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Does the parasitic freshwater pearl mussel *M. margaritifera* harm its host?

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Abstract Many parasites have strong negative impacts on their hosts, but the effects of single-host, non-trophically transmitted parasites can be subtle and are not well understood. We examined the physiological response of juvenile brown trout (*Salmo trutta*) to encystment by the parasitic larvae (glochidia) of the freshwater pearl mussel, *Margaritifera margaritifera*. Glochidia abundance was positively correlated to host body size and was accompanied by significant spleen enlargement at 31 days postexposure, but not before (15 days) or after (160 days). Compared to controls, encysted gill lamellae were significantly thicker and longer, and tended to have fewer mucous cells which may have facilitated encystment. There were no significant difference in mean blood haematocrit between encysted and uninfected trout, but encysted trout took c. 6 h longer to reach basal ventilation rate than controls suggesting that glochidiosis may impose a respiratory burden to brown trout. These findings may have implications for the artificial propagation of the freshwater pearl mussel because the effects of glochidia on host respiratory performance appear to be additive. Therefore, aiming for high glochidia loads may not be the best option for mussel propagation programmes, if this compromises host fitness and hence the probability of successful glochidia excystment.

Keywords Single-host parasite · Freshwater mussel · Physiology · Splenomegaly · Respiration · Haematocrit

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Introduction

Freshwater mussels (Bivalvia: Unionoidea) rank amongst the most endangered aquatic organisms in the world (Lydeard et al., 2004; Strayer et al., 2004). They have an obligate parasitic stage (glochidia) attached to the gills or fins of freshwater fish (Meyers & Millemann, 1977) and are increasingly the target of conservation programmes that rely on the infection of suitable fish hosts for propagation (Thomas et al., 2010). Yet, knowledge of fish vulnerability and resistance to glochidia is very scant, despite its obvious importance for Unionid mussel conservation.

It is assumed that glochidia represent some form of parasitic burden (Treasurer & Turnbull, 2000; Treasurer et al., 2006) because fish often mount an immune response that results in the sloughing of large numbers

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of glochidia (Hastie & Young, 2003; Rogers-Lowery et al., 2007) and can acquire immunity following experimental exposure to a range of freshwater mussels (O'Connell & Neves, 1999; Rogers & Dimock, 2003; Dodd et al., 2005, 2006). On the other hand, because glochidia are obligate, single-host parasites, their fate is inexorably linked to that of their hosts, and a strong negative impact on fish hosts would ultimately decrease parasite fitness. Indeed, the parasitic status of freshwater mussels has been called into question, and the relationship of glochidia with their fish hosts has been described as one of phoresy (Barnhart et al., 2008), or even as a form of symbiosis-protocooperation (Ziuganov & Nezlin, 1988; Geist, 2010), although this has never been tested.

Salmonids are the definitive, obligate hosts for the glochidia of *Margaritifera margaritifera* (L.), the freshwater pearl mussel, and here we examined a suite of physiological responses of brown trout (*Salmo trutta* L.) to glochidia encystment at various times postexposure in order to shed light on the nature of the salmonid-freshwater pearl mussel host–parasite system. We examined the spleen of infected and control trout because the vertebrate spleen is the location of soluble antigen recognition (Rowley et al., 1999) and can also act as an erythrocyte reservoir (e.g., Yamamoto, 1987). Spleen enlargement (splenomegaly) following parasite infection has been reported for many species, including birds (Brown & Brown, 2002) and fish (Seppänen et al., 2009), making the relative size of the spleen a potentially useful indicator of host immune-competence. However, stress can also cause a change in the relative size of the spleen of fish (Manning, 1994). For example, Kortet et al. (2003) observed a decrease in the relative size of the spleen of *Rutilus rutilus* as a result of spawning stress in the wild. The spleen of encysted trout, therefore, could become enlarged due to an immunological response, or contracted due to a stress response.

Many organisms show reduced blood haematocrit following infection by blood parasites. That is the case, for example, with blackeye thicklip infected by gnathid isopods (Jones & Grutter, 2005), or rabbitfish *Siganus luridus* infected by the microcotylid *Allobivagina* spp. (Paperna, 1984), but it is unclear if this is also the case for glochidia-infected trout. Glochidia become encapsulated in the secondary fish gill lamellae and the cysts often pierce through the fish's blood vessels (Karna & Millemann, 1978; Araujo & Ramos, 1998; Araujo et al., 2002), so there is clearly potential for altering host blood parameters. Histological examination of fish gills, and data on red blood counts, therefore, could reveal potential glochidia impacts. Finally, because glochidia of *M. margaritifera* are exclusively gill parasites (Wächtler et al., 2000), we expected that they might have an impact on salmonid host respiration. Fusion of secondary lamellae, nodule formation, and thickening or scarring of the gills have sometimes been noted in other glochidia–fish systems, and it has been suggested that this might increase resistance to gas diffusion, and perhaps decrease respiratory performance (Meyers et al., 1980). Some fish respond to glochidia encystment by hyperventilating (Crane et al., 2011), but whether this represents a response to impaired gas exchange is not known. No information is available on the salmonid–freshwater pearl mussel system, and we employed opercular beat rate as a measure of ventilation frequency (Hawkins et al., 2004; Millidine et al., 2008) to test if glochidia encystment-affected salmonid respiration. Our general expectation was that the effects of glochidia on salmonid physology would be mild or short lived in order to ensure host survival and maintain parasite fitness.

Methods

Sources of fish and estimation of glochidia loads

Studies were conducted at the Cynrig Fish Culture Unit of the Environment Agency Wales (Powys, Wales) and at the Freshwater Research Unit, Swansea University. Juvenile 0+ brown trout (*Salmo trutta*) used in this study (fork length 54–265 mm) were derived from R. Usk broodstock maintained at the EAW hatchery, as part of the Environment Agency (Wales) captive breeding program for *M. margaritifera.* Approximately 1,000 trout were transferred to a 1.5 diameter tank which was connected to a holding tank containing 50 adult mussels at least 2 months before glochidia spatting during the autumns of 2008 and 2009. A similar number of trout was kept in other tanks unconnected to the mussels to serve as unexposed controls. Fish were fed in excess by means of automatic feeders according to standard hatchery procedures.

A sample of 27–90 juvenile brown trout were humanely killed by an overdose of anaesthesia

(2-phenoxyethanol, Sigma Chemical Co., St Louis, MO) at four different time intervals over the course of glochidia encystment. The fish were weighed (wet weight, 0.1 g), measured (fork length, mm) and their gill arches dissected and mounted on glass slides. Glochidia found on each of the eight gill arches were counted under a dissection microscope (Leica) at 40× magnification and these were summed up to provide total glochidia loads for each fish. Glochidia numbers were counted on two occasions separated several weeks apart to provide data on the reliability of glochidia counts. Approximate dates of spatting (glochidia release) were estimated from visual observations of adult mussels and detection of encysted glochidia in the trout gills. Days postexposure (d.p.e.) were calculated as the mid-point between the date of the last negative sampling occasion (when no glochidia were found) and the first positive sampling event.

Gill histology

At 15 days postexposure, the first left gill arch of 15 trout was placed in an excess of freshwater Bouin's fixative (Humason, 1979). Histologically-fixed gill arches were dehydrated in a series of graded ethanol baths (70, 80, 90, and 100%), and cleared with Histo-Clear (National Diagnostics, USA) before mounting in paraffin wax. Serial sections (6 µm) were made using a 52164 Kent Cambridge rotary microtome and at least ten slides per individual were stained using the haemotoxylin-eosin method (Lillie, 1965). Gill sections were then photographed using an Olympus C500 digital camera mounted on an Olympus BX41 microscope at 40× magnification. The width and length of one control (unencysted) and one encysted secondary lamellae, as well as the thickness at glochidia cysts at 0°, 180°, and 270° axes were measured for each individual host from high resolution digital photographs using Image-J (Fig. 1). The presence or absence of epitheliocystis-like colonies (Nowak & LaPatra, 2006) was noted, and the number of mucous cells in a 200 µm^2 area centred on the cyst was counted.

Haematocrit determination

To determine haematocrit values, whole blood from the caudal veins of freshly killed trout (exposed $n = 21$; unexposed $n = 23$) was drawn into capillary tubes (75 × 1.5 mm) at 30 days postexposure, centrifuged at 3,000*g* for 5 min and the total packed red blood cell volume read from a haematocrit graduated scale (Hawksley Scientific) as per Woo (1969).

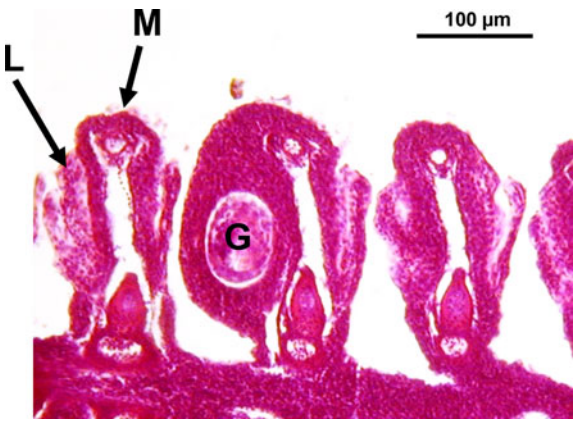

Fig. 1 Encysted *M. margaritifera* glochidium in the gills of brown trout host 15 days postexposure (corresponding to 176 cumulative temperature units). Key: *G* glochidium, *L* secondary lamellae, *M* mucous cells. H & E stain, ×40 magnification

Splenomegaly

Spleens were dissected from trout hosts at 15 days ($n = 27$), 30 days ($n = 27$) and 160 days postexposure ($n = 30$), weighed (0.001 g) and photographed with a Canon EOS D40 fitted with a SIGMA EM-140 DG ringflash and a macro lens (TAMRON SP DI 90 mm 1:2.8, 1:1 magnification), mounted on a copy stand at a fixed 40 cm height from the object. Spleen areas were subsequently digitized from high resolution TIFF images using Image-J (Abramoff et al., 2004) in order to quantify the extent of glochidia-induced splenomegaly (enlargement of the spleen).

Ventilation frequency

Ventilation frequency of trout hosts was estimated from measurements of opercular beat rate (OBR) as per Roberts et al. (2011). A total of 50 exposed brown trout were haphazardly collected from the EAW hatchery at 160 d.p.e., transported to Swansea University and allowed to acclimatize in a 1 m diameter recirculation tank for 1 week. Individual fish were then placed at random in six 3-l aquaria (25 × 15 × 18 cm) fitted with a constant air supply. A wooden frame and dividers isolated the aquaria and prevented the fish

Table 1 Variation in glochidia prevalence and abundance in 0+ brown trout hosts sampled at various days postexposure (d.p.e)

Sampling year	Spat release	D.P.E	No. of trout examined	FL (mm) (SE)	Prevalence (%)	Mean glochidia (SE)
2008	22/09	15	27	91.3 (±3.68)	100.0	100.7 (±18.62)
		31	27	98.4 (±2.74)	100.0	150.9 (±2.94)
		160	49	107.3 (±3.52)	55.0	36.7 (±7.94)
2009	28/09	167	90	174.4 (±1.98)	47.7	54.1 (±7.66)

from seeing each other. Small observation holes allowed an observer to view the fish without being seen. OBR was recorded with the aid of a stopwatch at 6 min intervals during the first hour, then at hourly intervals for 4 h, before a final reading was taken 15 h after introducing the fish, which was considered to be the baseline value (Brydges et al., 2008; Roberts et al., 2011).

Statistical analysis

We employed general linear models to examine the effect of body size (fork length) and days postexposure on trout glochidia loads, and tested for glochidia-induced changes in spleen size and haematocrit at various times postexposure by ANCOVA, using fork length as a covariate. To test for glochidia-induced changes in gill morphology, we employed a paired *t* test to compare the length and width of encysted and unencysted (control) secondary lamellae from the same individuals, as well as the number of mucous cells per 200 μm^2 section. Repeated measures ANOVA was used to compare OBR of uninfected and infected trout with different glochidia loads using fork length as a covariate to control for variation in body size. Where Mauchly's test for sphericity could not be met, Greenhouse-Geisser corrected probability values were used. We used SPSS 16.0, and SYSTAT v. 10 for all statistical tests, and applied the logarithmic or square root transformations to improve normality and homogeneity of variances, as required.

Ethics statement

Trout used in this study had been exposed to glochidia of the freshwater pearl mussel by EAW staff as part of Environment Agency (Wales) captive breeding program for *M. margaritifera,* and were part of the routine monitoring of encystment rates at the hatchery. Fish were humanely killed in accordance to HO schedule 1 and carried out by trained personnel.

Results

Effect of body size and time since encystment on glochidia abundance

Glochidia counts were reliable, as there were no false negatives and repeatability in glochidia counts was very high (intraclass-correlation coefficient = 0.999, Cronbach's Alpha = 1.000). Glochidia abundance generally decreased with days postexposure (Table 1). Multiple regression ($F_{2,190} = 33.927$, $P < 0.001$) indicated that variation in glochidia counts (square-root transformed values) was positively associated with trout body size ($t = 2.517$, $P = 0.013$) and negatively associated with days-postexposure ($t = -7.703$, $P < 0.001$; Fig. 2). However, the positive effect of host body size on glochidia abundance, which was evident at 15 days ($F_{1,25} = 4.88$, $P = 0.037$) and 30 days postexposure ($F_{1,25} = 280.02$, $P < 0.001$), was no longer significant at 160 d.p.e. ($F_{1,47} = 2.404$, $P = 0.128$) or 167 d.p.e. ($F_{1,88} = 0.837$, $P = 0.363$).

Gill histology

Encysted lamellae were significantly thicker (mean = 251.6 μm, SD = 46.8) and longer (mean = 298.9 μm, SD = 78.34) than un-encysted (control) lamellae from the same fish (mean thickness = 141.4 μm, SD = 50.7, $t_{14} = 12.560$, $P < 0.001$; mean length = 245.2 μm, SD = 76.2, $t_{14} = 6.222$, $P < 0.001$). The number of mucous cells per 200 μm^2 gill area was also higher in control lamellae (mean 3.5, SD = 3.0) than in encysted lamellae (mean = 0.4, SD = 0.5), but the difference was not significant ($P = 0.054$).

Splenomegaly

As with glochidia counts, repeatability of spleen area measurements was very high (intraclass-correlation =

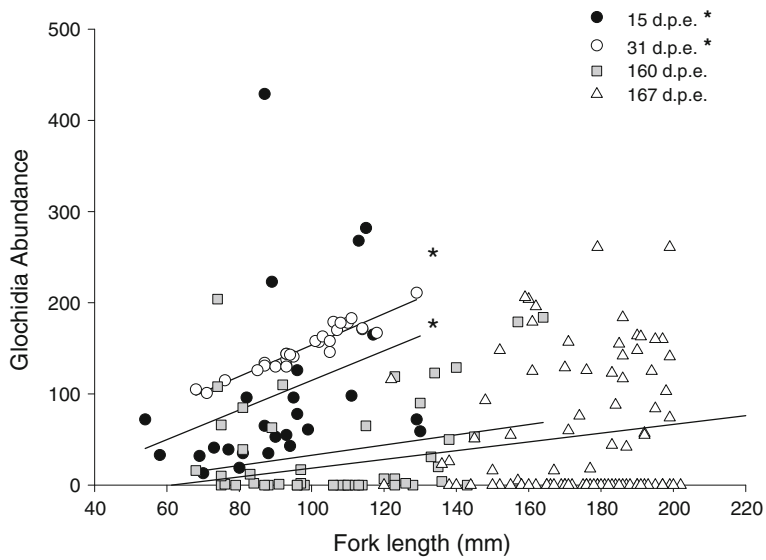

Fig. 2 Relationship between trout body size (fork length, mm) and glochidia abundance over the course of encystment. *Asterisk* denotes a significant positive relationship

0.999, Cronbach's Alpha = 1.000), indicating that digital photography can be used to obtain precise measurements of spleen area. At 15 and 160 days postexposure glochidia encystment did not result in any detectable enlargement of the spleen, and all the observed variation in spleen size was explained by differences in host body size. However, at 30 d.p.e. there was a significant enlargement of the spleen of infected fish compared to controls ($t = 8.442$, $P < 0.001$), once the effects of host body size had been statistically controlled for (multiple regression $F_{2,24} = 94.461$, $P < 0.001$).

Haematocrit

Mean haematocrit values were not related to glochidia abundance at 30 d.p.e. ($F_{2,18} = 1.959$, $P = 0.170$), nor was there a significant difference in mean haematocrit between exposed (16.42% ± 4.47, $n = 21$) and unexposed trout (16.47% ± 3.78, $n = 29$) when the effect of body size had been accounted for (ANCOVA exposure status $F_{1,41} = 0.240$, $P = 0.627$; fork length $F_{1,41} = 4.702$, $P = 0.036$).

Ventilation frequency

As data violated the assumption of sphericity for repeated measures analysis, the Greenhouse-Geisser correction was applied. Ventilation frequency was elevated immediately after transferring the fish to the observation aquaria, and declined over time to reach basal levels 15 h later (Fig. 3). Univariate repeated measures analysis indicated a strong between subjects effect of log-transformed glochidia loads on ventilation frequency ($F_{1,42} = 13.805$, $P = 0.001$), as well as significant within subject effects of time ($F_{11,462} = 21.806$, $P < 0.001$), and interactions between time and body size ($F_{11,462} = 3.601$, $P = 0.016$) and between and time and glochidia loads ($F_{11,462} = 4.943$, $P = 0.003$). These results were confirmed by multivariate repeated measures analysis, which again indicated a strong effect of elapsed time on ventilation frequency (Wilks' Lambda = 0.302, $F_{11,32} = 6.735$, $P < 0.001$), as well as significant interactions between time and body size (Wilks' Lambda = 0.392, $F_{11,32} = 4.504$, $P < 0.001$) and between time and glochidia load (Wilks' Lambda = 0.511, $F_{11,32} = 2.784$, $P = 0.012$). The presence of such interactions indicates that the rate at which ventilation frequency approaches basal levels depends on host body size (large fish take longer) and glochidia loads (more heavily encysted fish also take longer). Changes in ventilation frequency over time were well described by a logarithmic decay curve (Fig. 3, control trout $R^2 = 0.949$, $P < 0.001$; encysted trout, $R = 0.985$, $P < 0.001$) and indicated that trout encysted with glochidia took on average almost 6 h. longer to reach basal levels (predicted time = 941 min.) than control trout without glochidia (predicted time = 584 min.), despite having a similar mean body size ($t_{46.7}$,

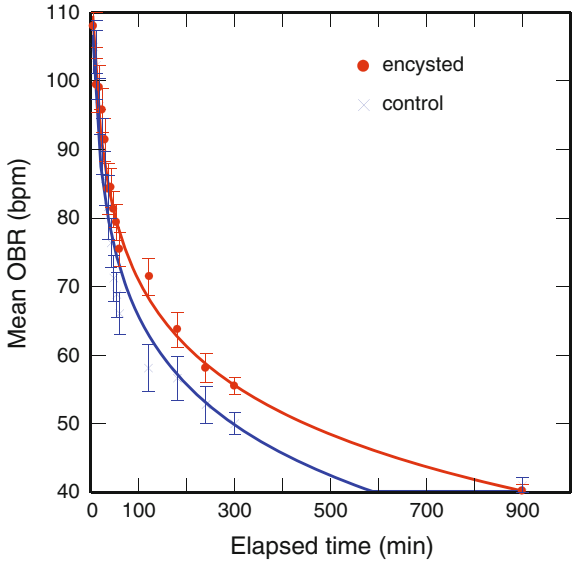

Fig. 3 Logarithmic decay function describing the time to reach basal ventilation rate (OBR, bpm) of glochidia-encysted and control trout following hand-netting (control trout, OBR = −14.54LogN (time) + 132.61, $R^2 = 0.949$, $P < 0.001$; encysted trout, OBR = −14.07LogN (time) + 136.33, $R^2 = 0.985$, $P < 0.001$)

$P = 0.622$). On the other hand, glochidia load (log transformed) had no statistical effect on basal ventilation rate 15 h after netting (glochidia effect $t = 0.090$, $P = 0.929$), once the effects of host body size had been accounted for (fork length effect $t = -8.229$, $P < 0.001$; multiple regression $F_{2,46} = 34.816$, $P < 0.00$). Large trout displayed lower basal ventilation rates than small trout, independently of glochidia load, and encysted trout showed elevated ventilation rates than controls at all times until they reached basal levels (Fig. 4).

Discussion

The results of this study, based on two different cohorts of juvenile brown trout, support the view that glochidia of the freshwater pearl mussel may impose some form of respiratory burden on their salmonid host, although other physiological effects are probably mild and transitory, and appear to peak at 1 month postexposure.

Thus, we failed to find any effect of glochidia on packed red blood cell counts, while spleen enlargement appears to have been short lived, possibly reflecting an immunological host response to antigenic material (Contamin et al., 2000; Morand & Poulin, 2000; Brown & Brown, 2002; Stanley & Engwerda, 2007; Cowan et al., 2009). Splenomegaly in brown trout was only observed after 1 month postexposure, when it was positively related to glochidia abundance. No reduction in relative spleen size was found, which would be indicative of stress (Kortet et al., 2003), therefore suggesting that the observed splenomegaly was likely a host immune response. Humoral and tissue reaction to *M. margaritifera* glochidia have previously been described in brown trout (Bauer, 1987a, b; Bauer & Vogel, 1987), and also in coho salmon (*Oncorhynchus kisutch*) encysted with *M. falcata* glochidia (Fustish & Millemann, 1978). In the bluegill sunfish, *Lepomis macrochirus*, a humoral and mucosal antibody response against glochidial antigens of *Utterbackia imbecillis* was found at 20 and 60 days postexposure (Rogers-Lowery et al., 2007). In previously challenged fish, anti-glochidial antibodies have been identified in host blood much sooner following a repeated glochidial challenge, indicating the existence of acquired immunity. For example, Bauer & Vogel (1987) noted the production of *M. margaritifera*-specific anti-glochidial antibodies in previously challenged brown trout as early as 7 days postexposure. The same results were obtained by O'Connell & Neves (1999), who detected anti-glochidia antibodies in previously exposed *Ambloplites rupestris* 7 days after a repeated challenge by glochidia of *Villosa iris*.

We found no significant effect of glochidiosis on trout haematocrit values 1 month postexposure. In a previous study, glochidia abundance was also found to be unrelated to salmonid host condition or plasma lactate levels (Treasurer et al., 2006). However, plasma chloride levels in glochidia-encysted juvenile salmon were found to be significantly higher 10 days after sea transfer (Treasurer & Turnbull, 2000), suggesting that glochidiosis may affect the ability of salmon to adapt to the marine environment.

In contrast, the respiratory system of brown trout appears to have been affected by glochidia encystment, resulting in some swelling and thickening of secondary gill lamellae, although this effect was localized around the cyst (as evidenced by the lack of swelling in unencysted lamellae). Gill parasites such as Unionid glochidia may be expected to have an impact on the host's respiration performance due to impaired gas exchange of encysted lamellae, as seen

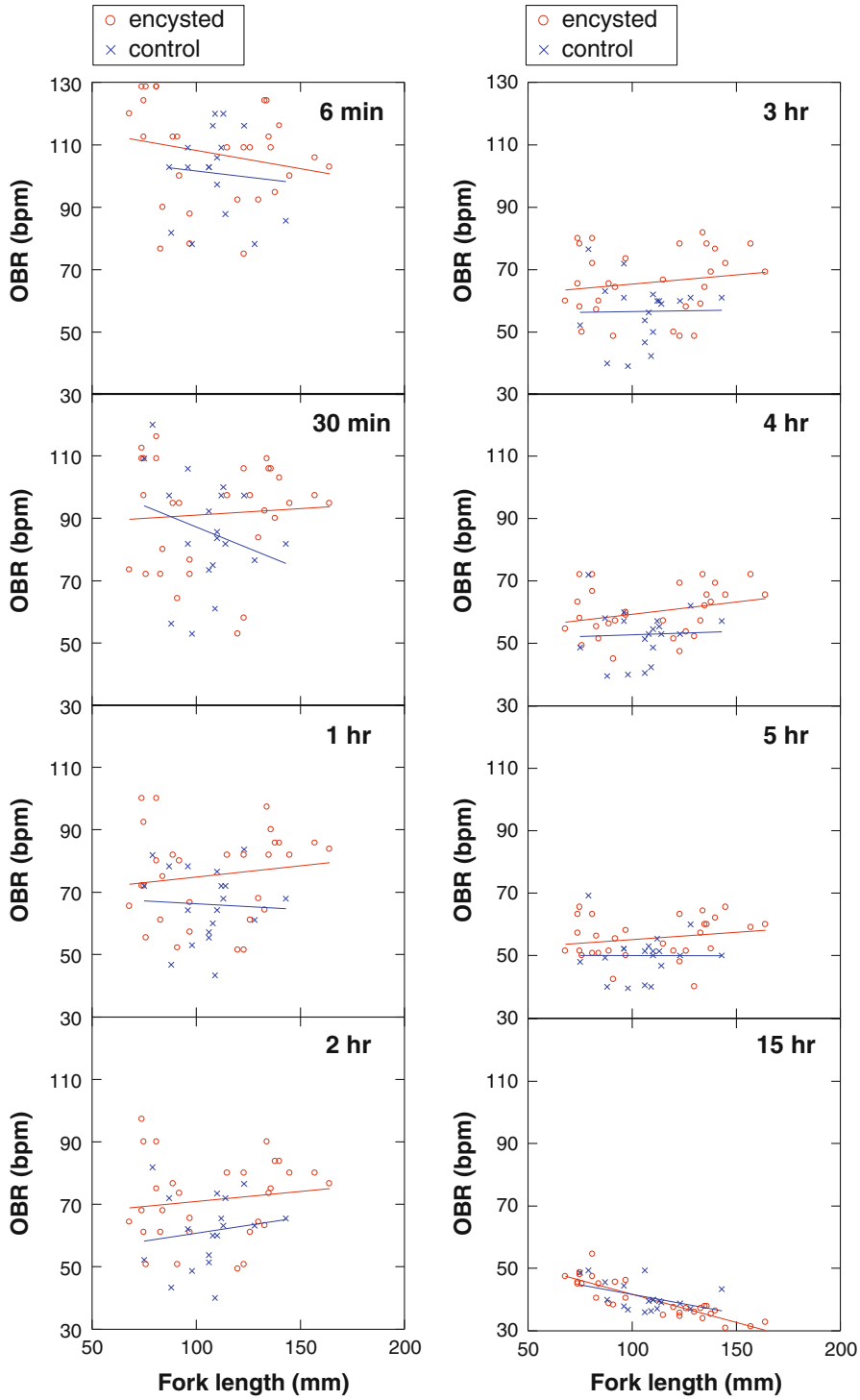

Fig. 4 Relationship between host body size (fork length, mm) and ventilation rate (OBR, bpm) of glochidia-encysted and control trout at various times following hand-netting. Basal ventilation rate was reached after c. 15 h

by the elevated ventilation frequency of *Micropterus salmoides* infected by glochidia of *Lampsilis reeveiana*, even several months postglochidial excystment (Kaiser, 2005). Although basal ventilation frequency was unrelated to glochidia loads in our study, encysted trout took almost 6 h longer than uninfected trout to

reach basal levels following netting. It would thus appear that, within the range of glochidia loads found in this study (1-204 glochidia per fish), glochidia encystment may have impaired the ability of trout to recover from hyperventilation in response to a mild stressor. A recent study (Kaiser, 2005) also found an impact of glochidiosis on host respiratory performance, though at much greater glochidia loads (mean 632 glochidia/fish) than those in our study (mean 37 glochidia/fish). Our results show, for the first time, that glochidia of *M. margaritifera* can impair the respiratory performance of its salmonid hosts even at low abundances.

The relationship between glochidia abundance and host body size is unclear. Although a positive association has been noted in salmonids (Young & Williams, 1984; Bauer & Vogel, 1987), lack of association (Cunjak & McGladdery, 1991), or even negative association between glochidia abundance and host size (Bauer, 1987b) have also been reported. However, many of the earlier studies did not discriminate between different age classes (i.e., 0+, 1+, etc.) of the fish hosts, which were segregated by body size alone. Therefore, it is likely that some of these contradictory results are probably due to acquired immunity caused by previous exposure of older fish, rather than by a genuine effect of host body size. In contrast, we found a positive relation between glochidia abundance and host body size based on fish of the same age (0+), which had never been in contact with glochidia, and which could not, therefore, have developed acquired immunity. This effect was also transitory and restricted to the initial stages of encystment, as found by others (Hastie & Young, 2001).

The pathogenicity of Unionid glochidia to their various hosts is not clear; whilst perhaps not truly pathogenic, the symbiosis-protocooperation explanation (Geist, 2010), or the phoretic description of this relationship (Barnhart et al., 2008) do not fully explain all the observed effects of glochidiosis. The transitory spleen enlargement observed in this study, along with the observed temporal changes in the effect of host body size on glochidia abundance, and the acquired immunity reported by others, strongly suggest that the impacts of glochidia on the hosts are slight. Results for *M. margaritifera* and other freshwater mussels (Fustish & Millemann, 1978; Bauer, 1987b; Bauer & Vogel, 1987; O'Connell & Neves, 1999; Rogers-Lowery et al., 2007) suggest that there is an advantage to be gained from shedding glochidia, at least during the initial period of encystment, thereby providing a strong argument against the relationship being phoretic or a form of symbiosis-protocooperation. However, our study—as well as that of Treasurer et al. (2006), indicates that glochidia have little or no impact on the hosts' haematological condition, at least within the range of glochidia numbers commonly seen in the wild. However, the effects of glochidiosis on respiratory performance, measured here as the ability to recover from hyperventilation, appear more significant. Taken together, our results indicate that although glochidia of *M. margaritifera* may have a small and transitory effect on salmonid physiology, they may have an effect on respiratory performance and that this may compromise the capacity of the host to recover from even a mild form of stress. Such an impact is perhaps unexpected from a single-host parasite whose fitness depends on that of its host. However, given that individual salmonid fish can differ greatly in parasite resistance (Consuegra & Garcia de Leaniz, 2008), it is possible that freshwater pearl mussels might produce glochidia of varying virulence as a bet hedging strategy to infect hosts of varying immune-competence (Fenton & Hudson, 2002). If so, some degree of host–parasite mismatch might be expected, and some impacts on the host might become inevitable.

The results of our study have several implications for the conservation and artificial propagation of the critically endangered freshwater pearl mussel. Firstly, glochidia load was found to increase with host body size during the initial stages of encystment, suggesting that glochidia attachment is, at least initially, probably a function of surface area, as reported for other fish (Poulin, 2000). Captive breeding programmes for freshwater mussels usually aim for high encystment rates (Thomas et al., 2010), and our study suggests that it may be beneficial to select the largest fish as hosts. However, as no relationship existed between body size and glochidia loads at 160 and 167 days postexposure, this may indicate that large trout are more efficient at shedding glochidia than small ones. A potential trade-off may therefore exist between encystment rates and transformation success, and this would merit further study.

The observed relationship between glochidia load and ventilation rates has also conservation implications. Encysted trout and control trout showed the same size-adjusted basal ventilation rate, but trout

encysted with glochidia took significantly longer to reach basal ventilation levels than controls. Moreover, the existence of an interaction between elapsed time and glochidia load indicates that the effect of glochidia is cumulative, and that the more glochidia trout have the longer it takes for them to recover from even a mild form of stress. The fitness implications of these findings are unclear, but it is possible that encysted trout may be more susceptible to stress or perhaps even more vulnerable to predation if released into the wild (Poulin, 1995).

Artificial encystment typically results in glochidia loads many times higher than those commonly found in the wild (Karna & Millemann, 1978; Hruska, 2001), and although loads up to 800 glochidia per fish result in little ($\sim$1%) or no mortality (Hastie & Young, 2001, 2003; Preston et al., 2007) our results suggest that they may affect fish hosts in other, more subtle ways. If so, aiming for high glochidia loads may not be the best option for mussel propagation programmes if this compromises host fitness, and thus the probability that glochidia will survive until excystment.

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References

Abramoff, M. D., P. J. Magelhaes & S. J. Ram, 2004. Image processing with Image J. Biophotonics International 11: 36–42.

Araujo, R. & M. A. Ramos, 1998. Description of the glochidium of *Margaritifera auricularia* (Spengler, 1793) (Bivalvia, Unionidae). Philosophical Transactions Royal Society London Series B 353: 1553–1559.

Araujo, R., N. Camara & M. A. Ramos, 2002. Glochidium metamorphosis in the endangered freshwater mussel *Margaritifera auricularia* (Spengler, 1793): a histological and scanning electron microscopy study. Journal of Morphology 254: 259–265.

Barnhart, M. C., W. R. Haag & W. N. Roston, 2008. Adaptations to host infection and larval parasitism in Unionoida. Journal of the North American Benthological Society 27: 370–394.

Bauer, G., 1987a. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.). II. Susceptibility of brown trout. Archiv für. Hydrobiologie 76(4): 403–412.

Bauer, G., 1987b. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.). III. Host relationships. Archiv für Hydrobiologie 76: 413–423.

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.

Brown, C. R. & M. B. Brown, 2002. Spleen volume varies with colony size and parasite load in a colonial bird. Proceedings of the Royal Society of London B 269: 1367–1373.

Brydges, N. M., P. Boulcott, T. Ellis & V. A. Braithwaite, 2008. Quantifying stress responses induced by different handling methods in three species of fish. Applied Animal Behaviour Science 116: 295–301.

Consuegra, S. & C. Garcia de Leaniz, 2008. MHC-mediated mate choice increases parasite resistance in salmon. Proceedings of the Royal Society B: Biological Sciences 275: 1397–1403.

Contamin, H., C. Behrb, O. Mercereau-Puijalonb & J.-C. Michela, 2000. *Plasmodium falciparum* in the squirrel monkey (*Saimiri sciureus*): infection of nonsplenectomised animals as a model for exploring clinical manifestations of malaria. Microbes and Infection 2: 945–954.

Cowan, K. M., D. Shutler, T. B. Herman & D. T. Stewart, 2009. Splenic mass of masked shrews, *Sorex cinereus*, in relation to body mass, sex, age, day of the year, and bladder nematode, *Liniscus* (=*Capillaria*) *maseri*, infection. Journal of Parasitology 95: 228–230.

Crane, A. L., A. K. Fritts, A. Mathis, J. C. Lisek & M. C. Barnhart, 2011. Do gill parasites influence the foraging and antipredator behaviour of rainbow darters, *Etheostoma caeruleum*? Animal Behaviour 82: 817–823.

Cunjak, R. A. & S. E. McGladdery, 1991. The parasite-host relationship of glochidia (Mollusca: Margaritiferidae) on the gills of young-of-the-year Atlantic salmon (*Salmo salar*). Canadian Journal of Zoology 69: 353–358.

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

Dodd, B. J., M. C. Barnhart, C. L. Rogers-Lowery, T. B. Fobian & R. V. Dimock Jr, 2006. Persistence of host response against glochidia larvae in *Micropterus salmoides*. Fish and Shellfish Immunology 21: 473–484.

Fenton, A. & P. J. Hudson, 2002. Optimal infection strategies: should macroparasites hedge their bets? Oikos 96: 92–101.

Fustish, C. A. & R. E. Millemann, 1978. Glochidiosis of salmonid fishes. II. Comparison of tissue response of coho and Chinook salmon to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae). Journal of Parasitology 64: 155–177.

Geist, J., 2010. Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera* L.): a synthesis of conservation genetics and ecology. Hydrobiologia 644: 69–88.

Hastie, L. C. & M. R. Young, 2001. Freshwater pearl mussel (*Margaritifera margaritifera*) glochidiosis in wild and farmed salmonid stocks in Scotland. Hydrobiologia 445: 109–119.

Hastie, L. C. & M. R. Young, 2003. Conservation of the freshwater pearl mussel I: captive breeding techniques. Conserving Natura 2000 Rivers Ecology Series No. 2, English Nature, Peterborough.

Hawkins, L. A., J. D. Armstrong & A. E. Magurran, 2004. Predator-induced hyperventilation in wild and hatchery Atlantic salmon fry. Journal of Fish Biology 65: 88–100.

Humason, G. L., 1979. Animal Tissue Techniques (4th ed). W.H. Freeman & Co, San Francisco.

Hruska, J., 2001. Experience of Semi-natural Breeding Programme of Freshwater Pearl Mussels in the Czech Republic. In Anon (ed.), The Freshwater Pearl Mussel in Europe: Population Status and Conservation Strategies. Freiburg: 69–75.

Jones, C. M. & A. S. Grutter, 2005. Parasitic isopods (*Gnathia* sp.) reduce haematocrit in captive blackeye thicklip (Labridae) on the Great Barrier Reef. Journal of Fish Biology 66: 860–864.

Kaiser, B. E., 2005. The Effects of Glochidiosis on Fish Respiration. Unpublished MSc thesis, Missouri State University, USA. http://www.worldcat.org/title/effects-of-glochidiosis-on-fish-respiration/oclc/066473455.

Karna, D. W. & R. E. Millemann, 1978. Glochidiosis of salmonid fishes III. Comparative susceptibility to natural infection with *Margaritifera margaritifera* (L.) (Pelycypods: Margaritanidae) and associated histopathology. Journal of Parasitology 64: 528–537.

Kortet, R., J. Taskinen, T. Sinisalo & I. Jokinen, 2003. Breeding-related seasonal changes in immunocompetence, health state and condition of the cyprinid fish, *Rutilus rutilus*, L. Biological Journal of the Linnean Society 78: 117–127.

Lillie, R. D., 1965. Histopathologic Technic and Practical Histochemistry, 3rd ed. McGraw-Hill Book Co., New York.

Lydeard, C., R. H. Cowie, W. F. Ponder, A. E. Bogan, P. Bouchet, S. A. Clark, K. S. Cummings, T. J. Frest, O. Gargominy, D. G. Herbert, R. Hershler, K. E. Perez, B. Roth, M. Seddon, E. E. Strong & F. G. Thompson, 2004. The global decline of nonmarine molluscs. BioScience 54: 321–330.

Manning, M. J., 1994. Fishes. In Turner, R. J. (ed.), Immunology: A Comparative Approach. John Wiley, Chichester: 69–100.

Meyers, T. R. & R. E. Millemann, 1977. Glochidiosis of salmonid fishes. I. Comparative susceptibility to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae). Journal of Parasitology 63: 728–733.

Meyers, T. R., R. E. Millemann & C. A. Fustish, 1980. Glochidiosis of salmonid fishes IV. Humoral and tissue responses of coho and Chinook salmon to experimental infection with *Margaritifera margaritifera* (L.) (Pelycypoda: Margaritanidae). Journal of Parasitology 66: 274–281.

Millidine, K. J., N. B. Metcalfe & J. D. Armstrong, 2008. The use of ventilation frequency as an accurate indicator of metabolic rate in juvenile Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 65: 2081–2087.

Morand, S. & R. Poulin, 2000. Nematode parasite species richness and the evolution of spleen size in birds. Canadian Journal of Zoology 78: 1356–1360.

Nowak, B. & S. LaPatra, 2006. Epitheliocystis in fish. Journal of Fish Diseases 29: 573–588.

O'Connell, M. T. & R. J. Neves, 1999. Evidence of immunological responses by a host fish (*Ambloplites rupestris*) and two non-host fishes (*Cyprinus carpio* and *Carassius auratus*) to glochidia of a freshwater mussel (*Villosa iris*). Journal of Freshwater Ecology 14: 71–78.

Paperna, I., 1984. Chemical control of *Amyloodinium ocellatum* (Brown 1931) (Dinoflagellida) infections: in vitro tests and treatment trials with infected fishes. Aquaculture 38: 1–18.

Poulin, R., 1995. "Adaptive" changes in the behaviour of parasitized animals: a critical review. International Journal of Parasitology 25: 1371–1383.

Poulin, R., 2000. Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. Journal of Fish Biology 56: 123–137.

Preston, S. J., A. Keys & D. Roberts, 2007. Culturing freshwater pearl mussel *Margaritifera margaritifera*: a breakthrough in the conservation of an endangered species. Aquatic Conservation Marine and Freshwater Ecosystems 17: 539–549.

Rogers-Lowery, C. L., R. V. Dimock Jr. & R. E. Kuhn, 2007. Antibody response of bluegill sunfish during development of acquired resistance against the larvae of the freshwater mussel *Utterbackia imbecillis*. Developmental and Comparative Immunology 31: 143–155.

Rogers, C. L. & R. V. Dimock Jr., 2003. Acquired resistance of bluegill sunfish *Lepomis macrochirus* to glochidia larvae of the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) following multiple infections. Journal of Parasitology 89: 51–56.

Roberts, L. J., J. Taylor & C. Garcia de Leaniz, 2011. Environmental enrichment reduces maladaptive risk-taking behavior in salmon reared for conservation. Biological Conservation 144: 1972–1979.

Rowley, A. F., T. C. Hunt, M. Page & G. Mainwaring, 1999. Fish. In Rowley, A. F. & N. A. Ratcliffe (eds), Vertebrate Blood Cells. Cambridge University Press, Cambridge: 18–127.

Seppänen, E., H. Kuukka, A. Voutilainen, H. Huuskonen & N. Peuhkuri, 2009. Metabolic depression and spleen and liver enlargement in juvenile Arctic charr *Salvelinus alpinus* exposed to chronic parasite infection. Journal of Fish Biology 74: 553–561.

Stanley, A. C. & C. R. Engwerda, 2007. Balancing immunity and pathology in visceral leishmaniasis. Immunology and Cell Biology 85: 138–147.

Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton & S. J. Nichols, 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. BioScience 54: 429–439.

Thomas, G. R., J. Taylor & C. Garcia de Leaniz, 2010. Captive breeding of the endangered freshwater pearl mussel *Margaritifera margaritifera*. Endangered Species Research 12: 1–9.

Treasurer, J. W. & T. Turnbull, 2000. The pathology and seawater performance of farmed Atlantic salmon infected with glochidia of *Margaritifera margaritifera*. Journal of Fish Biology 57: 858–866.

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, 2000. Larval Types and Early Postlarval Biology in Naiads (Unionoida). In Bauer, G. & K. Wächtler (eds), Ecology and Evolution of the Freshwater Mussels Unionoida. Springer, New York, NY: 93–125.

Woo, P. T., 1969. The haematocrit centrifuge for the detection of trypanosomes in blood. Canadian Journal of Zoology 47: 921–923.

Yamamoto, K., 1987. Contraction of spleen in exercised cyprinid. Comparative Biochemistry and Physiology A 87: 1087–1093.

Young, M. R. & J. C. Williams, 1984. The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* (Linn.) in ScotlandI. Field studies. Archiv für Hydrobiologie 99: 405–422.

Ziuganov, V. V. & L. P. Nezlin, 1988. Evolutionary Aspects of Symbiosis of Pearl Mussels and Salmonid Fishes. In: The problems of macroevolution. Moscov. Nauka: 110–111.