Trematode infections reduce clearance rates and condition in blue mussels *Mytilus edulis*

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Abstract

Suspension feeders are important players in coastal food webs by coupling pelagic primary and benthic secondary production but the potential interference of parasite infections with this pivotal role is not well investigated. We experimentally determined the effect of infections with metacercariae of the common trematode *Renicola roscovita* on clearance rates and condition of blue mussels (*Mytilus edulis*). We also investigated whether there were differences in the effects of infections between different size classes of mussels. Using controlled infections under lab conditions, our experiment showed that infections significantly reduced the clearance rates of mussels. This effect was less pronounced in small (42 % reduction) than in large mussels (71%). In addition, infections also significantly reduced mussel body mass index in large (23% reduction) and small (17 %) mussels compared to uninfected mussels. These reductions most likely resulted from a combined effect of an interference of the parasites with the mussel's feeding apparatus (with the palps being the preferred infection site of the parasite) and of the energetic demands of the parasites (with metacercariae growing in size after initial infection). These negative effects on mussel filter feeding capacity indicate that parasites can alter a crucially important functional trait of marine suspension feeders.

Introduction

Benthic suspension feeders play a key role in coastal food webs by coupling pelagic primary and benthic secondary production and thus mediating the energy transfer through coastal ecosystems (Gili & Coma 1998). Bivalves constitute a particularly abundant part of benthic suspension feeders (Gili & Coma 1998, Newell 2004). Enormous volumes of water pass through their gills, enabling them to alter the abundance and composition of seston and plankton (Dame 1993). With their feeding activity bivalves may even act as natural eutrophication controllers which can significantly improve marine water quality (Officer et al. 1982, Wilkinson et al. 1996, Lindahl et al. 2005, Shumway 2011). In addition, through the production of faeces and pseudofaeces bivalves increase the localized sedimentation rate of suspended matter (Dame 2011) and change the benthic community composition (van der Zee et al. 2012).

The mechanisms underlying bivalve suspension feeding are particularly well understood for mussels (*Mytilus* spp., for a review see Gosling 2003). In the processing of food, mussels share the main mechanical steps with other filter feeders: interception of particles, transport, selection, and ingestion volume control (Beninger et al. 1995). After the water enters a mussel via the inhalant siphon it is filtered by the gills. The captured particles are then transported to the labial palps. The labial palps are complex structures (Beninger et al. 1995) which are important for ingestion volume control and particle selection (Beninger & St-Jean 1997). Two measures are commonly used to quantify mussel feeding activity: the *filtration rate* is defined as the pumping or volume flow rate and the *clearance rate* is defined as the volume of water cleared of suspended particles per unit of time. Clearance rate is identical to the filtration rate when all particles are retained (Riisgård 2001). Both measures are known to be influenced by various environmental factors like water temperature, particle concentration, quality and

quantity of available food, salinity and ambient water flow rate (Gosling 2003). In addition, mussel size and weight are well known intrinsic factors determining filtration rates in mussels and the interplay of intrinsic and environmental factors has long been studied (Newell 1970, Bayne 1998, Gosling 2003).

Very little is known about how parasitism affects mussel filtration rates. Mussels serve as hosts to a variety of different parasite taxa, including shell boring polychaetes, intestinal copepods, and tissue-inhabiting trematodes (Lauckner 1983). In particular the latter can occur in vast numbers of up to several thousand individuals per mussel (Lauckner 1983, Svaerdt & Thulin 1985, Zens 1999). One of the dominant trematode species in mussels along northern European shores is *Renicola roscovita* (Lauckner 1983). Like all trematodes it has a complex life cycle including several sequential hosts. Periwinkles (*Littorina littorea*) serve as first intermediate hosts from which free-living infective stages (cercariae) emerge that infect the second intermediate host (mussels). Here, the infective stages encyst as metacercariae and await ingestion of their host by a definitive host (gulls and other birds feeding on mussels). In the bird, the adult parasites reproduce and shed eggs with the bird's faeces that infect new snails (Werding 1969). In mussels, *R. roscovita* preferably settles in the palps but can also be found encysting in the gills, the digestive gland and other body tissues at higher infection levels (Fig. 1; Lauckner 1983, Svaerdh & Thulin 1985). Given the often high infection levels in mussels and the preferred location in the palps, some effect of *R. roscovita* infections on mussel feeding appears very likely. Indeed, infected mussels show slower growth than un-infected conspecifics in field experiments (Thieltges 2006). However, it is not known whether this is due to a reduction in particle processing ability, and if so, what the magnitude of such a reduction might be.

In this study, we experimentally determined the effects of infections with *R. roscovita* on mussel clearance rates and body condition by using controlled infections under lab conditions. We also investigated whether there were size-related differences in the effects of such infections.

Material and Methods

Parasite and host collection

Infected periwinkles (*Littorina littorea*) were used as cercariae donors to artificially create trematode infections. Several hundred snails were collected from an intertidal mussel bed close to the island of Texel (Balgzand, Netherlands, N 52.944, E 4.903) in October 2010 and acclimated in aerated flow through tanks (50 x 30 x 35cm) with natural sea water and kept constantly dark at 15 °C (to reduce shedding of cercariae). After two days the snails were screened for infections by placing them in 6-well plates (filled with 10-15ml of seawater) in an incubator at approximately 25 °C for 3-5 hours while exposing them to intense light to induce shedding of cercariae. Then the wells were checked for the presence of cercariae of *R. roscovita* under a dissection microscope. Infected and uninfected snails were kept separately in small aerated seawater tanks (13 x 21 x 13cm) at a constant temperature of 15 °C until the controlled infections of the mussels were performed. The water was exchanged daily and snails were fed *ad libitum* with *Ulva lactuca*.

Mussel hosts (*Mytilus edulis*) where collected from beach groin constructions at the west coast of the island of Texel (Netherlands, N 53.023, E 4.707) in November 2010. Mussels from this locality were assumed to be free of *Renicola roscovita* infections because the first intermediate host, the periwinkle *Littorina littorea*, is not present due to high wave energy exposure (confirmed by screening 50 mussels for the presence of metacercariae). For the

experiment, a total of 24 mussels was used, 12 from each of two shell length categories: "small" (19-22 mm) and "large" (46-49 mm). After carefully removing (a few) barnacles from their shells, all mussels were acclimated in an aerated flow-through sea water tank (50 x 30 x 35cm), filled with natural sea water and kept in a climate chamber at a constant temperature of 15 °C. During this time a diet of *Isochrysis galbana* was provided by adding 500-1000ml of algal suspension (approx. 10^6 cells ml^{-1}) once every day.

Experimental infections

To infect mussels with *R. roscovita*, the 24 experimental mussels were distributed equally in six small aerated plastic aquaria (13 x 21 x 13cm). Each aquarium contained two small and two large mussels which were numbered individually with nail polish. Half of the aquaria also contained 15 snails (in each aquarium) infected with *R. roscovita* while the other half contained 15 un-infected snails. All six aquaria were placed in a climate chamber with a constant temperature of 20 °C and constant light to initiate shedding of cercariae (Thieltges & Rick 2006). 100 ml (ca. 10^6 cells ml^{-1}) of *I. galbana* were added to each aquarium daily. After a total of four days all periwinkles were removed and all mussels were kept together in the same flow-through tank (50 x 30 x 35 cm) in a climate chamber at a constant temperature of 15 °C for 2 days to ensure encystation of metacercariae and acclimation to the same conditions as in the experiment. During this time mussels were fed with *I. galbana* by adding 500-1000ml of algae (approx. 10^6 cells ml^{-1}) once per day.

Clearance rate measurements & experimental design

We used the *indirect clearance method* to measure the clearance rate as the volume of water cleared of suspended particles per unit of time (Riisgård 2001). We determined the algae concentrations (*I. galbana)* of samples taken after certain time intervals by counting algal

cells with a CASY® Cell Counter and Analyser System (Schärfe System GmbH, Germany). The clearance rate could then be determined from the exponential decrease in algal concentration as a function of time (see details under data analysis). In general, the proper measuring of filtration and clearance rates in mussels is known to be difficult (Riisgård 2001) since the rates strongly depend on many biotic and abiotic factors (reviewed by Gosling 2003). No laboratory method can be directly generalized to field conditions (Beninger 2009). The *indirect clearance method* we used is generally considered valid (Riisgård 2001); however, a notable shortcoming of this technique is the confounding influence of decreasing algal cell concentrations during the experiment. We mitigated this possible source of bias by replenishing the algal concentration (Riisgård 2001) during the incubation period (see below).

The experimental setup consisted of 24 individual 2 l plastic containers filled with one litre of seawater (UV-sterilized, 0,2µm filtered) and provided with an airstone for a constant air supply and uniform mixture of the water. In each container we placed one of the 24 experimental mussels 15 minutes before measurements were started (pilot experiments had shown that mussels started with filtration at constant rates within this time). In addition to the 24 experimental containers, we set up 6 containers without mussels as controls to detect potential changes in algal concentration caused by factors other than the presence of mussels (e.g. intrinsic algal mortality). The experiment was performed at a temperature of 15 °C and under constant light. Prior to the experiment the cell concentration of our algal culture stock was measured to calculate the volume needed to produce a starting concentration (t_0) of around 13-14 x 10^3 algal cells per ml in the tanks (following Riisgård & Randløv 1981). Immediately after adding the algae, a sample of 10ml was taken out of each container and the algal concentration measured and set as the 'input concentration' per respective tank. Afterwards samples were taken every 15min for two hours. After each measurement, samples

were cast back into the respective container to avoid a decrease of the water volume during the experiment. Algal cell counts of each sample were performed twice to minimize potential effects of measuring inaccuracy. If the counted cell number decreased below 1 x 10^3 cells ml^{-1},or if it was expected to decrease below this value before the next measuring point, another dose of algal culture was added to bring the number back to the starting concentration (following Riisgård 2001). The experiment was conducted during one day in three sequential runs with 2 replicates of each treatment combination (and 2 control containers), resulting in a randomized (temporal) block design.

Dissection, condition index and palp size

Immediately after completing all the measurements, the mussels were frozen at -18°C until dissection. At this point, 7 days since starting the infections had passed (4 days infections, 2 days acclimation, 1 day experiment, see above). Procedures for infected and uninfected mussels were exactly the same to avoid any bias, particular in tissue dry mass measurements. Prior to dissection, mussel shell length (maximum anterior-posterior dimension) was measured to the nearest 0,01 mm with a digital vernier calliper. Then the posterior adductor mussel was cut, the shell opened and the tissue (palps and gills carefully separated) placed between two thick glass slides (compressorium). Under a stereomicroscope all metacercariae of *R. roscovita* were counted (or their absence verified in uninfected mussels) and their location noted (palps, gills, foot and rest of the body). Finally, the tissue was carefully transferred from the slides to crucibles and dried at 60 °C for three days to determine dry mass and to calculate the condition index (CI) of each mussel ($CI=W/L^3$; Dare 1976) following Riisgård (2001).

181 Data analysis

182 We used the mean of the two algal cell counts per sample for further analysis. Since the
183 control containers showed no agglomeration or loss of algae during the two hours of
184 measurements (see results below), we did not correct cell counts for algae loss other than by
185 filtration by the mussels. Using the number of algae in a container at each time point, we
186 calculated the clearance rate of each mussel for each period in between adding new algae. The
187 decline in algal cells was estimated by calculating the slope of the regression line describing
188 the ln-transformed cell numbers as a function of time. This offered a more reliable estimate
189 than the formula applied by Riisgård (2001) because it allowed to include more measurements
190 than only the first and the last one. To determine the clearance rate in ml min^{-1} we multiplied
191 the slope of each regression with -1000 (to account for the 1000ml volume the mussels were
192 kept in). Depending on how often new algal culture was added, there were 1-4 clearance rate
193 estimates per mussel achieved during the 2 hours. If there was more than one measurement,
194 we used the mean clearance rate for further analysis.
195
196 We tested for differences in mussel size (log-transformed) between infected and uninfected
197 mussels within each size class using separate t-tests. To test for differences in clearance rates
198 and condition between infected and uninfected and small and large mussels we used a fully
199 factorial ANOVA design with mussel size and infection status as fixed factors. In addition,
200 we added a block factor to test for potential differences among the three separate runs.
201 Clearance rates and condition indices were both log-transformed to meet the assumptions of
202 parametric tests (confirmed by visual inspection of residual plots). To test for potential
203 agglomeration or loss of algae during the two hours of measurements in the control containers
204 we used linear regressions (log algal concentration over time).
205

206 To compare relative infection levels between small and large mussels, we calculated palp
207 surface area (P) as $P=1,84*L^2$, with L=shell length in cm (Kiørboe & Møhlenberg 1981) and
208 determined the no. of metacercariae mm^{-2} palp surface area by dividing the total no. of
209 metacercariae found in a mussel by the calculated palp surface area. In addition, we calculated
210 the no. of metacercariae mm^{-2} mussel by dividing the total no. of metacercariae found in a
211 mussel by a measure for the surface area, its length (mm^{-2}).
212
213 **Results**
214 The final dissections showed that all controlled infections were successful, with small and
215 large mussels acquiring, on average, approximately 1500 and 3000 metacercariae,
216 respectively (Table 1). No other macroparasites were noticed in the experimental mussels. In
217 small mussels about half of the metacercariae were encysted in the palps and gills (48%); in
218 large mussels this proportion was higher (62%; Table 1). Although larger mussels carried a
219 higher total load of metacercariae, their relative parasite burden per mussel length was about
220 2.5 times lower than in small mussels (Table 2). The difference between parasite load per palp
221 surface between large and small mussels was less pronounced, with small mussels carrying
222 about 1.5 times higher numbers of metacercariae (Table 2).
223
224 While none of the small mussels needed a new addition of algae during the experiment, some
225 of the uninfected large mussels needed a new addition of algal culture up to four times. In
226 contrast, several of the infected large mussels did not need an extra dose. However all
227 individual declines in algal concentration for each period were still linear when new algae
228 were added, and algal concentrations were comparable in all trials. Furthermore, in trial
229 experiments we conducted, *M. edulis* did not show differences in filtration rates when
230 exposed to algal concentrations between 2-12 x 10^3 *I. galbana* cells ml^{-1}. Hence, we consider

231 our clearance rate experimental design to be robust. The control containers showed no
232 agglomeration or loss of algae during the two hours of measurements (no statistical effect of
233 time on algal concentration in linear regressions, p=0.98 (< ±5 % difference between start and
234 end value)). In general, there was no statistical difference in mussel size between infected and
235 non-infected mussels within each size class (t-tests; small mussels: $F_{1, 10}$=0,17; p=0,688; large
236 mussels: $F_{1, 10}$=0,6; p=0,469, Table 2), indicating that mussel size was not confounding the
237 measurements.
238
239 The experiment showed statistically significant effects of mussel size and infection status on
240 mussel clearance rate and condition and a marginally significant interaction term (Table 3).
241 There was no statistically significant effect of the (temporal) block factor for clearance rates
242 but the block factor was statistically significant when looking at mussel condition (Table 3).
243 Mean clearance rates of infected mussels were only 58% (small mussels) and 29% (large
244 mussels) of the ones observed in uninfected mussels (Fig. 2). In general, large mussels had
245 higher clearance rates than small mussels (Fig.2). Similarly, small mussels had a lower
246 condition than large mussels (Fig. 3) and the condition index was lower in infected compared
247 to uninfected mussels, both in small and in large mussels (77% and 83% of uninfected
248 mussels, respectively; Fig. 3). Since mussel length was not statistically different between
249 infected and uninfected mussels within the two size classes (see above), the difference in
250 condition resulted from higher body dry mass of uninfected mussels (small: 20.1±3.3 mg;
251 large 430.2± 33.8 mg) acquired (or maintained) during the 7 days from starting the infections
252 to the termination of the experiment (see above) compared to infected mussels (small:
253 14.5±1.4 mg; large: 346.3±25.9).
254
255

Discussion

The experiment showed that infections with the trematode *Renicola roscovita* significantly reduced the clearance rates of mussels. This effect was less pronounced in small (42 % reduction) than in large mussels (71%). In addition, in the short course of the experiment, infections also significantly reduced mussel condition in large (23% reduction) and small (17 %) mussels.

These results corroborate our hypothesis that the preferred location of the parasites in the gills and palps interferes with the filtration in infected mussels. This interference most likely results from a mechanical disturbance through the encapsulated cysts in the gills and palps. However, infections in other tissues (e.g. digestive gland) may have added to the reduced filtration activity by negatively affecting their hosts. Such negative effects may result from the fact that metacerariae of *R. roscovita* grow in size after the initial infection of their hosts (Lauckner 1983) and thus require increased energy resources from their hosts. However, the duration of our experiment may have been too short for such energy demands resulting from metacercarial growth to be relevant. An alternative source of parasite-induced energy drain may be the costs of host immune responses and tissue repair for the mussel hosts. The multiple penetration of mussel tissue in the course of infection may lead to loss of hemolymph and increased production of hemocytes, which could be energetically costly. Should this actually be the main driver of parasite-induced mussel energy drain, the effect of infections may only be transitory and decreasing in the long run. However, data from a previous field experiment on the effects of *R. roscovita* infections on mussel growth during a longer observation time since initial infection (10 weeks, Thieltges 2006) suggest that negative effects of metacercarial infections also continue over longer time periods. More detailed experiments will be needed to disentangle the effect of reduced food intake due to mechanical

281 interference of the filtration process, energy drain by energetic demands of metacercariae
282 during their growth and potential energetic costs of immune responses and tissue repair on
283 mussel filtration and condition.
284
285 The magnitude of the observed effects on condition after only 7 days since the start of
286 infections seems unlikely at first sight. However, mussels are well known to increase in body
287 mass much faster than in shell length under laboratory conditions. For example, Riisgård et al
288 (2012) reported an increase in mussel dry weight from 22.9 to 72.0 mg within 17 days at a
289 salinity of 30 and constant feeding, resulting in weight specific growth rate (μ) of 6.7 % d^{-1} (μ
290 = ln (W_t/W_o) t^{-1} × 100; with W_o and W_t being the initial and final body dry weight,
291 respectively; Riisgård et al (2012). Unfortunately, we do not have initial body tissue dry
292 weights for a similar calculation of μ. However, using the same formula the difference in
293 body weight between infected and uninfected mussels in our experiments amounts to a
294 difference of 4.7 % d^{-1} in small and 3.1 % d^{-1} in large mussels, suggesting that the differences
295 in body condition within such a short time are well within the range reported in the literature.
296 However, whether the difference in body weight resulted from a reduced growth of infected
297 compared to uninfected mussels or from a loss of weight in infected versus uninfected
298 mussels cannot be inferred from our experiment. The combined effect of interference with the
299 mussel's feeding apparatus and direct or indirect energetic demands imposed on the mussels
300 by the infections probably also underlies the observed reduction in shell growth of infected
301 mussels reported from an earlier field experiment (Thieltges 2006) and indicates that *R.*
302 *roscovita* infections translate into significant reductions of host filtration, condition and
303 growth. Reduced condition of infected hosts has also been observed in other bivalve-
304 trematode systems, e.g. in the brown mussel *Perna perna* infected with metacercaraie of the
305 genus *Proctoeces* (Calvo-Ugarteburu & McQuaid 1998). In contrast, another study did not

306 find an effect of infections with *Himastla interrupta* on cockle (*Cerastoderma edule*)
307 condition (de Montaudouin et al. 2012). Hence, it remains to be investigated how universal
308 negative effects of metacercarial infections on bivalve condition are and what the underlying
309 mechanisms are.
310
311 Since mussels constitute an important part of the suspension feeding compartment in many
312 coastal ecosystems, coupling pelagic primary and benthic secondary production (Gili & Coma
313 1998, Dame 2011), the observed effects may ultimately influence energy transfer in coastal
314 ecosystems. Such ecosystem wide effects will depend on the actual infection levels in the
315 field since the effects of metacercarial infections are considered to be density-dependent, i.e.
316 the impact on hosts increases with infection intensity (Fredensborg et al. 2004, Thieltges
317 2006). The laboratory infection rates with *R. roscovita* in our experiments are well within the
318 range of infection levels observed in European coastal waters where infection levels of up to
319 3000 to almost 6000 metacaercariae per mussel (depending on location) have been recorded
320 (Svaerdh & Thulin 1985, Zens 1999, Buck et al. 2005). However, the mean infection levels
321 on many natural mussel beds are often lower and future studies will be needed to estimate the
322 overall effect of infections on mussel filtration capacity and effects on energy flow in coastal
323 ecosystems. Such calculations will also need to integrate the effects of other parasites on
324 mussel filtration. For example, the parasitic copepod *Mytilicola intestinalis* also reduces the
325 filtration capacity of mussels (Meyer & Mann 1950). However, in this case the underlying
326 mechanism is not a direct interference with the filtration apparatus (the copepods inhabit the
327 mussels' intestines) but most likely an indirect effect resulting from negative effects on
328 mussel condition. Apart from effects on total filtration capacity, parasites may also interfere
329 with the sorting capability of their hosts, in particular when they inhabit the palps, which are
330 particle sorting organs (Beninger & St-Jean 1997). This limitation in sorting capacity will

reduce quality and/or quantity of the ingested food affecting mussel condition/growth/energy balance (and by that possibly feed back towards system dynamics).

In conclusion, our experiment showed that infections with the trematode *Renicola roscovita* significantly reduced the clearance rates of mussels, with subsequently negative consequences on individual condition. This suggests that parasites may also affect energy transfers in coastal systems via lowering molluscan secondary production. Our study shows that parasites can affect important traits for their hosts and suggest that further studies will be valuable to evaluate the overall direct and indirect effects of parasites in coastal ecosystems.

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409 **Table 1:** Mean number ± SD of *R. roscovita* metacercariae in different body tissues of
410 infected small and large mussels. Autopsies showed that all uninfected mussels carried no did
411 metacercariae of *R. roscovita*.
412

Mussel size	Labial palps	Gills	Foot	Other tissue	Total
Small	362 ± 95	397 ± 56	110 ± 71	627 ± 276	1559 ± 242
Large	1247 ±470	647 ± 341	218 ± 89	920 ± 526	3032 ± 1247

413
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Table 2: Mean shell length, palp surface area, no. of metacercariae mm^{-2} mussel length and no. of metacercariae mm^{-2} palp surface area (all ± SD) of small and large infected and uninfected mussels. For calculations see text.

Size & infection densities	**Small mussels**		**Large mussels**	
	infected	**uninfected**	**infected**	**uninfected**
Shell length (mm)	21.28 ± 1.19	21.03 ± 0.55	47.52 ± 0.89	47.12 ± 0.88
Palp size (mm^2)	8.35 ± 0.92	8.14 ± 0.43	41.56 ± 1.54	40.87 ± 1.63
No. metacerc. mm^{-2} mussel	3.53 ± 0.63	-	1.38 ± 0.64	-
No. metacerc. mm^{-2} palp	44.44 ± 9.99	-	30.51 ± 12.76	-

421 **Table 3:** Results of two-factorial analysis of variance (ANOVA) testing for differences in

422 clearance rate and condition (both log10 transformed) between uninfected and infected *M.*

423 *edulis* of two size classes (small and large).

424

Effect	df	MS	F	p
Clearance				
Size	1	1.357	35.503	<0.001
Infection	1	0.903	23.618	<0.001
Size*Infection	1	0.144	3.761	0.068
Block	2	0.084	2.192	0.141
error	18	0.038		
Condition				
Size	1	0.60	92.071	<0.001
Infection	1	0.06	8.605	0.009
Size*Infection	1	0.001	0.214	0.649
Block	2	0,027	4.099	0.034
error	18	0,007		

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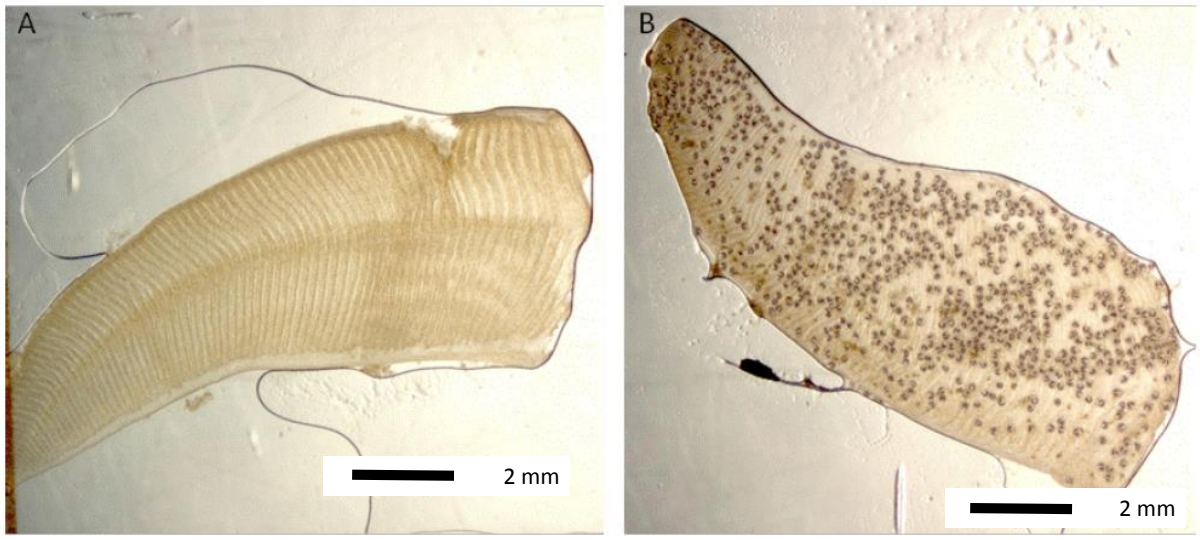
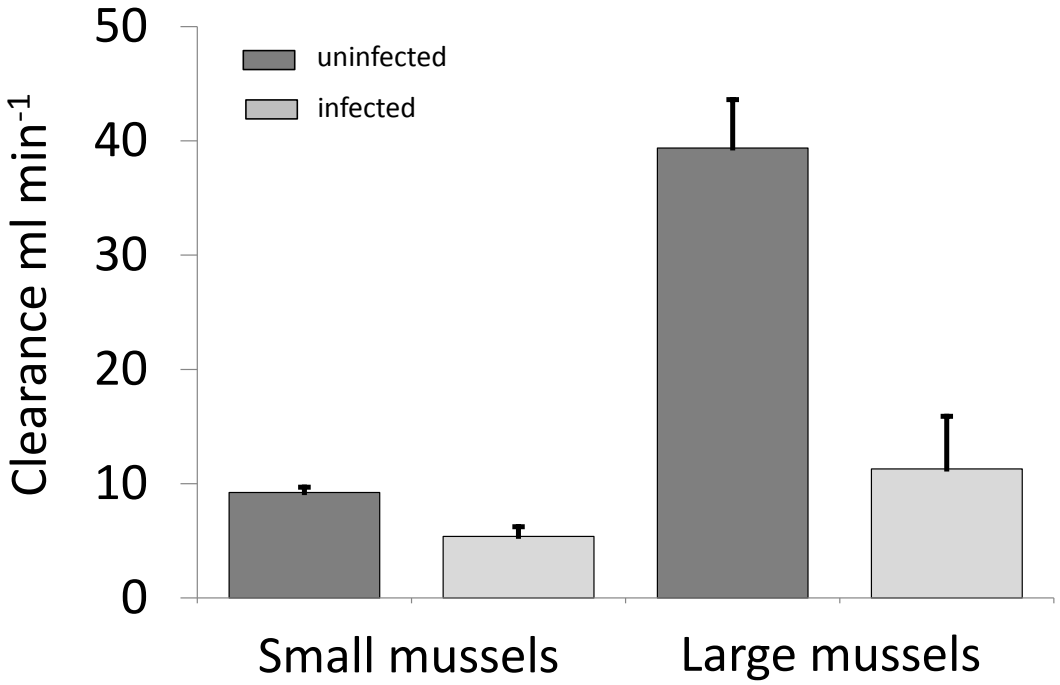


Fig. 1: a) Uninfected palp of a mussel (*Mytilus edulis*) and b) palp heavily infected with metacercariae of the trematode *Renicola roscovita*.



433

434 **Fig. 2:** Mean clearance rates (+SE) of small (19-22 mm) and large (46-49 mm) mussels with

435 (infected) and without (uninfected) metacercarial infections of *R. roscovita* in the laboratory

436 filtration experiment (back-transformed from log10) . n=6 per treatment combination.

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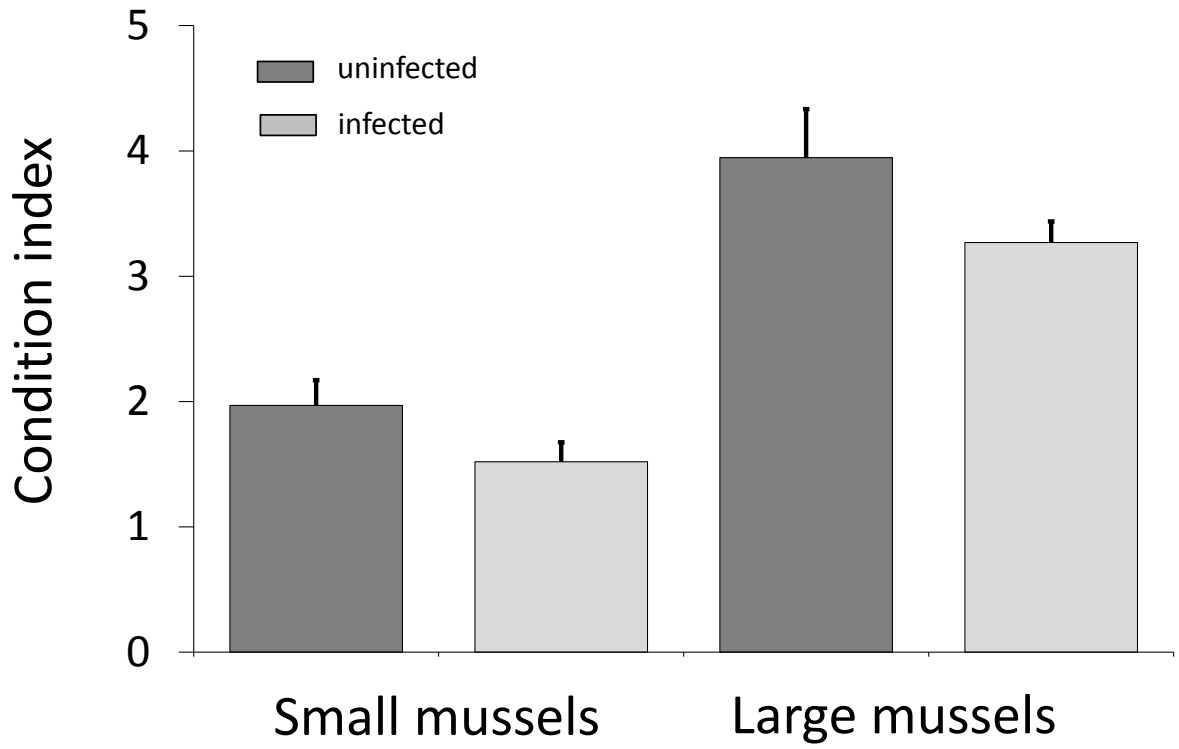

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441 **Fig. 3:** Mean condition index (+SE, calculated as dry weight x shell length^{-3}) of small (19-22
442 mm) and large (46-49 mm) mussels with (infected) and without (uninfected) metacercarial
443 infections of *R. roscovita* in the laboratory filtration experiment. n=6 per treatment
444 combination.

445