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RESEARCH

Parasitic freshwater mussels impact the shoaling behaviour and habitat use of their host fish

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Abstract
Freshwater mussels in the order Unionida are a highly endangered taxon of ecosystem engineers with a variety of conservation and reintroduction efforts around the world attempting to increase wild populations. The unionid lifecycle involves a parasitic larval life-stage (glochidia), primarily adapted for dispersal rather than growth. Little is known about the behavioural effects of the glochidia on their host fish. We investigated the shoaling behaviour and habitat preference of the Eurasian minnow (*Phoxinus phoxinus*) while infested with the glochidia of one of Europe's most endangered unionid mussels, the thick-shelled river mussel (*Unio crassus*). Behavioural assays were carried out in a hydrologically complex flow-through aquaria at two time points of the infestation period, a carrying stage and an excystment stage (14 and 28 days post infestation). We demonstrate that glochidiosis reduces minnow shoaling frequency and increases the number of isolated individuals. However, minnow shoal area and nearest neighbour distance remained unaffected. Infested minnows demonstrated a significant preference for slower flowing and less turbulent habitat. These results suggest that glochidiosis reduces host swimming efficiency, which likely increases predatory pressure on infested minnows. Simultaneously, this preference for calmer habitats may benefit the encysted mussels, as these habitats are better suited for juvenile mussel excystment. Our results highlight the importance of low-flow and low-turbulence habitats for unionid recruitment, as parasitized host individuals tolerate these conditions well.

Significance statement
Unionid mussels are an endangered order of parasitic bivalves with a variety of effects on both their hosts and the ecosystem more broadly. While there are many conservation efforts for this order, there are few studies on the ecological impacts of parasitism from unionids on host ecology and behaviour. To address this, we filmed artificially infested hosts in hydrologically complex aquaria to assess the effects of unionid infestation on the shoaling and swimming behaviour of the Eurasian minnow. We demonstrate that parasitized hosts shoaled less frequently but did not alter overall shoal morphology. Additionally, we demonstrate that parasitized hosts prefer slower flowing and less turbulent water. Our results indicate that unionids may manipulate host behaviour and alter host habitat preference to habitats well-suited for excysting juvenile mussels, highlighting the importance of restoring habitat when engaging inunionid conservation.

Keywords Cyprinidae · Unionidae · *Phoxinus phoxinus* · *Unio crassus* · Endangered species · Conservation

Introduction

Social behaviour is common across the animal kingdom (Avilés 1999; Silk 2007). A principal advantage of such behaviour is the reduced risk of predation, an effect which can occur through a variety of mechanisms such as the dilution effect (Foster and Treherne 1981), the many eyes effect (Lima 1995) or the confusion effect (Landeau and Terborgh 1986). The costs and benefits of sociality have been particularly well studied in fish, as most species spend at least a

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part of their life in shoals, forming either loosely aggregated swarms or polarized and synchronous schools, though these terms are often used interchangeably (Godin 1986; Pitcher 1993; Delcourt and Poncin 2012). Shoal formation and function are highly species dependent, with some fish only shoaling as juveniles or for mating, whereas others shoal for the majority their life. In all cases, individuals must coordinate to form functional social groups (Sirot and Touzalin 2009; Bartashevich et al. 2024).

Individual coordination between fish in a shoal can be influenced by a variety of biotic and abiotic factors such as individual health and the surrounding environment, which in combination can create complex sociobiological interactions with dynamic ecological consequences (Barber et al. 2000; Kasumyan and Pavlov 2023). Generally, fish have increased stamina and swimming performance when shoaling (Ligman et al. 2023). Fluvial fish species are subjected to highly variable flow regimes, both over time and space, which can alter their propensity to shoal in accordance to the initial function of a species' social behaviour (Garner 1997; Allouche and Gaudin 2001; Sneddon et al. 2006; Chicoli et al. 2014; Hockley et al. 2014). However, shoaling exposes an individual to a higher risk of parasite transmission from con-specifics (Côté and Poulin 1995; Reynolds et al. 2019; Walsman et al. 2022). As parasitized individuals typically experience reduced swimming efficiency (Barber et al. 2000), they may become more social to take advantage of the increased swimming efficiency provided by a shoal (Petkova et al. 2018; Ligman et al. 2023). On the other hand, non-parasitized individuals may avoid shoaling with parasitized conspecifics to avoid contracting an infection, particularly in the case of directly transmitted parasites where the rate of parasitism increases with flow velocity (Reynolds et al. 2019), often resulting in infested individuals being positioned at greater distance from their nearest neighbours and on the shoal periphery (Barber and Huntingford 1996; Tobler and Schlupp 2008; Krkošek et al. 2011; Hockley et al. 2014). As fish can rapidly transfer information about the environment to other members in the shoal, parasites can disrupt the anti-predator advantages of shoaling by reducing shoal cohesion (De Bie et al. 2020; Demandt et al. 2020; Miles et al. 2024).

Unionid mussels are a unique order of monoxenous parasites which spend the vast proportion of their life (decades to centuries) as free-living adults with a comparatively short parasitic period (days to months; Strayer 2008). Adult males release gametes into the water column which are filtered by females and used for internal fertilization. Larval mussels (glochidia) are brooded on marsupial pouches and later released, either directly onto a host with the use of host attraction mechanisms or loosely into the water column where they affix onto a host, typically the gills of fish, inducing glochidiosis. After a period of encystment and metamorphosis, juvenile mussels excyst and fall to the sediment to then develop into adults (Strayer 2008). In this system, parasites are not transmitted between host individuals, as glochidia are primarily adapted to be a dispersal vector for the adult life stage, typically growing very little while on the host despite some nutrient extraction (Taeubert et al. 2012a; Denic et al. 2015). As such, glochidia do not gain a fitness advantage by increasing the risk of predation for their hosts. Generally, glochidiosis causes limited impacts to host fish, though lower growth rates and osmotic potential, and increases to metabolic rate and mortality are observed at higher infestation rates (Douda et al. 2017; Filipsson et al. 2017; Chowdhury et al. 2019; Rock et al. 2022). Glochidiosis can additionally alter host feeding rates, swimming performance, habitat use and social interactions (Crane et al. 2011; Österling et al. 2014; Filipsson et al. 2016; Horký et al. 2019; Rock et al. 2025a, b).

Unionid mussels are highly endangered, in part due to their complex parasitic life cycle reliant on host fish, themselves also generally endangered (Strayer et al. 2004; Dudgeon et al. 2006; Lopes-Lima et al. 2017; Modesto et al. 2018). For example, the thick-shelled river mussel (*Unio crassus*), one of Europe's most endangered species has experienced an estimated 50% population decline in the past half century alone (Lopes-Lima et al. 2017). As adult unionids have a variety of well-described ecosystem functions there is noteworthy global interest in their conservation, typically in the form of breeding projects and habitat restoration (Strayer et al. 2004; Vaughn et al. 2008; Lopes-Lima et al. 2014; Vaughn and Hoellein 2018). Despite these broad conservation efforts, little is known about the impact of glochidiosis on host fish behaviour and ecology, an essential aspect of unionid reproductive biology (Rock et al. 2022).

We tested the impact of glochidiosis from the thick-shelled river mussel on the shoaling behaviour and habitat preference of the Eurasian minnow (*Phoxinus phoxinus*), a common host, in a flow-through stream aquarium with a complex hydrodynamic profile. As glochidia are not a directly transmissible parasite between fish, and do not gain a fitness benefit from decreasing shoal cohesion, we predict that minnow shoaling frequency (proportion of time spent in large groups) and morphology (size of shoal) should remain unchanged under glochidiosis. Hosts suffering from glochidiosis typically experience impaired swimming performance, an effect which is more highly expressed at higher infestation levels and during the period of excystment (Taeubert and Geist 2013; Filipsson et al. 2017; Slavík et al. 2017; Rock et al. 2022), and we therefore further predict that infested minnows should prefer slower flowing and less turbulent water more than their non-parasitized counterparts.

Methods

Animal husbandry

On May 5, 2021, approximately 600 minnows (47 ± 11 mm; mean ± SD) were electrofished from the river Vramsån (55°56'14.3"N 13°56'43.5"E) and transported to the Karlstad University aquarium facility, where they were housed in two 600 L holding tanks, fitted with mesh lids and floating aquaponics filter material for enrichment. Tank water was filtered with two large canister filters (EHEIM classic 1500 XL 2260) fitted with EHEIM universal 2400 return pumps. Water output from the filter was fed through a custom-made chiller system built into the facility to maintain the water at 14 °C. The aquarium facility light regime approximated the natural environment with a 14:10 light: dark regime. Minnows were fed to satiation on a daily basis with frozen red chironomid larvae. 50% water changes were performed weekly to maintain high water quality.

On June 6, 2021, fourteen gravid mussels were collected from the river Tommarpsån (55°32'57.4"N 14°08'16.0"E) and brought to the Karlstad University aquarium facility. Gravidity was determined by carefully opening the mussels with specialized tongs and visually inspecting the gill flaps for marsupial pouches (Beaver et al. 2019). Mussels were kept in four well-aerated 9 L plastic aquaria and maintained at 16 C° with a 14:10 light: dark regime in their native water. Daily water changes with increasing levels of lab-made water (RODI water, re-ionized to match native water parameters) were performed to slowly acclimate the mussels to lab conditions. On June 17, 2021 several mussels in one aquarium released glochidia which were used for the infestation procedure. Mussels were returned to their home stretch in the river Tommarpsån after glochidia release.

The number of viable glochidia (~26 000 glochidia) was determined by concentrating the glochidia into 500 ml of water, and then counting the number of glochidia in a 0.5 ml drop under a stereo microscope and then adding a few grains of table salt (NaCl) to initiate a characteristic 'snapping' stress reaction; this differentiated dead and living glochidia. All minnows in one of the two holding tanks were infested with living glochidia. The minnows from this tank were placed for 45 min into a heavily aerated 70 L infestation chamber which contained a concentration of 500 glochidia per L. After the infestation protocol, minnows were returned to their holding tank. Control minnow were subjected to a sham treatment by placing them into a similarly aerated 70 L infestation chamber (without added glochidia) for 45 min, in a procedure similar to Taeubert et al. (2012b). At 14 days post infestation (dpi), 100% of glochidia-treated minnows retained 6 ± 4 glochidia per fish (gl/f; mean ± SD), at 28 dpi, 69% of glochidia-treated minnows retained 2 ± 1 gl/f. The infestation rates used here are comparable to natural levels but can be an order of magnitude higher in laboratory conditions (Taeubert et al. 2012a; Lamand et al. 2016; Schneider et al. 2017).

Characterizing flow regimes

Flow velocity (m/s) and turbulent kinetic energy (TKE; m^2/s^2) within the flow-through tanks were modelled in 2 dimensions using the commercial computational fluid dynamics (CFD) software package ANSYS Fluent (2022 R1). Fluent is a general-purpose fluid dynamics simulator, commonly used in ecohydraulic studies (Andersson 2012; Marriner et al. 2016; Stamou et al. 2018), adopting a finite volume discretization method to solve the equations of motion. For these simulations, the Reynolds-Averaged Navier-Stokes equations (RANS) were solved, applying a viscous Shear Stress Transport k-ω model (SST k-ω) to close the flow turbulence rather than solving the equation directly (Tarena et al. 2025). A 2D planar transient pressure-based solver was used, with a SIMPLE (Semi-Implicit Method for Pressure-Linked Equations) scheme to solve the Pressure-Velocity coupling and a Second-Order-Upwind scheme for viscous and convective terms of the governing equations. An unstructured hexahedral mesh was generated using the ANSYS Workbench meshing tool. The average flow velocity of 0.5 m/s (measured at the outflow of the flume with an OTT HydroMet current meter, Z400, Germany) was used as boundary condition at the inlet, while a pressure outlet was imposed at the exit of the model. A no-slip lateral boundary condition was imposed to the walls. Numerical model validation was performed after the simulations, comparing the obtained flow field's velocity values in various parts of the flume with the ones measured in the actual flume. The test arena was subdivided into 100 rectangular sections measuring 17 cm × 9.8 cm, within which values for flow velocity and TKE were averaged.

Experimental procedure

Four identical flow-through tanks with maximum internal dimensions of 170 × 98 cm, filled with 10 cm of water and a layer of sand on the bottom were used for the behavioural analysis. These flow-through tanks provided a range of habitat conditions for the minnows to inhabit, with varying flow and turbulence characteristics (Fig. 1). Two behavioural analysis protocols were performed on the minnows at 14 and 28 dpi, corresponding to the periods of glochidia cyst maintenance and juvenile mussel excystment, respectively (Schneider et al. 2018). Six minnows chosen at random from

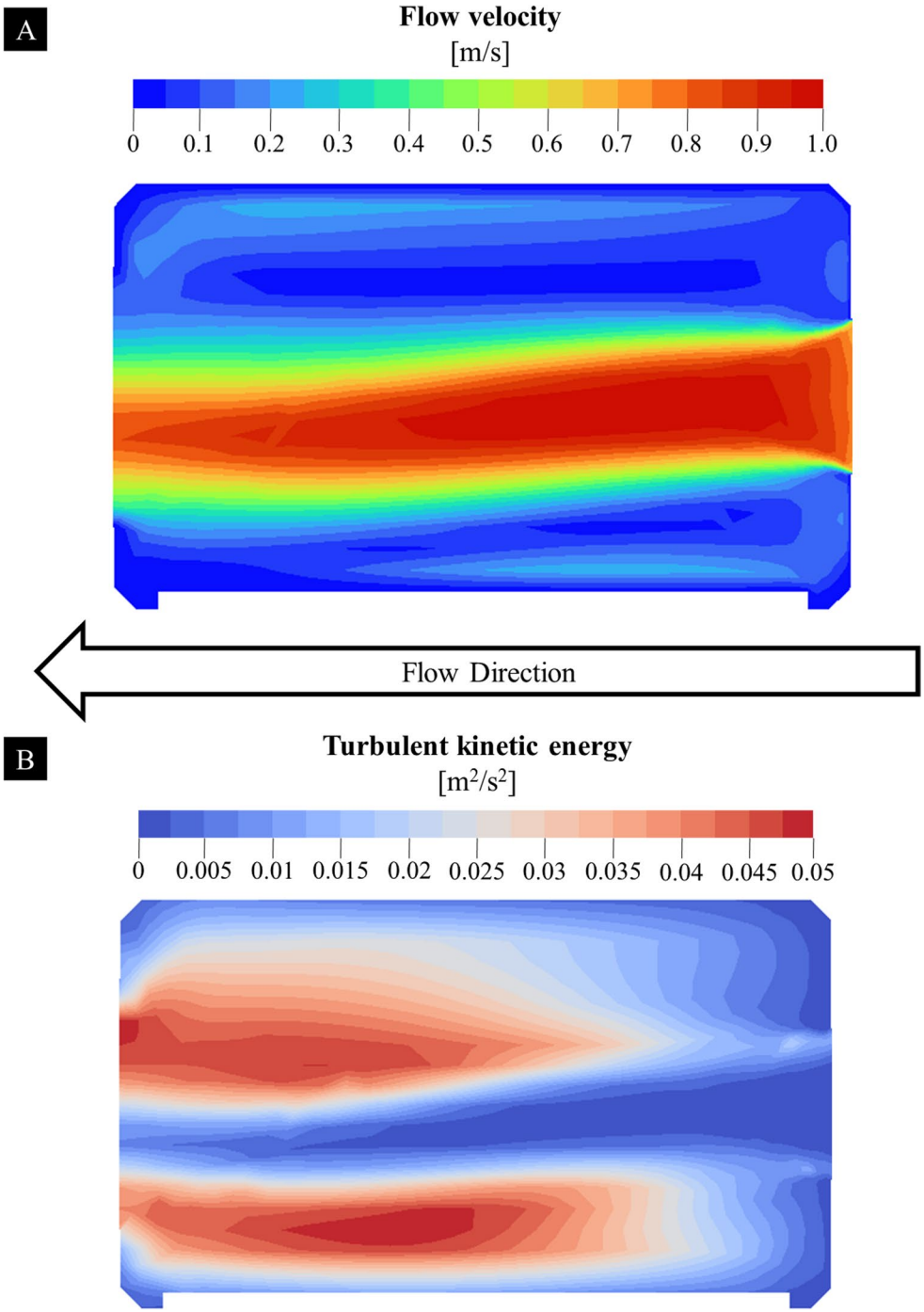

Fig. 1 Heat map indicating the 2-dimentional variation of flow velocity (**A**) and turbulent kinetic energy (**B**) within the experimental area from a top-down prospective, generated by the commercial Computational Fluid Dynamics (CFD) software package ANSYS in Fluent (2022 R1). Maximum internal arena dimensions measure 170 cm × 98 cm. Indentation on the bottom edge of the heatmap indicates a viewing panel into the arena

either infested or non-infested groups ($n = 192$ individuals in total), were placed in each flow-through tank and recorded from above with either a Canon XA10 or XA11 for 60 min. After each behavioural protocol, minnows were euthanized with a benzocaine overdose and stored in ethanol to assess minnow size and glochidia infestation intensity. A total of eight trials were run for each infestation category at both time points.

Data collection and analysis

Data on minnow behaviour was investigated by extracting ten frames from the video recordings. After a 30-min acclimation period, the first frame where the position of all six minnows could be determined marked the start of data.

collection; every 75 s (± 15 s based on fish visibility) one frame was extracted, for a maximum of 10 observations, and the position of each minnow marked (variation in frame

collection was necessary to account for poor visibility in some video recordings). Saved frames were uploaded to ImageJ (version: v1.53e) where several metrics for minnow shoaling behaviour were measured, calibrated with the internal dimensions of the experimental arena. The nearest neighbour distance (NND) of all minnows (average minnow NND) was measured first to allow for further metrics to be collected. A group of minnows was determined to be forming a shoal (Yes/No) if four or more individuals were within four average body lengths of their nearest neighbour (188 mm; Pitcher 1983). The number of isolated minnows, defined as an individual beyond 188 mm from its nearest neighbour, was also counted. From here, the NND of shoaling individuals (individuals within 188 mm of their NN) and shoal area were calculated, additionally, the area of all minnows (minnow area) was calculated. Both metrics of area were measured as the area of the convex polygon delineated by the peripheral members (Barber and Huntingford 1996). The position of each minnow within the experimental arena was determined by overlaying a grid pattern (cell size = 17 × 9.8 cm; L × H) over the frame and assessing into which section the minnow fell. This was used to assign flow velocity and TKE values to individual minnows. Average flow velocity and TKE of the shoal were calculated by averaging the flow velocity and TKE values of the shoaling individuals. It was not possible to record data blind because we placed tags on aquaria, viewable in-frame, to identify infested and non-infested recordings.

Unless otherwise specified, minnow behaviour data was averaged across every trial to provide one value, which were then analysed with a 2-way ANOVA using glochidia presence (Yes/No), days post infestation (14 or 28 dpi) and the interaction glochidia presence × days post infestation as predictors. Shoal area was analysed with a 2-way ANCOVA using glochidia presence (Yes/No), days post infestation (14 or 28 dpi) and the interaction glochidia presence × days post infestation as predictors, including the average number of minnows in the shoal was included as a covariate to account for different shoal sizes. A GLMM with binominal error distribution was fitted to the shoaling presence data (Yes/No) to determine shoaling frequency, using glochidia presence (Yes/No), days post infestation (14 or 28 dpi) and the interaction glochidia presence × days post infestation as predictors; trial ID was used as a random factor. A GLMM with Poisson error distribution was fitted to the count data of isolated minnows using glochidia presence (Yes/No), days post infestation (14 or 28 dpi) and the interaction glochidia presence × days post infestation as predictors; trial ID was used as a random factor.

Results

Shoal morphology

Infested minnows shoaled significantly less frequently than their non-infested counterparts (Fig. 2a) and had significantly more isolated individuals (Fig. 2b), which resulted in a significantly increased average minnow area (Table 1; Fig. 2c). Despite these differences, the average infested minnow NND did not differ from that of non-infested minnows (Table 1; Fig. 2d). Infested minnow shoal area and shoal NND did not differ significantly from those of non-infested minnows (Table 1; Fig. 2e, f).

Generally, minnows shoaled significantly more frequently at 28 dpi than at 14 dpi, however, there was no significant interaction effect between glochidia presence and days post infestation, indicating that the difference between infestation treatments persisted through time (Table 1; Fig. 2a). The number of isolated individuals and the average minnow area did not differ significantly over time, nor produced a significant interaction effect, indicating that the effect of glochidiosis was consistent through time (Table 1; Fig. 2b, c). All other metrics of shoal morphology did not differ significantly over time, and none of the models demonstrated a significant interaction effect between glochidia presence × days post infestation (Table 1; Fig. 2d-f).

Minnow habitat preference

On average infested minnows demonstrated a preference for significantly slower flowing water compared to their non-infested counterparts, a difference which did not vary significantly through time (Table 2; Fig. 3a). Similarly, shoals of infested minnows demonstrated a preference for significantly slower flowing water at 14 dpi, but did not differ from their non-infested counterparts at 28 dpi, demonstrated by a significant interaction term. (Table 2; Fig. 3b). Infested minnows demonstrated a near significant preference for less turbulent water on average (Table 2; Fig. 3c) and a significant preference for less turbulent water when shoaling (Table 2; Fig. 3d).

Discussion

Our study aimed to investigate the impact of glochidiosis on the shoaling behaviour and habitat preference of the Eurasian minnow. The results support our hypothesis that glochidiosis would have no impact on shoal morphology, but did not support our hypothesis that glochidiosis would have no impact on shoaling frequency. Additionally, we show

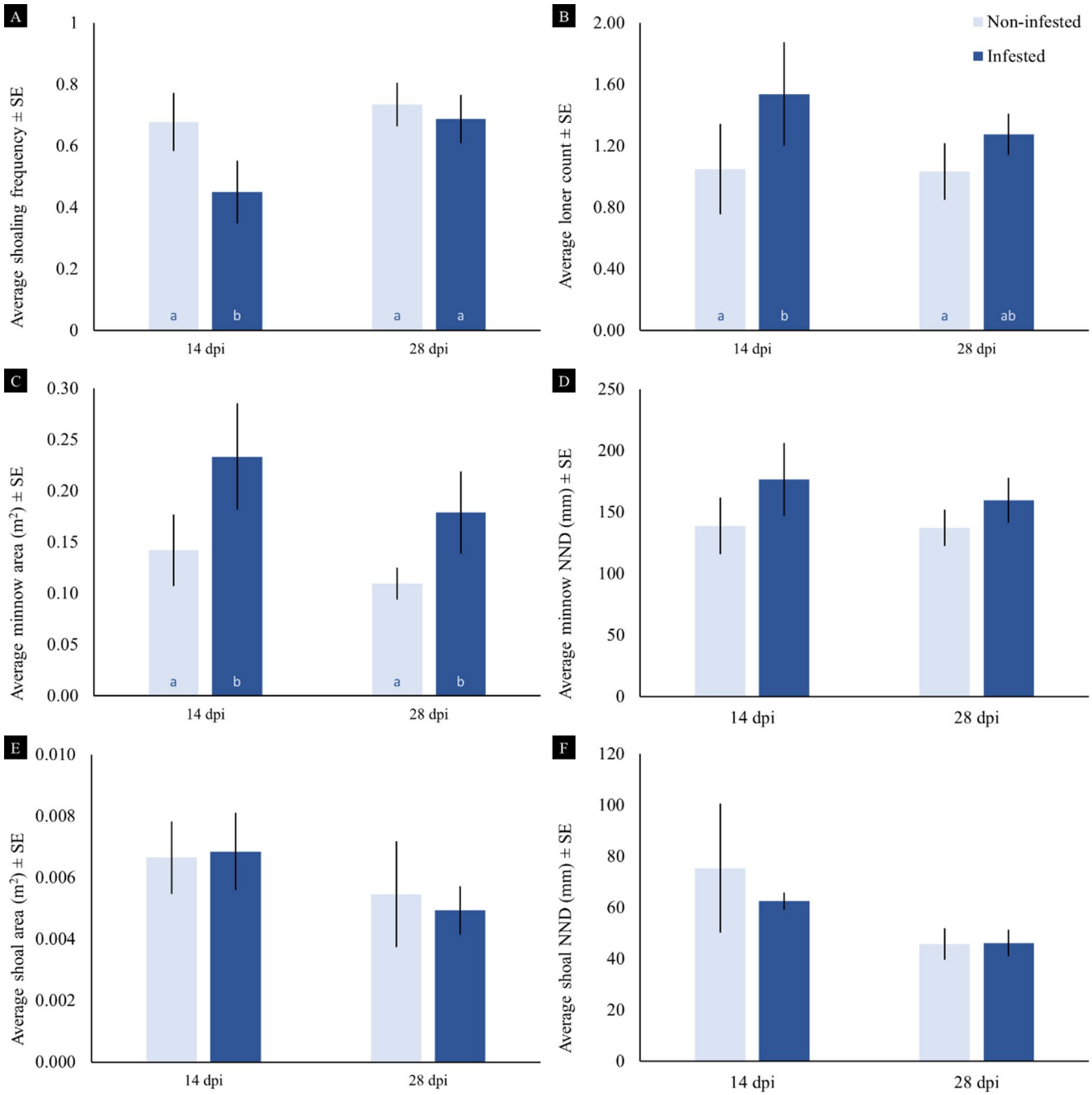

Fig. 2 Measures qualifying shoaling behaviour of infested and non-infested *P. phoxinus* at two points during the infestation, error bars indicate standard error. Average shoaling frequency (**a**), average loner count (**b**), average minnow area (**c**) average minnow NND (**d**), average shoal area (**e**) and average shoal NND (**f**). Letters on bars indicate significant differences between treatment groups of $p < 0.05$

that glochidiosis altered the use of flow velocities, likely as a consequence of decreased swimming performance. We propose that the changes observed to shoaling frequency are also closely linked to the changes observed in habitat preference, as infested minnows with poorer swimming performance could be more easily separated from the shoal as it moved through a variable flow environment. The increase in isolated minnows may be a disadvantageous effect for the encysted mussels as isolated minnows are more easily targeted by predators, thereby decreasing total unionid recruitment.

Prior studies have demonstrated a consistent glochidiosis-induced impact on swimming performance and activity levels (Rock et al. 2022). Glochidiosis typically reduces the swimming performance and activity levels of their host in the early stages of infestation, with the effects disappearing

Table 1 Degrees of freedom (DF), F-statistic and significance level of factors used in the statistical analysis of measures on minnow shoal morphology

Measure	Factor	DF	F	*p*
Average shoaling frequency	**Days post infestation**	**1, 314**	**7.715**	**0.006**
	Glochidia presence	**1, 314**	**7.661**	**0.006**
	Days post infestation × Glochidia presence	1, 314	1.725	0.190
Average loner count	Days post infestation	1, 314	0.910	0.341
	Glochidia presence	**1, 314**	**9.144**	**0.003**
	Days post infestation × Glochidia presence	1, 314	1.735	0.189
Average minnow area	Days post infestation	1, 28	1.321	0.260
	Glochidia presence	**1, 28**	**4.487**	**0.043**
	Days post infestation × Glochidia presence	1, 28	0.084	0.774
Average minnow NND	Days post infestation	1, 28	0.175	0.679
	Glochidia presence	1, 28	1.855	0.184
	Days post infestation × Glochidia presence	1, 28	0.119	0.733
Average shoal area	Days post infestation*	1, 26	3.374	0.078*
	Glochidia presence	1, 26	0.195	0.662
	Days post infestation × Glochidia presence	1, 26	0.975	0.333
	Number of minnows in shoal	1, 26	1.460	0.238
Average shoal NND	Days post infestation	1, 27	2.778	0.107
	Glochidia presence	1, 27	0.207	0.652
	Days post infestation × Glochidia presence	1, 27	0.231	0.634

Significant factors are shown in bold, near significant differences are marked with *

Table 2 Degrees of freedom (DF), F-statistic and significance level of factors used in the statistical analysis of measures on minnow habitat preference

Measure	Factor	DF	F	*p*
Average minnow flow preference	Days post infestation	1, 28	0.246	0.624
	Glochidia presence	**1, 28**	**7.637**	**0.010**
	Days post infestation × Glochidia presence	1, 28	1.892	0.180
Average shoal flow preference	Days post infestation	1, 27	0.299	0.589
	Glochidia presence	**1, 27**	**5.259**	**0.030**
	Days post infestation × Glochidia presence	**1, 27**	**4.343**	**0.043**
Average minnow turbulent kinetic energy	Days post infestation	1, 28	0.555	0.462
	Glochidia presence*	1, 28	3.990	0.056*
	Days post infestation × Glochidia presence	1, 28	0.035	0.853
Average shoal turbulent kinetic energy	Days post infestation	1, 27	0.207	0.590
	Glochidia presence	**1, 27**	**4.787**	**0.038**
	Days post infestation × Glochidia presence	1, 27	0.463	0.502

Significant factors are shown in bold, near significant differences are marked with *

later on (Horký et al. 2014; Slavík et al. 2017). However, the effect of glochidiosis can vary greatly between specific host-parasite species pairings, causing no change to host behaviour in some pairings, while others continually increase in effect over time (Crane et al. 2011). Generally, glochidiosis-induced decreases in activity level are associated with a reduced gas-exchange capacity and increased metabolic rate from damaged gills filaments, typical of glochidia encystment (Crane et al. 2011; Horký et al. 2014; Slavík et al. 2017; Rock et al. 2022). Here, infested minnows preferred habitats with slower flowing and less turbulent water at 14 dpi, but only for less turbulent water at 28 dpi when infestation intensity was lower, indicating that the negative impacts of glochidiosis decrease to some degree over time. During this later infestation period, minnows may have been able to take more advantage of the increased swimming efficiency provided by shoals, allowing them to tolerate faster flowing but not more turbulent water. Individual fish within a shoal are able to take advantage of the vortices produced by upstream individuals to improve swimming efficiency in linear flow environments, allowing the shoal as a whole to tolerate faster but not more turbulent flows (Ligman et al. 2023). Minnows typically form lose shoals in the low flow environments and tighter shoals as flow increases, up to a point when flow gets to a critical level where shoals often break apart as individuals or small shoal subsets seek shelter in calmer water demanding less energy to maintain their position within the water column (Pitcher 1973; Garner 1997; De Bie et al. 2020). The increase in isolated individuals observed here may thus be the result of two factors we cannot distinguish at present. Firstly, minnows may have been isolated from each other across space. Isolated individuals may have been unwilling or unable to cross the high flow area in the middle of the arena. Secondly, as minnows form loser shoals in slower flows, and infested minnows tended to prefer slower flows, the individuals marked as isolated may have simply swum away from the group while exhibiting natural exploratory behaviour.

The effects of parasite infestation on fish shoals can be highly variable, but generally result in less dense shoals, or with parasitized individuals at a greater distance from their conspecifics (Barber et al. 2000; Kasumyan and Pavlov 2023). These effects are generally considered to be part of an anti-parasite defence mechanism by non-infested shoal members as shoaling has been shown to increase parasite transmission rates (Côté and Poulin 1995; Reynolds et al. 2019; Walsman et al. 2022). Parasites, however, are known to manipulate host behaviour to increase their own transmission success (Poulin 2010). As parasites have evolved to be transmitted through a variety of mechanisms, manipulated hosts could become more or less prone to shoaling depending on what parasite they are infected by (Mikheev 2011).

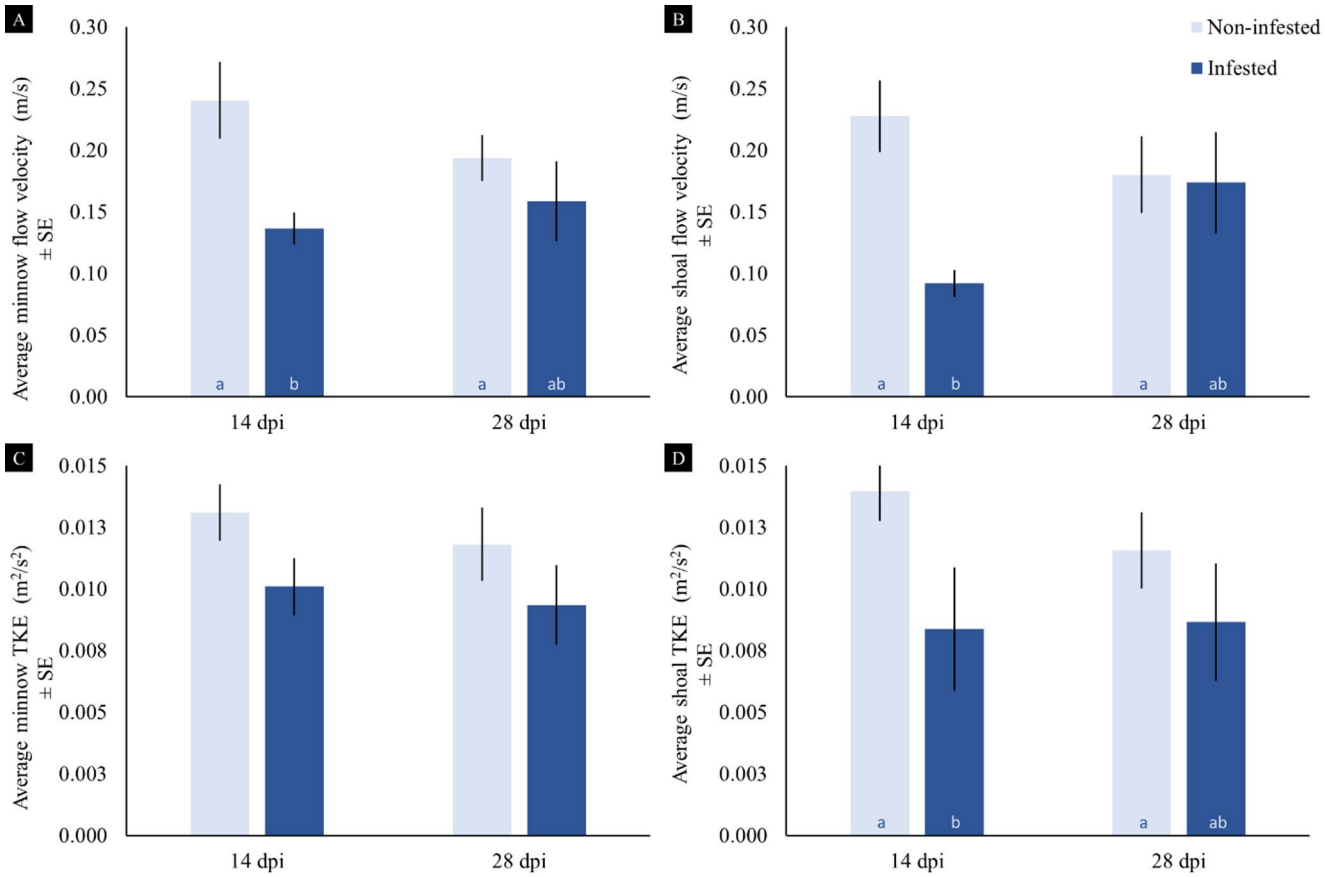

Fig. 3 Measures qualifying habitat preference of infested and non-infested *P. phoxinus* at two points during the infestation, error bars indicate standard error. Average minnow flow velocity (**a**), average shoal flow velocity (**b**), average minnow TKE (**c**), and average shoal TKE (**d**). Letters on bars indicate significant differences between treatment groups of $p < 0.05$

For example, trophically transmitted cestodes often increase shoal NND and decrease shoal cohesion and predator avoidance in both stickleback and minnow models – increasing the risk of predation and thereby transmission success of the parasite – despite not posting an infestation threat to conspecifics (Barber et al. 1995; Barber and Huntingford 1996; Demandt et al. 2020, 2021). However, when a dominant proportion of the population is infested, and non-parasitized individuals become the outgroup, it becomes disadvantageous for non-parasitized individuals to avoid shoaling as the (theoretical) risk of predation outweighs the risk of parasitism (Demandt et al. 2021). On the other hand, directly transmitted parasites may make their hosts more social and take dominant or leading positions in the shoal, increasing the risk of transmission to non-infested hosts, despite an often-increased NND (Ward et al. 2002, 2005; Krkošek et al. 2011; Petkova et al. 2018). Here, we show that despite reduced shoaling frequency, glochidiosis did not affect shoal area, or NND. The absence of glochidia-induced effects to shoal area or NND may be considered beneficial to the encysted glochidia as decreased shoal cohesion could increase risk of predation to the host minnow. Prior studies have suggested that glochidia may manipulate host behaviour by altering habitat preference for slower flowing waters where juvenile mussels are more capable of burying into the sediment after excysting from the host (Rock et al. 2025a, b).

This study into the effects of glochidiosis provides valuable information to both unionid conservation practitioners and parasitologists more generally. The study may be limited by a potential tank effect as the two minnow groups were kept in separate, adjacent tanks for the duration of the experimental period, however, we have no reason to believe that the tanks affected minnow behaviour in the experimental arenas as they were treated equally and were subjected to the same environmental variations. Our results indicate that minnows respond to glochidiosis in a manner similar to other parasites, exhibiting reduced shoaling frequency, suggesting the presence of a generalized anti-parasite behavioural response. Moreover, we demonstrate a glochidia-induced change to habitat preference, indicating reduced swimming performance in infested minnows. Our results highlight the importance of low-flow and low-turbulence habitats for unionid recruitment, as parasitized

host individuals do not tolerate the opposite conditions well. Future habitat restoration for the purpose of unionid conservation should ensure the creation of these calm sections of river as a refuge for infested minnows. These habitats would not only attract infested minnows to recently restored areas, but provide ideal habitat for juvenile mussels, increasing unionid conservation success. However, additional research is required as the global understanding of the effects of glochidia on their host fishes is currently underdeveloped, and there are few studies exploring host manipulation in directly transmitted parasites.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00265-026-03736-2.

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Author contributions SLR and MÖ conceptualized the study design and methodology. SLR and MB collected the data. FT ran the hydrological models. SLR led statistical analysis with support from JW and PAN. SLR led the writing of the manuscript supported by all other authors, including OC.

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Data availability Data are available as a Supplementary file.

Declarations

Ethical approval This study was performed following ethical permit 001673 (Göteborgs djurförsöksetiska nämnd), all applicable institutional and national guidelines for the use of animals were followed.

Competing interests The authors declare no competing interests.

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References

Allouche S, Gaudin P (2001) Effects of avian predation threat, water flow and cover on growth and habitat use by chub, *Leuciscus cephalus*, in an experimental stream. Oikos 94:481–492. https://doi.org/10.1034/j.1600-0706.2001.940310.x

Andersson B (ed) (2012) Computational fluid dynamics for engineers. Cambridge University Press, Cambridge

Avilés L (1999) Cooperation and non-linear dynamics: an ecological perspective on the evolution of sociality. Evol Ecol Res 1:459–477

Barber I, Huntingford FA (1996) Parasite infection alters shoaling behaviour: deviant positioning of helminth-infected minnows in conspecific groups. Proc R Soc Lond B 263:1095–1102. https://doi.org/10.1098/rspb.1996.0161

Barber I, Huntingford FA, Crompton DWT (1995) The effect of hunger and cestode parasitism on the shoaling decisions of small freshwater fish. J Fish Biol 47:524–536. https://doi.org/10.1111/j.1095-8649.1995.tb01919.x

Barber I, Hoare D, Krause J (2000) Effects of parasites on fish behaviour: a review and evolutionary perspective. Rev Fish Biol Fisher 10:131–165. https://doi.org/10.1023/A:1016658224470

Bartashevich P, Herbert-Read JE, Hansen MJ, Dhellemmes F, Domenici P, Krause J, Romanczuk P (2024) Collective anti-predator escape manoeuvres through optimal attack and avoidance strategies. Commun Biol 7:1586. https://doi.org/10.1038/s42003-024-07267-2

Beaver CE, Geda SR, Johnson NA (2019) Standardizing a non-lethal method for characterizing the reproductive status and larval development of freshwater mussels (Bivalvia: Unionida). J Vis Exp 152:e60244. https://doi.org/10.3791/60244

Chicoli A, Butail S, Lun Y, Bak-Coleman J, Coombs S, Paley DA (2014) The effects of flow on schooling *Devario aequipinnatus*: school structure, startle response and information transmission. J Fish Biol 84:1401–1421. https://doi.org/10.1111/jfb.12365

Chowdhury MMR, Marjomäki TJ, Taskinen J (2019) Effect of glochidia infection on growth of fish: freshwater pearl mussel *Margaritifera margaritifera* and brown trout *Salmo trutta*. Hydrobiologia 848:3179–3189. https://doi.org/10.1007/s10750-019-03994-4

Côté IM, Poulin R (1995) Parasitism and group size in social animals: a meta-analysis. Behav Ecol 6:159–165. https://doi.org/10.1093/beheco/6.2.159

Crane AL, Fritts AK, Mathis A, Lisek JC, Barnhart MC (2011) Do gill parasites influence the foraging and antipredator behaviour of rainbow darters. Etheostoma caeruleum? Anim Behav 82:817–823. https://doi.org/10.1016/j.anbehav.2011.07.015

De Bie J, Manes C, Kemp PS (2020) Collective behaviour of fish in the presence and absence of flow. Anim Behav 167:151–159. https://doi.org/10.1016/j.anbehav.2020.07.003

Delcourt J, Poncin P (2012) Shoals and schools: back to the heuristic definitions and quantitative references. Rev Fish Biol Fisher 22:595–619. https://doi.org/10.1007/s11160-012-9260-z

Demandt N, Praetz M, Kurvers RH, Krause J, Kurtz J, Scharsack JP (2020) Parasite infection disrupts escape behaviours in fish shoals. Proc R Soc B 287:20201158. https://doi.org/10.1098/rspb.2020.1158

Demandt N, Bierbach D, Kurvers RH, Krause J, Kurtz J, Scharsack JP (2021) Parasite infection impairs the shoaling behaviour of uninfected shoal members under predator attack. Behav Ecol Sociobiol 75:148. https://doi.org/10.1007/s00265-021-03080-7

Denic M, Taeubert JE, Geist J (2015) Trophic relationships between the larvae of two freshwater mussels and their fish hosts. Invertebr Biol 134:129–135. https://doi.org/10.1111/ivb.12080

Douda K, Velíšek J, Kolářová J, Rylková K, Slavík O, Horký P, Langrová I (2017) Direct impact of invasive bivalve (*Sinanodonta woodiana*) parasitism on freshwater fish physiology: evidence and implications. Biol Invasions 19:989–999. https://doi.org/10.1007/s10530-016-1319-7

Dudgeon D, Arthington AH, Gessner MO et al (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. Biol Rev 81:163–182. https://doi.org/10.1017/S1464793105006950

Filipsson K, Petersson T, Höjesjö J, Piccolo JJ, Näslund J, Wengström N, Österling EM (2016) Heavy loads of parasitic freshwater pearl mussel (*Margaritifera margaritifera* L.) larvae impair foraging, activity and dominance performance in juvenile brown trout (Salmo trutta L). Ecol Freshw Fish 27:70–77. https://doi.org/10.1111/eff.12324

Filipsson K, Brijs J, Näslund J, Wengström N, Adamsson M, Závorka L, Österling ME, Höjesjö J (2017) Encystment of parasitic freshwater pearl mussel (*Margaritifera margaritifera*) larvae coincides with increased metabolic rate and haematocrit in juvenile brown trout (*Salmo trutta*). Parasitol Res 116:1353–1360. https://doi.org/10.1007/s00436-017-5413-2

Foster WA, Treherne JE (1981) Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. Nature 293:466–467. https://doi.org/10.1038/293466a0

Garner P (1997) Effects of variable discharge on the velocity use and shoaling behaviour of *Phoxinus phoxinus*. J Fish Biol 50:1214–1220. https://doi.org/10.1111/j.1095-8649.1997.tb01649.x

Godin J-GJ (1986) Anti-predator function of shoaling in teleost fishes: a selective review. Nat Can 113:241–250

Hockley FA, Wilson CAME, Graham N, Cable J (2014) Combined effects of flow condition and parasitism on shoaling behaviour of female guppies *Poecilia reticulata*. Behav Ecol Sociobiol 68:1513–1520. https://doi.org/10.1007/s00265-014-1760-5

Horký P, Douda K, Maciak M, Závorka L, Slavík O (2014) Parasite-induced alterations of host behaviour in a riverine fish: the effects of glochidia on host dispersal. Freshw Biol 59:1452–1461. https://doi.org/10.1111/fwb.12357

Horký P, Slavík O, Douda K (2019) Altered thermoregulation as a driver of host behaviour in glochidia-parasitised fish. J Exp Biol 222:jeb184903. https://doi.org/10.1242/jeb.184903

Kasumyan AO, Pavlov DS (2023) Influence of environmental factors and the condition of fish on schooling behavior. J Ichthyol 63:1362–1373. https://doi.org/10.1134/S0032945223070123

Krkošek M, Connors BM, Ford H et al (2011) Fish farms, parasites, and predators: implications for salmon population dynamics. Ecol Appl 21:897–914. https://doi.org/10.1890/09-1861.1

Lamand F, Roche K, Beisel JN (2016) Glochidial infestation by the endangered mollusc *Unio crassus* in rivers of north-eastern France: *Phoxinus phoxinus* and *Cottus gobio* as primary fish hosts. Aquat Conserv Mar Freshw Ecosyst 26:445–455. https://doi.org/10.1002/aqc.2603

Landeau L, Terborgh J (1986) Oddity and the 'confusion effect' in predation. Anim Behav 34:1372–1380. https://doi.org/10.1016/S0003-3472(86)80208-1

Ligman M, Lund J, Fürth M (2023) A comprehensive review of hydrodynamic studies on fish schooling. Bioinspir Biomim 19:011002. https://doi.org/10.1088/1748-3190/ad1335

Lima SL (1995) Back to the basics of anti-predatory vigilance: the group-size effect. Anim Behav 49:11–20. https://doi.org/10.1016/0003-3472(95)80149-9

Lopes-Lima M, Teixeira A, Froufe E, Lopes A, Varandas S, Sousa R (2014) Biology and conservation of freshwater bivalves: past, present and future perspectives. Hydrobiologia 735:1–13. https://doi.org/10.1007/s10750-014-1902-9

Lopes-Lima M, Sousa R, Geist J et al (2017) Conservation status of freshwater mussels in Europe: state of the art and future challenges. Biol Rev 92:572–607. https://doi.org/10.1111/brv.12244

Marriner BA, Baki ABM, Zhu DZ, Cooke SJ, Katopodis C (2016) The hydraulics of a vertical slot fishway: A case study on the multi-species Vianney-Legendre fishway in Quebec, Canada. Ecol Eng 9:190–202. https://doi.org/10.1016/j.ecoleng.2016.01.032

Mikheev VN (2011) Monoxenous and heteroxenous fish parasites manipulate the behavior of their hosts in different ways. Biol Bull Rev 1:446–457. https://doi.org/10.1134/S2079086411050045

Miles J, Vowles AS, Kemp PS (2024) The role of collective behaviour in fish response to visual cues. Behav Process 220:105079. https://doi.org/10.1016/j.beproc.2024.105079

Modesto V, Ilarri M, Souza AT, Lopes-Lima M, Douda K, Clavero M, Sousa R (2018) Fish and mussels: importance of fish for freshwater mussel conservation. Fish Fish 19:244–259. https://doi.org/10.1111/faf.12252

Österling EM, Ferm J, Piccolo JJ (2014) Parasitic freshwater pearl mussel larvae (*Margaritifera margaritifera* L.) reduce the drift-feeding rate of juvenile brown trout (*Salmo trutta* L). Environ Biol Fish 97:543–549. https://doi.org/10.1007/s10641-014-0251-x

Petkova I, Abbey-Lee RN, Løvlie H (2018) Parasite infection and host personality: *Glugea*-infected three-spined sticklebacks are more social. Behav Ecol Sociobiol 72:173. https://doi.org/10.1007/s00265-018-2586-3

Pitcher TJ (1973) The three-dimensional structure of schools in the minnow, *Phoxinus phoxinus* (L). Anim Behav 21:673–686. https://doi.org/10.1016/S0003-3472(73)80091-0

Pitcher TJ (1983) Heuristic definitions of fish shoaling behaviour. Anim Behav 31:611–613. https://doi.org/10.1016/S0003-3472(83)80087-6

Pitcher TJ (1993) Functions of shoaling behaviour in teleosts. In: Pitcher TJ (ed) The behaviour of teleost fishes. Springer US, Boston, MA, pp 294–337. https://doi.org/10.1007/978-1-4684-8261-4_12

Poulin R (2010) Parasite manipulation of host behavior: an update and frequently asked questions. Adv Stud Behav 41:151–186. https://doi.org/10.1016/S0065-3454(10)41005-0

Reynolds M, Hockley FA, Wilson CA, Cable J (2019) Assessing the effects of water flow rate on parasite transmission amongst a social host. Hydrobiologia 830:201–212. https://doi.org/10.1007/s10750-018-3863-x

Rock SL, Watz J, Nilsson PA, Österling M (2022) Effects of parasitic freshwater mussels on their host fishes: a review. Parasitology 149:1958–1975. https://doi.org/10.1017/S0031182022001226

Rock SL, Nilsson A, Watz J, Calles O, Österling M (2025a) Parasitic mussels induce upstream movement in their fish hosts: early evidence of extended phenotype. Behav Ecol 36:araf043. https://doi.org/10.1093/beheco/araf043

Rock SL, Elmlund AM, Nilsson PA, Watz J, Calles O, Österling M (2025b) A habitat and a parasite: adult and larval parasitic freshwater mussels impact habitat choice and predator–prey interactions of a host fish and its prey. Ecol Evol 15:e72601. https://doi.org/10.1002/ece3.72601

Schneider LD, Nilsson PA, Höjesjö J, Österling EM (2017) Local adaptation studies and conservation: Parasite–host interactions between the endangered freshwater mussel *Unio crassus* and its host fish. Aquat Conserv Mar Freshw Ecosyst 27:1261–1269. https://doi.org/10.1002/aqc.2816

Schneider LD, Nilsson PA, Österling EM (2018) Evaluating temperature-and host-dependent reproduction in the parasitic freshwater mussel *Unio crassus*. Hydrobiologia 810:283–293. https://doi.org/10.1007/s10750-017-3217-0

Silk JB (2007) The adaptive value of sociality in mammalian groups. Phil Trans R Soc B 362:539–559. https://doi.org/10.1098/rstb.2006.1994

Sirot E, Touzalin F (2009) Coordination and synchronization of vigilance in groups of prey: the role of collective detection and predators' preference for stragglers. Am Nat 173:47–59. https://doi.org/10.1086/593358

Slavík O, Horký P, Douda K, Velíšek J, Kolářová J, Lepič P (2017) Parasite-induced increases in the energy costs of movement of host freshwater fish. Physiol Behav 171:127–134. https://doi.org/10.1016/j.physbeh.2017.01.010

Sneddon LU, Hawkesworth S, Braithwaite VA, Yerbury J (2006) Impact of environmental disturbance on the stability and benefits of individual status within dominance hierarchies. Ethology 112:437–447. https://doi.org/10.1111/j.1439-0310.2005.01192.x

Stamou AI, Mitsopoulos G, Rutschmann P, Bui MD (2018) Verification of a 3D CFD model for vertical slot fish-passes. Environ Fluid Mech 18:1435–1461. https://doi.org/10.1007/s10652-018-9602-z

Strayer DL (2008) Freshwater mussel ecology: a multifactor approach to distribution and abundance, vol 1. University of California Press, Oakland

Strayer DL, Downing JA, Haag WR, King TL, Layzer JB, Newton TJ, Nichols JS (2004) Changing perspectives on pearly mussels, North America's most imperiled animals. Bioscience 54:429–439. https://doi.org/10.1641/0006-3568(2004)054[0429:CPOPMN]2.0.CO;2

Taeubert JE, Geist J (2013) Critical swimming speed of brown trout (*Salmo trutta*) infested with freshwater pearl mussel (*Margaritifera margaritifera*) glochidia and implications for artificial breeding of an endangered mussel species. Parasitol Res 112:1607–1613. https://doi.org/10.1007/s00436-013-3314-6

Taeubert JE, Martinez AMP, Gum B, Geist J (2012a) The relationship between endangered thick-shelled river mussel (*Unio crassus*) and its host fishes. Biol Conserv 155:94–103. https://doi.org/10.1016/j.biocon.2012.06.005

Taeubert JE, Gum B, Geist J (2012b) Host-specificity of the endangered thick-shelled river mussel (*Unio crassus*, Philipsson 1788) and implications for conservation. Aquat Conserv Mar Freshw Ecosyst 22:36–46. https://doi.org/10.1002/aqc.1245

Tarena F, Nyqvist D, Katopodis C, Comoglio C (2025) Computational fluid dynamics in fishway research-a systematic review on upstream fish passage solutions. J Ecohydraulics 10:107–126. https://doi.org/10.1080/24705357.2024.2363772

Tobler M, Schlupp I (2008) Influence of black spot disease on shoaling behaviour in female western mosquitofish, *Gambusia affinis* (Poeciliidae, Teleostei). Environ Biol Fish 81:29–34. https://doi.org/10.1007/s10641-006-9153-x

Vaughn CC, Hoellein TJ (2018) Bivalve impacts in freshwater and marine ecosystems. Annu Rev Ecol Evol S 49:183–208. https://doi.org/10.1146/annurev-ecolsys-110617-062703

Vaughn CC, Nichols SJ, Spooner DE (2008) Community and foodweb ecology of freshwater mussels. J N Am Benthol Soc 27:409–423. https://doi.org/10.1899/07-058.1

Walsman JC, Janecka MJ, Clark DR, Kramp RD, Rovenolt F, Patrick R, Mohammed RS, Konczal M, Cressler CE, Stephenson JF (2022) Shoaling guppies evade predation but have deadlier parasites. Nat Ecol Evol 6:945–954. https://doi.org/10.1038/s41559-022-01772-5

Ward AJ, Hoare DJ, Couzin ID, Broom M, Krause J (2002) The effects of parasitism and body length on positioning within wild fish shoals. J Anim Ecol 71:10–14. https://doi.org/10.1046/j.0021-8790.2001.00571.x

Ward AJ, Duff AJ, Krause J, Barber I (2005) Shoaling behaviour of sticklebacks infected with the microsporidian parasite, *Glugea anomala*. Environ Biol Fish 72:155–160. https://doi.org/10.1007/s10641-004-9078-1

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