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scientific reports

OPEN

Evidence for dilution effect by *Gobio gobio*, a dead-end host in the *Unio crassus*–Cyprinidae coevolutionary system

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Freshwater mussels (Unionidae) depend on specific fish hosts to complete their life cycle. Glochidia, their parasitic larvae, must attach to the gills or fins of suitable fish species to metamorphose. However, non-host fish may intercept glochidia, reducing larval availability for competent hosts—a phenomenon known as the dilution effect. We investigated this mechanism in a natural population of the endangered mussel *Unio crassus*, focusing on the interaction between the dominating host *Phoxinus phoxinus* and the non-host *Gobio gobio*. Field surveys across three separate reaches of the Warkocz River (2015–2016) and a controlled infestation experiment demonstrated that *G. gobio* removes a substantial proportion of glochidia without supporting their metamorphosis. Co-occurrence analysis showed a negative relation between infestation levels of *G. gobio* vs. *P. phoxinus*, with a significant interaction modulated by *U. crassus* density. At low mussel densities, the impact of *G. gobio* on parasitic success was strongest. *Gobio gobio* was recorded at 90% of the known *U. crassus* localities in Poland, and in all of these sites it formed a dominant component of the fish assemblage. Our findings provide direct evidence of a context-dependent dilution effect and highlight the importance of fish community composition and behaviour in conservation of unionid mussels. The presence of non-host fish in habitats with low mussel abundance may undermine recruitment and increase extinction risk in fragmented populations.

Adaptations and counter-adaptations create a tightly coupled coevolutionary feedback loop during host–parasite evolution[1]. As a consequence, dependent organisms such as parasites, commensals, and mutualists are at risk of coextinction when their associated partners decline, potentially triggering extinction cascades and rapid biodiversity loss[2]. Ongoing large-scale changes in biodiversity and community structure may prevent many parasitic transmission stages from encountering suitable hosts[3]. The abundance of one partner in a co-evolving system can be influenced by factors external to the system, such as interactions with other species. When habitat functionality deteriorates, community composition may become simplified[4], disrupting the favourable-to-unfavourable host species ratio.

It has been demonstrated that local biodiversity can reduce infection risk across a range of freshwater host–parasite systems[5], an effect known as the “dilution effect”[6], which is also referred to in parasitology as the “decoy effect”[7]. This mechanism posits that multiple species may distract free-living parasite stages from suitable hosts, leading to unsuccessful transmission events involving so-called “dead-end” hosts. While the dilution effect is often associated with high biodiversity, it may also arise in species-poor communities if a dominant fish species acts as a dead-end host, thereby reducing transmission success and negatively affecting parasite fitness.

Freshwater mussels of the order Unionida (hereafter referred to as naiads) exhibit a specialized life cycle that includes an obligatory parasitic larval stage. Females deposit eggs into brood sacs located in modified gill tissue called marsupia, where fertilization occurs and the embryos develop into parasitic larvae known as glochidia. These are released into the water column and must attach to a suitable fish host to complete development into the pediveliger stage. Following transformation, the juvenile mussel detaches and begins its benthic life as a free-living individual[8]. The primary ecological role of fish hosts is thought to be the dispersal of mussel larvae over greater distances[9]. Because mussels exist as metapopulations, host-mediated dispersal enables range expansion and colonization of new habitats within river systems[10].

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Parasitism begins when glochidia are released into the water. Given that the fitness of naiads is entirely dependent on their fish hosts—without a reciprocal dependency—numerous adaptations have evolved to enhance host infection. In *Unio crassus*, the only known European species that actively attracts fish, females move toward the riverbank, immerse their inhalant siphon, draw in water, and then contract their valves to increase internal pressure. Upon opening the exhalant siphon, they expel jets of water containing glochidial conglutinates. These jets are directed onto the water surface and visually attract fish, which become infested while attempting to consume the apparent prey item[11], including the larvae themselves[12].

The number of glochidia released by adult mussels is generally consistent and size-dependent[13], with individuals typically reaching an asymptotic size in a given habitat[14]. Glochidia are released in intermittent spurts—approximately every 91 s—for 3 to 6 h from mid-morning to mid-afternoon. During this period, the marsupium is emptied, releasing up to 1,127 glochidia, which can disperse over approximately 0.5 m² of the water surface[11]. Each spurt attracts small fish, typically fry (mean = 6.4, SD = 5.8, *N* = 32). Although the total number of glochidia in the marsupium is substantial (1.47 g in a 70 mm mussel; T. Zając, unpubl. data), their availability in the water column is limited in time. Glochidial conglutinates sink to the bottom, and their viability decreases significantly after 12 h[11].

Importantly, mussels are adapted to infect specific fish species; glochidia cannot successfully metamorphose on all potential hosts[15,16]. Therefore, when glochidia encounter multiple fish species—including non-hosts—scramble competition may occur. This process, irrespective of whether fish actively consume glochidia or passively remove them via surface attachment, can lead to a strong dilution effect. The strength of this effect depends on the relative abundance of host versus decoy fish species.

In this study, we aim to test whether a dilution effect driven by decoy species occurs within the habitat of the threatened freshwater mussel *Unio crassus*, to assess the magnitude of this effect, and to identify the factors that influence it. We hypothesize that the infestation rate of the main fish host decreases in the presence of infested decoy species.

Results

Dynamics of fish community structure and *Unio crassus* abundance

In the studied river, the dominant species were *Phoxinus phoxinus* (54%), *Gobio gobio* (19%), and *Barbatula barbatula* (17%), which together accounted for 90% of all captured individuals—representing the majority of species available for infestation by *Unio crassus*. The remaining species comprised a marginal fraction of the catch (Table 1); however, 8 out of the 11 recorded species were capable of intercepting glochidia.

	Years	**2015-2016**					**2020**	**2021**	**2023**	**Total 2020 2021 2023 (N)**		
		Plot 1	**Plot 2**	**Plot 3**	**Total**		**All plots**	**All plots**	**All plots**			
	Species	***(N)***	***(N)***	***(N)***	***(N)***	**%**	***(N)***	***(N)***	***(N)***		**%**	**Change**
1	*Alburnus alburnus*	0	1	0	1	0.0	0	0	0	0	0	0.0
2	*Barbatula barbatula*	93	113	207	413	17.1	58	8	27	93	7.1	**−10.0**
3	*Carassius gibelio*	0	1	0	1	0	0	1	0	1	0.1	0.1
4	*Cottus gobio*	0	0	7	7	0.3	3	0	0	3	0.2	**−0.1**
5	*Eudontomyzon mariae**	49	22	12	83	3.4	7	5	3	15	1.1	−2.3
6	*Gobio gobio*	92	119	250	461	19	234	92	128	454	34.6	**15.6**
7	*Leucaspius delineatus*	2	0	3	5	0.2	0	0	0	0	0	**−0.2**
8	*Phoxinus phoxinus*	422	244	638	1304	53.9	303	66	74	443	33.8	**−20.1**
9	*Rhodeus amarus*	32	32	24	88	3.6	102	112	40	254	19.4	**15.8**
10	*Salmo trutta*	19	6	12	37	1.5	28	2	14	44	3.3	**1.8**
11	*Squalius cephalus*	3	0	19	22	1	0	4	1	5	0.4	**−0.6**
	Total	712	538	1172	2422	100	735	290	287	1312	100	**0.0**
12	*Unio crassus*											
	Monitoring cycle	2006-2008		2013-2014		2017-2018		2020-2021		Change 2020/2006		
	Site 1	18.2		6.1		2.2		0.5		**0.03**		
	Site 2	4.2		4.2		3.6		1.9		**0.45**		
	Site 3	2.4		0.0		0.6		2.6		**1.08**		
	Mean	8.3		3.4		2.1		1.7		**0.20**		

Table 1. Abundance and temporal changes in the studied species—fish, lamprey (*), and *Unio crassus*—in the Warkocz River. For fish and lamprey, values are expressed as the number of captured individuals and their percentage of the total catch; for *U. crassus*, values represent mean density derived from three cross-channel transects, each surveyed at three separate localities. Fish data are pooled across the two sampling years (2015 and 2016) and, in subsequent monitoring cycles, across all study plots. Species observed to intercept glochidia (regardless of whether successful metamorphosis occurred) are shown in bold. The quantitative structure of the fish community changed in subsequent years: the abundance of *P. phoxinus*, the main host of *U. crassus*, decreased by 20%, whereas the abundances of *G. gobio* and *Rhodeus amarus* each increased by almost 16%.

Infestation of *Gobio gobio* in comparison to *Phoxinus phoxinus* and *Barbatula barbatula*

Among the dominant fish species, 37.3% of *P. phoxinus* individuals (*N* = 1304) were found to be infested with glochidia. In contrast, infestation prevalence was only 9.8% in *G. gobio* (*N* = 461) and 1% in *B. barbatula* (*N* = 413). For the remaining fish species combined, the prevalence was 8.5%.

The mean intensity of infestation among infected *P. phoxinus* individuals was 3.5 glochidia per fish—twice as low as in *G. gobio* (6.8), and more than twice as high as in *B. barbatula* (1.5). The variation in infestation was much greater in *G. gobio* (SD = 11.26) than in *P. phoxinus* (SD = 3.20). Maximum infestation levels further highlight these differences: the highest number of glochidia on a single *P. phoxinus* was 23, whereas in *G. gobio* it reached 57. In comparison, only six glochidia were found across all *B. barbatula* specimens, indicating the negligible role of this species in glochidial attachment. Therefore, subsequent analyses focused exclusively on *P. phoxinus* and *G. gobio*.

When comparing the total number of glochidia found on both species, *P. phoxinus* accounted for 82.1% of all glochidia observed on fish, while *G. gobio* accounted for only 14.6% (Table 2). The maximum relative contribution of *G. gobio* to the glochidial pool occurred in 2015 at plot 3, where it carried 33.8% of all observed glochidia.

Gobio gobio as a dead-end host

On day five after artificial infestation, a total of 57 glochidia were observed attached to the fins, body, and opercular regions of ten *G. gobio* individuals. However, none of these larvae successfully metamorphosed into the pediveliger stage (the next juvenile phase) during the standard excystation period of 44 days. In contrast, the same protocol applied to ten *P. phoxinus* individuals yielded 389 attached glochidia and 249 viable, metamorphosed juveniles within the same period (Fig. 1A).

Co-occurrence

In the Warkocz River we analysed 234 buffer zones (5 m segments), of which 66 contained no fish, to investigate whether the number of glochidia found on *P. phoxinus* within each buffer was affected by the number attached to *G. gobio*. Results (Table 3) indicate that an increase in glochidia attached to *G. gobio* was associated with a decrease in the number attached to *P. phoxinus*. Moreover, a significant interaction was found between the number of *U. crassus* mussels present and the number of glochidia attached to *G. gobio*.

At a broader spatial scale, among 158 fish surveys conducted in 49 rivers with known *U. crassus* localities, *G. gobio* was recorded in 129 surveys (81.6%) within 5 km of mussel sites. When the distance threshold was reduced to 200 m, co-occurrence was detected in 47 out of 52 surveys (90.4%) (Fig. 1C). Considering the quantitative structure of fish assemblages, *G. gobio* accounted for 23.8% of individuals among the seven most abundant species at sites located within 5 km of *U. crassus* localities, and for 17.4% at sites within 200 m (Fig. 1D).

Discussion

In all Unionidae, fish infestation constitutes an obligatory parasitic phase in their ontogeny and is under strong selective pressure. This has driven the evolution of numerous behavioural adaptations, such as releasing glochidia in confined areas of still water[11] or employing species-specific lures[17] to direct the larval suspension toward attracted hosts. Consequently, any dilution of the glochidial concentration in the environment is likely to be detrimental to parasitic success.

Glochidia lack the ability to actively select hosts[18]. Instead, they behave like particulate matter temporarily suspended in water, with their concentration measurable in quantitative terms[19]. Under such conditions, infestation can be viewed as a continuous depletion of glochidia in the water column due to three mechanisms:

Season	Site	Species	N	Sum of glochidia	Percentage of all glochidia attached to fish	Minimum	Maximum
2015	1	*Gobio gobio*	64	19	3.1	0	12
		Phoxinus phoxinus	295	548	90.6	0	23
	2	*Gobio gobio*	66	54	16.8	0	42
		Phoxinus phoxinus	156	263	81.7	0	14
	3	*Gobio gobio*	185	222	33.8	0	57
		Phoxinus phoxinus	370	419	63.8	0	20
2016	1	*Gobio gobio*	28	0	0.0	0	0
		Phoxinus phoxinus	127	187	99.5	0	13
	2	*Gobio gobio*	53	2	2.0	0	1
		Phoxinus phoxinus	88	100	98.0	0	11
	3	*Gobio gobio*	65	7	3.3	0	3
		Phoxinus phoxinus	268	198	92.1	0	11
2015–2016	total	*Gobio gobio*	461	304	14.6	0	57
	total	*Phoxinus phoxinus*	1304	1715	82.1	0	23

Table 2. Total number of glochidia found on individuals of each species per site and year, along with the percentage contribution to the total pool of attached glochidia, and observed range (min–max) of glochidia per fish.

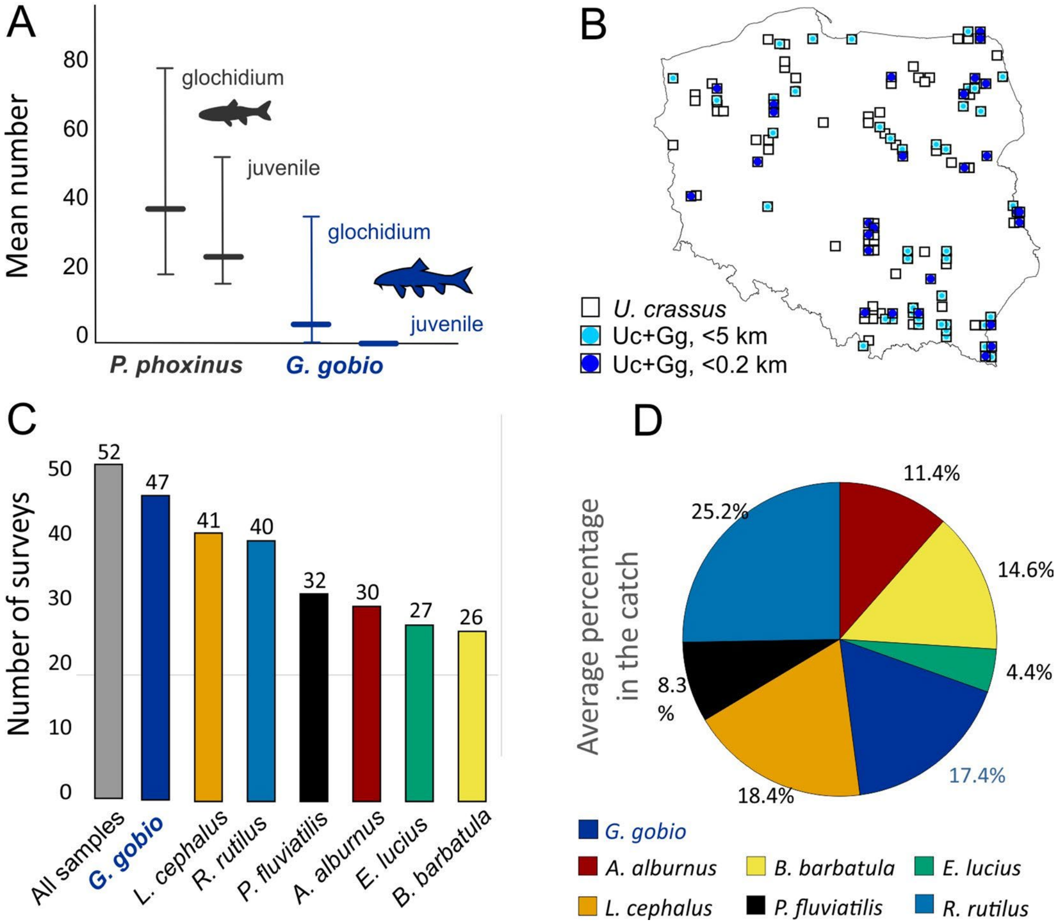

Fig. 1. The role of *G. gobio* as a decoy species. (**A**) Results of the infestation experiment: mean number of glochidia attached to fish five days after artificial infestation and mean number of successfully metamorphosed juveniles recovered over 40 days for *P. phoxinus* and *G. gobio*. Whiskers show observed ranges. (**B**) Spatial co-occurrence of *U. crassus* and *G. gobio* in Poland. Each square represents a 10 × 10 km grid cell. White squares indicate sites where *U. crassus* was recorded; light-blue squares denote cells where *U. crassus* and *G. gobio* occurred within < 5 km along the river; dark-blue squares mark cells where records of both species were within < 200 m. (**C**) Number of surveys in which the seven most frequent fish species occurred sympatrically with *U. crassus* (matched within < 200 m). (**D**) Mean percentage of these seven species in the catch across the same set of surveys.

Effect	Estimate	SE	Wald	*p*
Intercept	2.94	0.027	11,469	< 0.00001
G. gobio Sum glochidia	−0.0133	0.0023	34.5	< 0.00001
N Uc	−0.0002	0.0005	0.12	0.726
N Uc**G. gobio* Sum glochidia	0.0007	0.00006	117.9	< 0.00001
Site 1	0.084	0.024	11.9	0.0006
Site 2	0.138	0.034	16.4	< 0.00005
Season (2015)	0.327	0.018	317.4	< 0.00001
Season*site 1	−0.129	0.024	27.7	< 0.00001
Season*site 2	0.238	0.028	70.7	< 0.00001

Table 3. Generalized linear model (GLZ) of the relationship between the number of glochidia found on *P. phoxinus* within each 5 m buffer (Poisson distribution, log link) and the sum of glochidia on *G. gobio*, number of *U. crassus* individuals, site, and season.

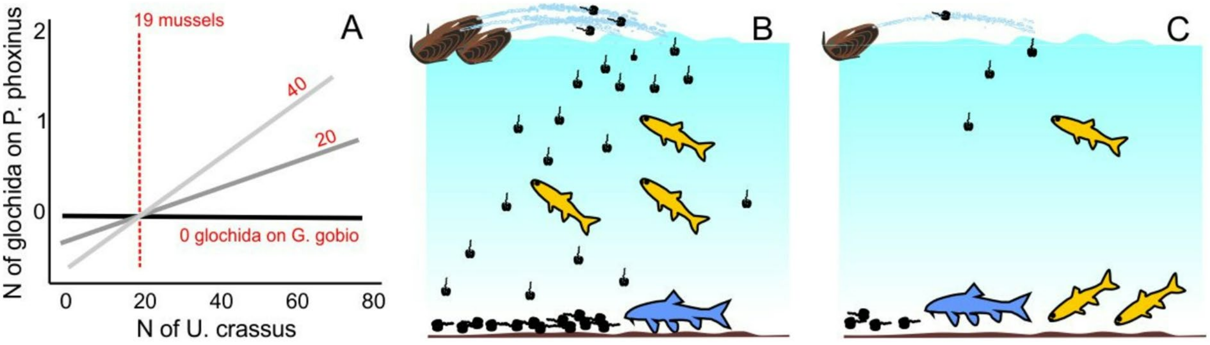

Fig. 2. Conceptual model of "foraging-based" dilution: (**A**) graphical representation of the interaction between *U. crassus* density and glochidia load on the two dominant fish species; (**B**) at high mussel densities, large quantities of glochidia are released over a limited water surface, *P. phoxinus* consumes them in the water column, while remaining larvae settle and might be intercepted by *G. gobio*; (**C**) at low mussel densities, fewer glochidia are intercepted in the water column, forcing *P. phoxinus* to forage on the bottom, where it competes with the better-adapted *G. gobio*.

(1) sinking to the bottom, (2) passive, random attachment to fish fins or gills, and (3) active consumption by fish[20–22]. A similar process occurs when glochidia settle on the bottom, where foraging fish reduce their density on the substrate.

In all these cases, the number of infested fish and the number of glochidia attached per individual should be proportional to the local glochidial density per unit of water volume or benthic area (e.g., within a buffer zone), and should correlate across species. In such a framework, the dilution effect is expected to be directly proportional to the relative abundance of dead-end hosts within a given spatial unit.

In the present data, *P. phoxinus* was not only the dominant species in terms of abundance, but its individuals also showed a higher prevalence of infestation compared to *G. gobio*, the second most abundant species. However, the mean number of glochidia per fish was higher in *G. gobio*, suggesting that this species may remove a disproportionately large share of the glochidial pool despite its lower relative abundance. In some cases, such as plot 3 in 2015, *G. gobio* comprised 26.8% of all fish but carried 33.8% of the total glochidia load, indicating a substantial potential for impact under favourable ecological conditions. These data indicate that infestation probability decreased over time, as the abundance of *P. phoxinus*—the primary host in the river—declined, while the abundance of the dead-end host *G. gobio* increased (Table 1).

Glochidial availability is also expected to depend on mussel density, as it serves as the larval source. If certain fish species co-occur with mussels at sites of high density, while others are more common at sites with sparse mussel populations, these differences should be reflected in glochidial prevalence and intensity. A necessary condition for the dilution effect to manifest is the spatial co-occurrence of both competent and dead-end hosts, implying the potential for scramble competition[23].

To test this, we assumed that within a defined spatial unit (e.g., a buffer), a finite pool of glochidia—produced by a local assemblage of spurting females—was partitioned between *P. phoxinus* and *G. gobio*. Our results (Table 3) revealed not only that glochidial attachment to *G. gobio* reduced infestation of *P. phoxinus* within the same spatial unit, but also that this interaction was modulated by mussel density. Specifically, at high mussel densities (and therefore high glochidial concentrations), *G. gobio* had no measurable effect on the infestation of *P. phoxinus*. However, when mussel density within a buffer fell below 19 individuals, the impact of *G. gobio* became significant (Fig. 2A).

This finding is particularly relevant in the context of declining populations of *U. crassus*, where low mussel densities may render the species especially vulnerable to the dilution effect—posing an additional threat and potentially contributing to an Allee effect[24]. Recent monitoring data from Poland (2023–2024) confirms that in 34.4% of the 64 surveyed sites across 34 rivers, fewer than 19 individuals were found per 5 m buffer (Zając K., unpubl. data). The decline of *U. crassus* was also confirmed in the Warkocz River in later years (Table 1), including evidence of increased mortality and reduced glochidia production[25]. This has direct implications for conservation planning: extensive low-density areas may contribute little to species demography compared to highly concentrated populations.

The observed interaction can be interpreted by considering the microhabitat preferences of *U. crassus*, which favours still water at channel margins and fine sediments[14,26,27]—conditions indicative of reduced flow. Glochidia expelled near the surface sink within seconds to minutes (unpubl. data), depending on water depth, while their overall lifespan ranges from 1 to 3 days, depending on temperature[11]. This implies that glochidia are accessible to pelagic fish for only a brief period but remain available to benthic fish for a significantly longer time.

P. phoxinus is known to forage in both the water column and on the substrate[28,29], whereas *G. gobio*, a benthivorous species with an inferior mouth and sensory barbels[30], is specialised for foraging on the bottom. At high mussel densities, glochidial concentrations in the water column are sufficient for *P. phoxinus* to forage effectively in both strata (Fig. 2B). In contrast, at low mussel densities (< 19 individuals per buffer), glochidia become scarce in the water column but remain abundant on the substrate. This shifts the zone of competition to the benthic habitat, where *G. gobio*, being better adapted, may outcompete *P. phoxinus* (Fig. 2C). This explains how 26.8% of the *G. gobio* population may intercept up to 33.8% of the available glochidia.

Some studies directly report that fish may actively pursue and consume glochidia[11,12]. Although little is known about glochidial release in other naiad species, our observations of *Pseudanodonta complanata* suggest that they passively expel glochidia, which sink quickly and become available to benthic fish. Other species, such as *U. pictorum* and *U. tumidus*, release mature glochidia attached to mucous threads resembling a pearl necklace. These threads may reach up to 15 cm in length, which is substantial compared to the larval thread of *U. crassus*, the entire length of which fits within the field of view of a microscope. A very long thread carrying approximately 500 glochidia could be highly effective for infecting bottom-dwelling fish inhabiting the sub-habitats used by those mussel species[31]. However, a species that evolved a strategy to release glochidia higher in the water column, prolonging their suspension time, would provide more opportunity for pelagic fish—such as the abundant and competent *P. phoxinus*—to become hosts.

The data collected from across Poland indicate that *G. gobio* co-occurs with *U. crassus* and constitutes a significant proportion of fish assemblages at sites occupied by the mussel. This suggests that the changes observed in the fish community of the Warkocz River may also occur in other rivers, potentially affecting *U. crassus* populations at larger spatial scales. However, both infestation success and the strength of the dilution effect will depend on the local environmental context[27]: it is the complete set of available fish—both hosts and dead-end hosts—that determines infestation success, which implies that other bottom-foraging species, such as the common nase (*Chondrostoma nasus*), may substantially reduce the dilution effect exerted by bottom-dwelling dead-end hosts. Recent changes in fish community composition due to restocking efforts[32,33], biological invasions[34], and other anthropogenic pressures[35] may profoundly influence host–parasite dynamics. While declines in freshwater mussels are often attributed to reductions in host fish populations[36], alterations in the structure of non-host communities may also play a role by reducing the availability of glochidia to competent hosts. Although the importance of host species composition in completing the complex unionid life cycle is well established[37], our findings suggest that host behaviour—and in particular the behavioural interactions between competent and dead-end species—is also important.

If a small subset of the fish community can intercept and remove a large share of glochidia due to specific foraging behaviours, then such behavioural traits become a key determinant of parasitic success and, consequently, of conservation outcomes for endangered mussels in low-density populations. Emerging evidence from other systems supports this view: in trematodes, the invasive snail *Potamopyrgus antipodarum* intercepts miracidia passively through its benthic behaviour, reducing infection in native snails[3]. Similarly, *Schistosoma mansoni* larvae may be diverted toward resistant or evasive snail species[7]. In terrestrial systems, the Virginia opossum (*Didelphis virginiana*) eliminates large numbers of ticks via grooming, acting as an ecological sink[38].

These examples highlight the need to consider not only species richness and host competence, but also host behaviour and spatial ecology when investigating parasite transmission dynamics and developing conservation strategies for parasitic taxa such as *Unio crassus*.

Methods

The study was conducted in 2015–2016 in the Warkocz River, located at the foothills of the Świętokrzyskie Mountains in central Poland. The Warkocz is a primary tributary of the Nida River (Vistula basin, Baltic Sea catchment). It is 17.5 km long and drains a 54 km² catchment consisting of forested and agricultural landscapes. The river retains a largely natural character along most of its length, exhibiting a pool–riffle and meandering structure, and is incised approximately 2 m into the surrounding terrain. Channel width ranges from 5 to 10 m, and water depth varies from 0.1 m in riffles to 1.5 m in pools during low-flow periods.

The riverbed is predominantly sandy, though patches of silt, fine sediment, loam, gravel, and rocks are also present. In-stream vegetation is generally sparse, with occasional occurrences of *Batrachium* spp., *Sagittaria sagittifolia*, and submerged bank-root systems; isolated individuals of *Nuphar luteum* were found in pools. The riparian zone is dominated by *Alnus glutinosa* stands, often adjacent to meadows. The Warkocz River is protected under the EU Habitats Directive as part of the Natura 2000 site "Dolina Warkocza" (PLH260021). The *Unio crassus* population within this site is monitored regularly as part of the national Natura 2000 monitoring programme and has been declining.

Three study plots (plot 1: 50°50'25.0"N, 20°45'27.0"E; plot 2: 50°50'22.9"N, 20°45'26.8"E; plot 3: 50°50'22.2"N, 20°45'22.3"E; all at 257.9–258.5 m a.s.l.) were selected within the Warkocz River to represent slightly distinct habitat types occupied by *U. crassus*. Each plot consisted of a river reach, 60–100 m in length, and the plots were separated by 100 m stretches that were not surveyed. In early March of each study year, immediately after ice melt, each plot was precisely mapped using a regular grid of measurement points (1 m intervals longitudinally, 0.2 m crosswise, and 2 cm vertical resolution for depth).

The Warkocz River supports a viable, though fluctuating, population of *Unio crassus* with evidence of active recruitment. As this is the only unionid species present, any glochidia observed on fish could be unequivocally attributed to *U. crassus*.

All *U. crassus* individuals were mapped within each plot. Each year, substrates and riverbanks were carefully hand-searched to locate mussels, which were marked on detailed plot maps. Beginning in early April, individuals were examined for the presence of glochidia in the marsupia, to determine their phenology according to[39]. Once fully swollen marsupia were detected, samples of 20–30 gravid females were examined every 1–2 weeks to determine whether glochidia were mature and ready for release (i.e., displaying "snapping" behaviour). This was used to determine the appropriate timing for fish sampling.

Fish sampling and data collection

Each year, fish were sampled during three or more events coinciding with the expected peak glochidial release period in *U. crassus* (May–June): 5 May, 5 June, 18 June, and 2 July 2015; 11 May, 9 June, and 24 June 2016. The mean water temperature recorded in the study plots during May–June was 14.7 °C. According to[40], glochidia remain attached for 16–28 days at 17 °C. Thus, if release occurred in mid-May, glochidia would be detectable on fish until mid-June. Since the dilution effect was expected only under high glochidial densities, analyses were restricted to the first 10 days of June in both years.

Each sampling event consisted of a single upstream pass using a battery-powered backpack electrofishing unit (IUP-12, Radet, Poland). Fish were immediately passed to staff responsible for recording the number of glochidia attached to fins. Specimens were identified to species, measured to the nearest 0.5 cm to minimize handling time, and examined for glochidial attachment. All fish were released after full recovery from electronarcosis, in areas unaffected by the electric field.

Spatial location of each captured individual was recorded during sampling. Prior to data collection, when the earliest signs of infestation were detected, a subset of fish from the site (three *G. gobio* and three *P. phoxinus*) was sacrificed to verify the presence of glochidia under a microscope and confirm the identification of Unio larvae[12]. Whenever doubts arose, we verified them directly in the field using a pen microscope; however, this was done only rarely, as the extended procedure could potentially harm the examined fish.

Random buffers and spatial distribution in the Warkocz river

Using QGIS (v3.14 “Pi”, Open Source Geospatial Foundation), random points were generated within a shapefile representing the Warkocz riverbed. These were used to define 5 m buffers along the channel. Spatial data layers for *U. crassus*, *G. gobio*, and *P. phoxinus* were intersected with each buffer, resulting in a geospatial dataset assigning occurrence values to each buffer. In total, 50 random buffers were generated per plot, resulting in 150 buffers, repeated across two years (2015 & 2016), for a total of 300 records.

Large-scale spatial analysis for Poland and its data sources

Occurrence records of *Unio crassus* were obtained from a curated distribution dataset[41], comprising 424 georeferenced records from 71 rivers. Fish assemblage data originated from the Polish State Environmental Monitoring programme (GIOŚ) and included 4,867 surveys conducted in 2,259 rivers between 2011 and 2024 under the EU Water Framework Directive. Each record contained precise site coordinates, species composition, and abundance.

Spatial matching of *U. crassus* records with fish survey sites was performed in QGIS (v3.14). Vector layers of mussel localities and fish monitoring sites were intersected with the national hydrographic network (MPHP). Fish surveys located within 5.0 km and 0.2 km along the river network from *U. crassus* records were retained, yielding 158 surveys from 122 sites in 46 rivers (< 5 km) and 52 surveys from 35 sites in 24 rivers (< 0.2 km). Records referring to different watercourses or exceeding distance thresholds were excluded.

Co-occurrence frequency of the seven most frequently associated fish species was calculated as the proportion of surveys in which a species was present. For each survey, the percentage contribution of individuals belonging to these seven species was computed, and mean values were derived for both spatial categories. Results are presented in Fig. 1.

Controlled laboratory experiment

To assess host suitability for *U. crassus*, artificial infestations were conducted. Fish were collected via electrofishing in May 2022 from the same study plots. Only individuals free of visible glochidia on fins or opercula were selected. Ten *G. gobio* and ten *P. phoxinus* were transported in oxygenated river water to the lab. Gravid female mussels were collected by hand from the same locations. Marsupia were punctured with syringes and inspected under a microscope for the presence of active, snapping glochidia[39]. Five gravid females were transported in aerated water to a 96-L tank without mechanical filtration. The tank was monitored daily for larval release.

Once conglutinates were visible, fish were introduced for inoculation. Water was vigorously aerated and hand-stirred to prevent sedimentation and encourage fish movement. Exposure lasted 20 min.

Post-inoculation, fish were placed individually in 13-L tanks within a recirculating aquaculture system, with surface overflow and filtered, aerated water. A 2 mm mesh grid at the tank bottom prevented fish from consuming settled juveniles. On day 5, fish were examined for attached glochidia on fins and opercula. Every 3–4 days, tanks were flushed through plankton nets and juveniles were counted under stereomicroscopes. Juvenile counts were recorded per individual. Sampling continued until no further juveniles were observed (day 44). Laboratory temperature was maintained at 16 °C. All animals were released at their collection sites upon experiment completion.

Statistical analysis

Differences in abundance between dominant fish species were tested using proportion Z-tests. To assess the effects of *G. gobio* infestation level, *G. gobio* abundance, and *U. crassus* density on glochidial load in *P. phoxinus*, a Generalized Linear Model (GLZ; Poisson distribution, log link) was constructed. The response variable was the number of glochidia attached to *P. phoxinus* individuals. Predictors included: the number of *G. gobio*, number of *U. crassus*, and the number of glochidia on *G. gobio*, plus their interactions. The best-fitting model was selected using Akaike’s Information Criterion (AIC).

The threshold at which the effect of *G. gobio* on *P. phoxinus* infestation became neutral was calculated analytically from fixed-effect estimates in the GLZ model. The model included a main effect of glochidial load on *G. gobio* and an interaction with *U. crassus* abundance. The value of *U. crassus* density (N) at which the effect was neutral was derived by solving the equation:

$$\beta Gobio + \beta \text{interaction} \times N = 0$$

Rearranged:

$$\mathrm{N} = -\beta \mathrm{Gobio}/\beta \mathrm{interaction}$$

This value represents the density of mussels at which increased glochidial supply fully offsets the negative effect of *G. gobio* on *P. phoxinus*, effectively neutralizing the dilution effect.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 12 May 2025; Accepted: 11 December 2025

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Acknowledgements

The study was supported by statutory funds of the Institute of Nature Conservation, Polish Academy of Sciences. The study was conducted on the basis of permit WNP.6401.190.2014.RN-2, granted to study a protected species (*U. crassus*). J.D. and K.T. holds a license for conducting electrofishing in accordance with Polish legal requirements.

Author contributions

J.D. and T.A.Z. conceived the idea and designed the study. A.M.Ć., J.D., A.L., K.T., K.Z. and T.A.Z. collected the data. J.D., T.A.Z. A.M.Ć., and K.Z. analysed, interpreted and visualised the data. J.D. and T.A.Z. wrote the main text of the manuscript. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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