphical presentation of the data.

RESULTS

Removal of cercariae by mussels

The repeated-measures ANOVA revealed a significant interaction between the treatment (*Anodonta* presence/absence) and the time (start/end of the experiment) on the cercariae count (Table 1, Fig. 1). *Post hoc* comparisons showed that *A. anatina* presence in the environment significantly decreased (4-fold on average) the concentration of cercariae after 2 h incubation. In contrast, cercariae numbers in the control treatment were similar at the beginning and at the end of the experiment. The initial numbers of cercariae were similar in the experimental and control treatments (Table 1, Fig. 1).

The individual clearance rates of mussels calculated from changes in cercariae numbers in *Anodonta* treatment were 0·6–3·7 L per hour (mean ± S.E. = 1·9 ± 0·6 L), meaning that all tested mussels filtered the water volume in the 2 L containers more than once during the 2 h incubation. On average, mussels reduced the number of cercariae from 6520 ± 369 to 1773 ± 628 cercariae L^{-1} in 2 h (Supplementary Table S1), while two individuals decreased cercariae numbers by 18- and 35-fold. Maximum individual removal rates varied from 256 to 22 563 (mean ± S.E. = 7406 ± 4357) cercariae h^{-1}. Numbers of cercariae removed did not correlate with the mussel length (Pearson correlation, $r = 0·39$, $P = 0·51$).

Infection experiments

Negative binomial GLMs demonstrated a significant influence of the treatment on the infection intensities in rainbow trout in all three experiments (Table 2). Generally, mean infection intensities were 30–40% lower in *Anodonta* treatment (Table 2, Fig. 2, Supplementary Table S2). When outliers were excluded, the results were still significant ($P < 0·05$). Mussel size did not significantly correlate with the infection intensity in fish (Pearson correlation, $r < 0·12$, $P > 0·75$ in all cases). There was no significant difference in fish mass between the treatments in any of the three experiments (t-test, $P > 0·40$ in all cases).

DISCUSSION

The removal of free-living parasite larvae by various aquatic predators has recently been recognized as an important factor limiting transmission of parasites and hence their infection success (Thieltges *et al.* 2008a; Johnson *et al.* 2010; Orlofske *et al.* 2015). Our experiments showed that the common freshwater mussel *A. anatina* can also reduce parasite abundance in water by filtering cercariae of *D. pseudospathaceum*. In addition, these bivalves significantly reduced (by 30–40%) the success of cercariae transmission to the rainbow trout, which is
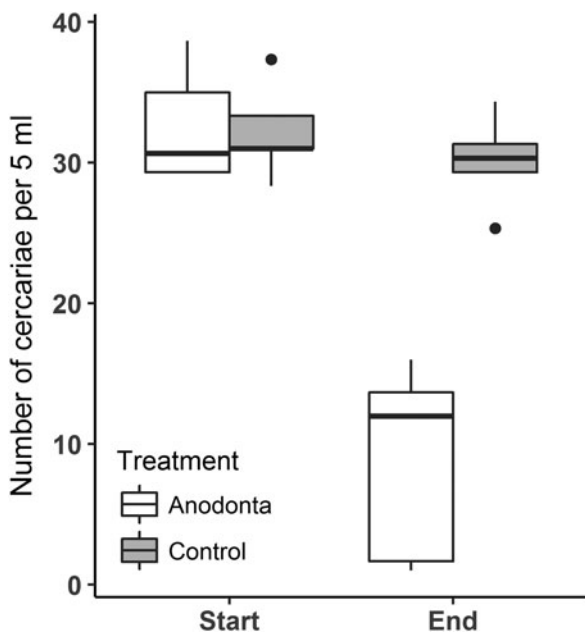
Fig. 1. Numbers of *Diplostomum pseudospathaceum* cercariae significantly and strongly (almost 4-fold) decreased in the presence of *Anodonta anatina*, while in the control treatment no decrease was found. 'Boxes' on the plot represent a median with the interquartile range (IQR). Whiskers extend from the highest to lower values within 1·5*IQR.

Table 2. Results of negative binomial GLM in high-, medium- and low-infection experiments. The effect of the treatment (absence/presence of *Anodonta anatina* in the environment) on the intensity of the *Diplostomum pseudospathaceum* infection in rainbow trout, *Oncorhynchus mykiss*, was significant in all three experiments

Experiment	Estimate	S.E.	z value	P value	N
High	0·29	0·13	2·20	0·028	22
Medium	0·31	0·12	2·58	0·010	22
Low	0·39	0·18	2·17	0·030	22

vulnerable to eye fluke infection both in natural conditions and at fish farms. These findings were confirmed in three independent experiments with different exposure doses. Since the mean density of *Anodonta* mussels in the lake littoral zone can be 15 individuals m^{-2} (Hanson *et al.* 1988), and the maximum density of unionoid mussels in a river can be over 1000 individuals m^{-2} (Oulasvirta, 2011), the effect of freshwater mussels on diplostomatid cercariae concentration could be considerable. However, further laboratory and field studies are needed to test how removal of cercariae by mussels varies under different environmental conditions (e.g. temperature, light, turbidity, biotic factors) and between different mussel species.

The reduction of trematode infection in mussel hosts has earlier been reported for marine bivalves (Thieltges *et al.* 2008*a*; Thieltges *et al.* 2009; Goedknegt *et al.* 2015), whereas the evidence for reduction of trematode infections in fish hosts has been lacking. Furthermore, this is the first evidence for the limitation of trematode transmission by (non-host) bivalves in freshwater environments, as the only previous study focusing on this topic did not reveal an effect of freshwater fingernail clams *Sphaerium* on cercariae abundance (Orlofske *et al.* 2012). However, the duck mussels tested in our study were considerably larger than fingernail clams, with higher filtration capacities, which could explain the observed differences in their removal of cercariae.

Our study indicated the ability of bivalves to reduce diplostomosis, which commonly causes problems at fish farms (Karvonen *et al.* 2004*a*, *b*; Karvonen *et al.* 2006). Therefore, bivalves might be used to control macroparasitic infections like fish and human trematodoses. Their potential effectiveness for prevention of several viral, bacterial and protozoan infections has been suggested earlier (reviewed in Burge *et al.* 2016). A wide ecological tolerance and widespread distribution of certain bivalve species make them good candidates for this role. For example, dreissenids and oysters remove pathogens effectively in different aquatic ecosystems (Graczyk *et al.* 2004; Lucy *et al.* 2008; Thieltges *et al.* 2009; Conn *et al.* 2013; McLaughlan and Aldridge, 2013). Thus, unionid mussels collected from natural habitats or derived from captive breeding (Barnhart, 2006; Scriven *et al.* 2011) could be used to control trematodoses, such as diplostomosis in fish farms in an ecologically sustainable way. One possible way to bridge between our small-scale laboratory research and its potential application in aquaculture would be mesocosm experiments including fish, unionid mussels and parasites. Managing parasitic infections in fish is intensively studied in semi-natural conditions (Karvonen *et al.* 2016).

The ability of *A. anatina* to reduce the abundance of cercariae in the environment (on average an almost 4-fold decrease by an individual mussel in 2 h) indicates that even a small number of these biofilterers can diminish the transmission of fish parasites. The potential use of *Anodonta* mussels for water quality improvement and parasite control (bacterial and protist infections) has already been suggested (Hänninen *et al.* 2005; Lucy *et al.* 2008; Ismail *et al.* 2015; Słodkowicz-Kowalska *et al.* 2015), because their filtration capacities are among the highest in freshwater bivalves (Pusch *et al.* 2001; Stybel *et al.* 2009). The filtration rates obtained in our experiment were comparable with the values calculated for this species by Kryger and Riisgård (1988), but higher than values obtained in the experiments by McIvor (2004). However,
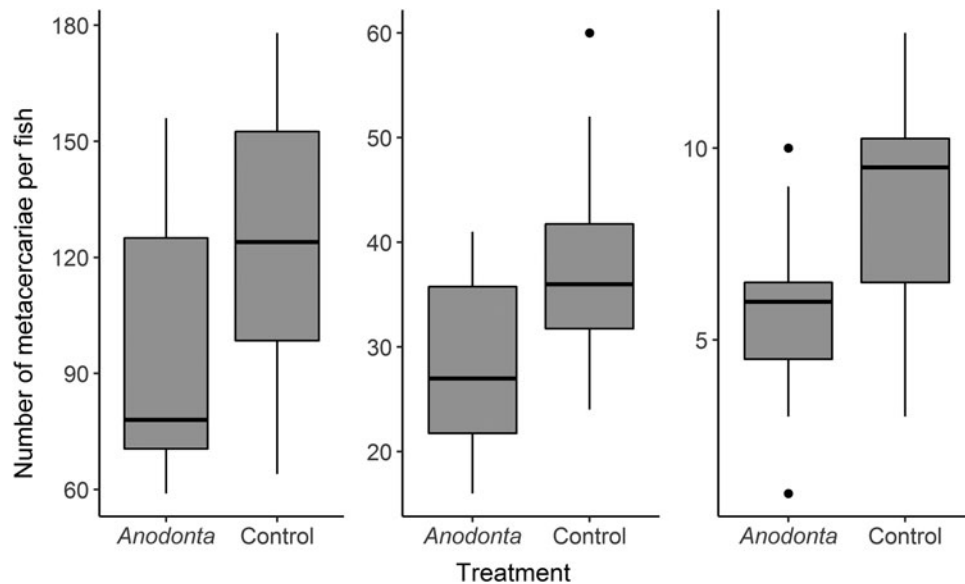
Fig. 2. Infection intensities in rainbow trout, *Oncorhynchus mykiss*, infected in the presence of *Anodonta* mussels were lower when high, medium and low amounts of *Diplostomum pseudospathaceum* cercariae were added to. 'Boxes' on plots represent a median with the interquartile range (IQR). Whiskers extend from the highest to lower values within 1·5*IQR. Outliers are plotted as black dots.

individual filtration activity of bivalves is very variable even under constant experimental conditions, following some endogenous rhythms (Kryger and Riisgård, 1988; Englund and Heino, 1996; McIvor, 2004; Huyvaert *et al*. 2012).

Clearance rates of bivalves are affected not only by endogenous rhythms, but also by various environmental factors, including water temperature, seston composition and concentration, light intensities, and age and size of the mollusc (Vanderploeg *et al*. 1995; Englund and Heino, 1996; Eversole *et al*. 2008; Kim *et al*. 2011). Biotic factors are also important; for example, the presence of fish can induce an increase in the filtration activity of *Anodonta* mussels (Jokela and Palokangas, 1993). Recently, the influence of temperature on parasite–predator interactions was discussed in the context of climate changes, and it was predicted that the temperature-driven filtration rates of bivalves will increase, leading to enhanced removal of free-living parasite stages and reduced parasite infectivity (Goedknegt *et al*. 2015; Burge *et al*. 2016).

In addition to the negative effect on parasite transmission, filtration of cercariae by bivalves could cause other important ecosystem effects. Parasites contribute significantly to aquatic food webs (Kuris *et al*. 2008; Lafferty *et al*. 2008), including the suggested important role of trematode cercariae in energy transfer (Morley, 2012). Thus, if cercariae are consumed by bivalves, trematodes could be involved in trophic pathways from bivalves to top consumers like fish and birds. However, it is not yet clear whether cercariae are damaged by filtration or/and ingested by mussels, because the gut contents of mussels was not analysed in our study or in

previous studies (Thieltges *et al*. 2008*a*; Goedknegt *et al*. 2015). These data are necessary to clarify the role of trematode cercariae in benthic food webs, which has earlier been assumed to be very important (Thieltges *et al*. 2008*b*). Moreover, fish could even be attracted by bivalves in the presence of cercariae, if they seek shelter against parasites. However, further studies are needed to test if bivalves could influence fish behaviour in such a way.

It should be noted that freshwater mussels do not only act as a sink for trematode cercariae (as shown in the present study) but also as a source of cercariae. The duck mussel, *A. anatina*, itself serves as the first intermediate host for bucephalid trematodes *Rhipdocotyle fennica* and *Rhipdocotyle campanula*, cercariae of which infect cyprinid fish, such as roach (*Rutilus rutilus*) (Taskinen *et al*. 1991; Gibson *et al*. 1992). Prevalences of *Rhipidocotyle* infection in *A. anatina* can be up to 30% and numbers of tissue-dwelling *Rhipidocotyle* metacercariae in roach up to 900 individuals fish^{-1} (Taskinen *et al*. 1991). Thus, presence of *A. anatina* can greatly influence trematode parasitism in the fish community, for example, by decreasing Diplostomatidae eye flukes in salmonids and increasing Bucephalidae parasitism in cyprinids. Some bivalve taxa (e.g. *Dreissena* and *Corbicula*) serve as intermediate hosts for echinostomatid trematodes (Fried *et al*. 1987; Conn and Conn, 1995), which are important as both animal and human disease agents. Thus, these bivalves may affect the population dynamics of echinostomatids, either enhancing transmission to molluscivore definitive hosts, or reducing transmission to definitive hosts that feed on fish or amphibians.

The effect of mussels on the abundance of trematode larvae is species-specific and may affect different freshwater organisms. Based on our findings, future research could aim: (i) to clarify whether cercariae are ingested (or simply damaged) by mussels and what impact such interactions have on aquatic food webs; (ii) to study factors (biotic, abiotic) limiting the filtering of cercariae by mussels; (iii) to conduct mesocosm experiments to test the use of mussels for preventing trematodoses in semi-natural systems; and (iv) to study indirect effects of mussels on fitness and behaviour of aquatic organisms through diverse effects on their parasites (elimination and/or production of cercariae). Knowledge of interactions between parasites, their hosts and predators may help to develop methods for the control of trematode transmission and management of public health and aquaculture.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0031182017001421.

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