

**Rola wybranych gatunków ryb jako żywicieli larw
chronionej skójki gruboskorupowej *Unio crassus* Philipsson, 1788**

**The role of chosen fish species as hosts for larvae
of the protected thick-shelled river mussel *Unio crassus* Philipsson, 1788**

Jacek Dołęga



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“In nature, nothing exists alone.”

- Rachel Carson

AUTOR:

MGR INŻ. JACEK DOŁĘGA

Instytut Ochrony Przyrody Polskiej Akademii Nauk

Al. Adama Mickiewicza 33, 31-120 Kraków

PROMOTOR:

DR HAB. TADEUSZ ZAJĄC, PROF. IOP PAN

Instytut Ochrony Przyrody Polskiej Akademii Nauk

Al. Adama Mickiewicza 33, 31-120 Kraków

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- (I) **Dolega, J.**, Zając, T.A., Ćmiel, A. Lipińska A., Tatoj K., Zając K. 2025. Evidence for dilution effect by *Gobio gobio*, a dead-end host in the *Unio crassus*–Cyprinidae coevolutionary system. *Sci Rep* 16, 2832 (2026). <https://doi.org/10.1038/s41598-025-32601-x>

- (II) **Dolega, J.A.**, Ćmiel, A.M., Halabowski, D., Zając, K., Mioduchowska, M., Zając, T. 2026. Baltic sculpin *Cottus microstomus* as a host for larvae of two species belonging to *Unio crassus* complex: *Unio crassus* Philipsson, 1788 and *Unio nanus* Lamarck, 1819. *Knowl Manag Aquat Ecosyst* 2026. <https://doi.org/10.1051/kmae/2026010>

- (III) **Dolega, J.A.**, Ćmiel, A.M., Zając, K., Zając, T. 202X. Host fish availability for the *Unio crassus* complex during glochidia release period

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Streszczenie

Wszystkie gatunki dużych małży słodkowodnych z rodziny Unionidae zostały zaliczone do Europejskiej Czerwonej Listy zwierząt Ginących i Zagrożonych. Coraz większego znaczenia nabiera zatem pogłębianie wiedzy o ich ekologii, co jest szczególnie ważne z punktu widzenia skutecznego zarządzania i ochrony tej grupy. Skójką gruboskorupowa *Unio crassus* Philipsson, 1788, od niedawna rozpatrywana jako kompleks gatunków pod wspólną nazwą *Unio crassus*, należała w czasach historycznych do najszerzej rozpowszechnionych i licznych gatunków, dzisiaj zanika w bardzo szybkim tempie. Skójki te znajdują się pod ochroną gatunkową w Polsce, na gruncie prawa krajowego i Dyrektywy Siedliskowej Rady Europejskiej, posiada status „zagrożona” EN wg Czerwonej Listy Gatunków IUCN.

Małże z rodziny Unionidae posiadają szczególny rodzaj rozrodu. Wytwarzają one larwy tzw. glochidia, które rozwijają w specjalnych workach skrzelowych zwanych marsupiami, a po osiągnięciu dojrzałości są one uwalniane do toni wodnej. Wymagają one krótkiego okresu pasożytowania na odpowiednich gatunkach ryb, aby skutecznie przejść metamorfozę i po kilku tygodniach odłączyć się od żywicieli jako wolnożyjące, młodociane osobniki. Oznacza to, że ekologii małży z tej grupy nie można w pełni rozpatrywać w oderwaniu od ichtiofauny, ponieważ to właśnie relacja z rybami warunkuje zamknięcie ich cyklu życiowego w środowisku naturalnym. Przedstawiony cykl artykułów podejmuje zagadnienia dotychczas słabo rozpoznane, a w niektórych przypadkach niemal nieobecne w badaniach nad ekologią i ochroną skójki gruboskorupowej.

Pierwszy artykuł, dotyczący tzw. „efektu rozcieńczenia”, analizuje sytuację w której obecność liczego, lecz nieskutecznego gospodarza może obniżać sukces reprodukcyjny *U. crassus*. Badania koncentrują się na relacji małża z dwoma dominującymi liczebnie gatunkami ryb: strzeblą potokową *Phoxinus phoxinus* Linnaeus, 1758, będącą głównym, skutecznym gospodarzem w badanej populacji, a kielbkiem *Gobio gobio* Linnaeus, 1758, który przechwytuje glochidia uniemożliwiając ich przeobrażenie. Praca łączy dane terenowe pochodzące z rzeki Warkocz z eksperymentem w warunkach kontrolowanych i pokazuje, że *G. gobio* może działać jak „ślepy zaułek”, czyli gospodarz usuwający część puli larw dostępnych dla właściwych gospodarzy. W eksperymencie potwierdzono, że glochidia przyczepiały się do *G. gobio*, ale nie przekształcały się w młode małże, podczas gdy na *P. phoxinus* larwy przechodziły skuteczną metamorfozę. Jednocześnie analiza współwystępowania w warunkach naturalnych wykazała negatywną zależność między liczbą glochidiów na *G. gobio* i *P. phoxinus*, szczególnie przy niskim zagęszczeniu małży. Oznacza to, że efekt rozcieńczenia jest kontekstowy: staje się szczególnie istotny w populacjach o małej liczbie osobników, gdzie pewna pula glochidiów może zostać przejęta przez gatunki nie zapewniające transformacji larw *U. crassus*, dodatkowo obniżając wskaźniki skuteczności rozrodu w zanikającej populacji.

Drugi artykuł podnosi problem jakości gospodarzy z innej perspektywy: taksonomicznej i biogeograficznej. Dotyczy on głowacza szczupłego *Cottus microstomus* Heckel, 1837 jako nie rozpoznanego wcześniej gospodarza larw dwóch gatunków należących do kompleksu *U. crassus*: *U. crassus* sensu stricto oraz *U. nanus* Lamarck, 1819. Dotychczas w literaturze za jednego z najważniejszych gospodarzy tego kompleksu uznawano głowacza białopłetwego *Cottus gobio* Linnaeus, 1758, jednak weryfikacje taksonomiczne wykazały, że w części zlewnisk Europy Środkowej i Wschodniej, m.in. w dorzeczu Wisły, razem z kompleksem *U. crassus* współwystępuje nie *C. gobio*, lecz *C. microstomus*. Powstała więc istotna luka poznawcza: nie było jasne, czy gatunek ten pełni funkcję skutecznego gospodarza. Przeprowadzony w warunkach kontrolowanych eksperyment wykazał, że *C. microstomus* umożliwia skuteczne przeobrażanie glochidiów w młode małże, a jego efektywność jest porównywalna z powszechnie uznawanym, skutecznym gospodarzem, *P. phoxinus*. Wynik ten jest ważny nie tylko z perspektywy biologii ewolucyjnej, ale i ochrony przyrody, ponieważ pokazuje, że skład gatunków ryb pełniących funkcję żywicieli kompleksu *U. crassus* może być regionalnie zróżnicowany, a jego właściwe rozpoznanie wymaga m.in. precyzyjnej identyfikacji taksonomicznej ryb. Przykład wskazanego kryptycznego gatunku głowacza oznacza również, że pokrewieństwo filogenetyczne może być traktowane jako wskaźnik potencjalnego dopasowania żywicieli w przypadku, gdy status żywiciela nie jest poznany.

Ostatni artykuł skupia się na kwestii faktycznej dostępności właściwych ryb żywicielskich w miejscu i czasie uwalniania glochidiów przez samice skójkę gruboskorupowej. Badania polegały na kilkukrotnym odłowieniu ryb w obrębie kilku rzek różniących się warunkami abiotycznymi i charakterem zespołów ryb: Warkocza, Cedronu, Nidy i Czarnej Włoszczowskiej, a dodatkowo, w Smudze Umianowickiej zastosowano monitoring znacznikami typu PIT-tag do oceny ekspozycji i potencjału dyspersyjnego ryb funkcjonujących w pobliżu ławicy małży. Równolegle w każdej z wymienionych lokalizacji, prowadzono obserwację fenologii skójkę gruboskorupowej oceniając termin występowania okresu uwalniania glochidiów. Wyniki wskazują, że fenologia uwalniania glochidiów jest zmienna między rzekami i latami, a udział ryb żywicielskich w okresie lęgów skójkę waha się nawet od 12,5% do 57%. Szczególnie ważnym wnioskiem tej pracy jest to, że w większości przypadków zespoły ryb były zdominowane przez gatunki niebędące żywicielami larw *U. crassus*. Skuteczna reprodukcja *U. crassus* powinna polegać na stałości dostępności gospodarzy w okresie uwalniania larw. Najbardziej stabilnie i powszechnie występującymi gatunkami zapewniającymi dostępność gospodarzy okazały się przede wszystkim *P. phoxinus* w rzekach podgórskich oraz *L. cephalus* w ciekach nizinnych. Badania z udziałem znakowania potwierdziły ponadto, że kleń obecny był przy ławicach skójkę w sposób stały, jednocześnie wykazując potencjał do przemieszczania się na znaczne odległości, co może umożliwiać daleką dyspersję glochidiów. Jednocześnie proporcja ryb żywicielskich była zwykle niższa poza okresem uwalniania glochidiów, co oznacza, że ocena siedlisk i monitoring ryb prowadzone poza właściwym oknem reprodukcyjnym małży mogą nie odzwierciedlać realnej dostępności żywicieli.

Summary

In view All species of large freshwater mussels of the family Unionidae are included on the European Red List of threatened animals. For this reason, improving our understanding of their ecology is becoming increasingly important, particularly from the perspective of effective conservation and management. The thick-shelled river mussel, *Unio crassus* Philipsson, 1788, which has recently been treated as a species complex under the collective name *Unio crassus*, was historically one of the most widespread and abundant freshwater mussel species in Europe. Today, however, it is declining at an alarming rate. In Poland, the species is legally protected under national legislation and the EU Habitats Directive, and it is classified as Endangered (EN) on the IUCN Red List.

Unionid mussels have a highly specialized reproductive strategy. They produce larvae, known as glochidia, which develop in specialized gill chambers called marsupia. Once mature, the glochidia are released into the water column. To complete their development, they must spend a short parasitic phase attached to suitable fish hosts. After several weeks, they detach from the host as free-living juvenile mussels. This means that the ecology of unionid mussels cannot be fully understood without considering fish communities, because interactions with fish are essential for completing their life cycle under natural conditions. The series of articles presented here addresses issues that have so far been poorly explored, and in some cases almost entirely overlooked, in studies on the ecology and conservation of the thick-shelled river mussel.

The first article focuses on the so-called “dilution effect”, examining a situation in which the presence of an abundant but ineffective host may reduce the reproductive success of *U. crassus*. The study centres on the relationship between the mussel and two numerically dominant fish species: the Eurasian minnow, *Phoxinus phoxinus* Linnaeus, 1758, which was the main effective host in the studied population, and the gudgeon, *Gobio gobio* Linnaeus, 1758, which intercepts glochidia but does not allow them to complete metamorphosis. Combining field data from the Warkocz River with a controlled experiment, the study shows that *G. gobio* may act as a “dead-end” host, removing part of the larval pool that would otherwise be available to suitable hosts. The experiment confirmed that glochidia attached to *G. gobio*, but did not transform into juvenile mussels, whereas larvae attached to *P. phoxinus* successfully completed metamorphosis. At the same time, field analyses of co-occurrence revealed a negative relationship between the number of glochidia recorded on *G. gobio* and *P. phoxinus*, particularly at low mussel densities. This indicates that the dilution effect is context-dependent: it becomes especially important in small, declining populations, where part of the glochidial pool may be intercepted by species that do not support larval transformation, further reducing reproductive efficiency.

The second article approaches host quality from a different angle: taxonomy and biogeography. It examines the smallhead sculpin, *Cottus microstomus* Heckel, 1837, as a previously unrecognized host

for larvae of two species belonging to the *U. crassus* complex: *U. crassus* sensu stricto and *U. nanus* Lamarck, 1819. Until now, the European bullhead, *Cottus gobio* Linnaeus, 1758, has been widely regarded as one of the most important hosts for this mussel complex. However, taxonomic revisions have shown that in parts of Central and Eastern Europe, including the Vistula River basin, the species co-occurring with the *U. crassus* complex is not *C. gobio*, but *C. microstomus*. This created an important knowledge gap: it was unclear whether *C. microstomus* could function as an effective host. A controlled experiment demonstrated that *C. microstomus* allows glochidia to successfully transform into juvenile mussels, and that its effectiveness is comparable to that of *P. phoxinus*, a widely recognized suitable host. This finding is important not only from the perspective of evolutionary biology, but also for conservation, as it shows that the composition of fish host species used by the *U. crassus* complex may vary regionally and requires precise taxonomic identification of fish hosts. The case of this cryptic sculpin species also suggests that phylogenetic relatedness may serve as a useful indicator of potential host suitability when the host status of a species is unknown.

The final article focuses on the actual availability of suitable fish hosts in the right place and at the right time, namely during the period when female thick-shelled river mussels release their glochidia. The study involved repeated fish sampling in several rivers that differed in abiotic conditions and fish community structure: the Warkocz, Cedron, Nida and Czarna Włoszczowska rivers. In addition, PIT-tag monitoring was used in the Smuga Umianowicka to assess the exposure and potential dispersal capacity of fish occurring near a mussel bed. In parallel, the reproductive phenology of *U. crassus* was monitored at each site to determine the timing of glochidial release. The results show that the timing of glochidial release varies among rivers and years, while the proportion of host fish present during the mussel breeding period ranged from 12.5% to 57%. One of the most important conclusions of this study is that, in most cases, fish communities were dominated by species that do not serve as hosts for *U. crassus* larvae. Effective reproduction in *U. crassus* therefore depends on the stable availability of suitable hosts during the larval release period. The most stable and widespread host species were *P. phoxinus* in foothill rivers and *L. cephalus* in lowland streams. Tagging data further confirmed that chub were consistently present near mussel beds and, at the same time, showed the potential to move over considerable distances, which may facilitate long-distance dispersal of glochidia. Importantly, the proportion of host fish was usually lower outside the glochidial release period. This means that habitat assessments and fish monitoring carried out outside the mussel's reproductive window may fail to reflect the true availability of suitable hosts.

Wprowadzenie do tematu badań

Procesy adaptacyjne organizmów żywych można podzielić na adaptacje do czynników środowiska nieożywionego, które w znacznym stopniu mogą być przewidywalne (np. sezonowość środowiska), oraz adaptacje do środowiska ożywionego, generującego dynamiczne relacje i sprzężenia zwrotne wynikające z interakcji między organizmami (Pianka 1978). Interakcje obejmują m.in. pasożytnictwo, które charakteryzuje się dość ścisłym powiązaniem pasożyta i gospodarza, którzy wchodzi w, na ogół ścisły, mechanizm koewolucji Ehrlich & Raven's (1964): pasożyt rozwija swoje przystosowania wiodące do zarażania żywiciela, podczas gdy żywiciel rozwija środki obrony przed zarażeniem, co wymaga z kolei rozwinięcia jeszcze bardziej specjalistycznego systemu zarażania przez pasożyta, prowadząc do całkowitego skupienia przystosowań na jednym lub niewielu gatunkach, w których pasożyt zaczyna się specjalizować. Niesie to jednak poważne niebezpieczeństwo uzależnienia się pasożytów od istnienia gospodarza, a w przypadku jego wymarcia, razem z nim wymiera pasożyt (kaskada wymierań, Strona 2015). Powstaje pytanie szczególnie interesujące z punktu widzenia koewolucji, czy pasożyt musi być powiązany z jednym gatunkiem lub ich grupą czy może też związek ten dotyczyć wielu gatunków stwarzając alternatywę w momencie zmian w liczebności gospodarza (Janzen, 1980)? Po drugie, czy pozostałe gatunki w danym ekosystemie mogą mieć wpływ na tę zależność, czy ze względu na koewolucję pozostają bez wpływu na powiązanie pasożyta z gospodarzem? Po trzecie, czy różnicowanie się gatunków niejako dziedziczy pasożyty, innymi słowy, czy gatunki spokrewnione są realną alternatywą dla wymierającego gospodarza w sytuacji kaskady wymierań? Pytania te stanowią bezpośrednie zastosowanie teorii ewolucji w teorii ochrony przyrody.

Istnieje dyskusja na temat ochrony pasożytów lub raczej siedlisk z nimi związanych. W ekologii i ochronie przyrody pasożyty nie są wyłącznie elementem negatywnym, lecz mogą stanowić istotny wskaźnik stanu ekosystemu. Szczególnie wysoka różnorodność pasożytów o złożonych cyklach życiowych wskazuje na dobrze zachowaną i funkcjonalną biocenozę, ponieważ ich cykle życiowe wymagają obecności wielu gatunków żywicieli oraz stabilnych powiązań troficznych. W tym sensie obecność pasożytów odzwierciedla spójność i złożoność sieci ekologicznych. Należy jednak podkreślić, że nie sama liczebność pasożytów, lecz ich różnorodność i struktura są kluczowe – wysoka intensywność infestacji jednego gatunku może bowiem świadczyć o zaburzeniach środowiskowych lub osłabieniu populacji gospodarzy. Dlatego pasożyty, właściwie interpretowane, mogą stanowić cenne narzędzie oceny jakości i stabilności ekosystemów (Hudson et al. 2006).

Koewolucja pasożytów i ich gospodarzy przyczynia się do zróżnicowania układów biologicznych bowiem należy do najbardziej dynamicznych procesów ewolucyjnych w przyrodzie. Dawniej zakładano, że w toku ewolucji zjadliwość pasożytów powinna stopniowo maleć, ponieważ nadmierne osłabienie lub śmierć gospodarza jest niekorzystna również dla samego pasożyta. Współcześnie wskazuje się jednak, że wirulencja pasożyta stanowi wynik kompromisu pomiędzy

efektywnością transmisji a stopniem eksploatacji gospodarza (Anderson & May 1982; Ewald 1994). Oznacza to, że zjadliwość nie musi zanikać, lecz stabilizuje się na poziomie trade-off, maksymalizującym sukces reprodukcyjny pasożyta i przeżycie gospodarza. W środowisku biotycznym powstają bowiem sprzężenia zwrotne: gospodarze rozwijają mechanizmy odpornościowe, podczas gdy pasożyty ewoluują strategie zwiększające skuteczność infekcji. Proces ten został opisany m.in. przez hipotezę Czerwonej Królowej (Van Valen 1973), zgodnie z którą organizmy muszą nieustannie ewoluować, aby utrzymać swoją pozycję w stale zmieniającym się środowisku biologicznym.

Niektóre przykłady koewolucji mają dość specyficzny charakter. Mięczaki to drugi co do liczebności gatunków typ w obrębie Animalia, zaraz po stawonogach. Jego liczną grupę stanowią duże słodkowodne małże z rzędu Unionida, które rozwinęły dość specyficzny sposób rozmnażania się, oparty na pasożytniczej larwie, glochidium. Glochidium małży z rzędu Unionida stanowi wyspecjalizowane stadium larwalne, które najprawdopodobniej wyewoluowało jako adaptacja do ograniczeń dyspersji w środowisku słodkowodnym. W przeciwieństwie do morskich małży, których larwy rozwijają się planktonicznie, przodkowie Unionida funkcjonowali w warunkach kierunkowego transportu larw (zawsze w dół rzeki), co sprzyjało ewolucji trochofory w kierunku wyspecjalizowanej larwy, zdolnej do przyczepiania się do innych organizmów i w ten sposób wykorzystujących ryby jako wektory dyspersji. Kolejne etapy ewolucji obejmowały rozwój struktur umożliwiających skuteczną infekcję oraz koewolucję z gospodarzami, prowadzącą do wysokiej specyficzności i złożonych strategii infekcyjnych (Kat 1984, Bauer & Wächtler 2001, Haag 2012, Barnhart et al. 2008). W przypadku małży z rzędu Unionida oraz ich rybich żywicieli prowadzi to do subtelnej równowagi pomiędzy skutecznością infestacji glochidiami a odpornością ryb (Rock et al. 2022).

Problem w ochronie przyrody, który szczególnie nasilił się w ostatnich dekadach to problem wymierania dużych małży słodkowodnych z rzędu Unionida (Bogan 1993). Wymienia się tutaj wiele przyczyn (Sousa et al. 2023, Ferreira-Rodríguez et al. 2019), jednakże chociaż stosunkowo wiele uwagi poświęca się problemowi koewolucji małży i ryb, to jednak badania tej relacji od strony dostępności ryb gospodarzy i składu gatunkowego fauny ryb na demografię małży są stosunkowo słabo analizowane. Zależność unionidów od ryb spowodowała, że priorytetem było analizowanie podatności ryb jako gospodarzy, co sprawdzano głównie w eksperymentach hodowlanych (Zale and Neves 1982, Geist *et al.* 2023), podczas gdy rzeczywista podatność na zarażanie i dostępność żywicieli w naturalnych siedliskach w Europie pozostawała nieznana.

Pojawiły się badania, które pokazywały znaczną zmienność podatności ryb żywicielskich na infestację glochidiami małży z rzędu Unionida. Zmienność ta występuje zarówno pomiędzy gatunkami ryb, jak i pomiędzy populacjami tego samego gatunku, co wskazuje na możliwość lokalnych procesów koewolucyjnych (Douda et al. 2017; dos Santos et al. 2024). Wykazano również, że wcześniejszy kontakt z glochidiami może prowadzić do rozwoju odporności immunologicznej gospodarzy,

ograniczającej sukces metamorfozy larw. Podatność ryb zależy ponadto od ich kondycji fizjologicznej i poziomu stresu, który może zwiększać skuteczność infekcji (Douda et al. 2018). Wyniki te wskazują, że relacja małża–ryba ma charakter dynamicznego układu koewolucyjnego, a sukces reprodukcyjny małży zależy nie tylko od obecności odpowiednich gatunków żywicieli, lecz także od ich lokalnej historii ewolucyjnej i stanu fizjologicznego. Pojawiło się zatem pytanie o skład gatunkowy ryb-gospodarzy, na ile kształtuje on sukces zarażeń larwami małży, jaki wpływ mają relacje filogenetyczne na podatność na zarażanie, pojawia się pytanie o odporność i wpływ stosunków ilościowych w składzie gatunkowym ryb na efektywność zarażania.

Efektywność przeobrażeń larw małży zależy oczywiście od możliwości infestacji właściwych gatunków ryb. Aby ta efektywność była najwyższa okres uwalniania glochidiów musi się pokrywać w czasie i przestrzeni z dostępnością ryb gospodarzy. Standardowe badania ichtiologiczne są na ogół wykonywane na jesieni, poza okresem rozrodu małży, co dodatkowo utrudnia analizę dostępności żywicieli. Brak tego rodzaju badań w Europie, a pojedyncze istniejące badania, wskazują na ścisłą specjalizację małży, wynikająca nie tyle z ich przystosowań co z ograniczonej dostępności zróżnicowanych gatunków żywicieli (Lemand et al. 2016, Schneider et al. 2019).

W nawiązaniu do powyższych problemów, niniejszej rozprawie podjęto się ich analizy u ginącego gatunku małża słodkowodnego, *Unio crassus*, poszukując odpowiedzi na następujące pytania:

- (1) Jaki jest poziom zarażenia larwami ginącego gatunku małża w odniesieniu do gatunków ryb żywicielskich i nieżywicielskich oraz jaki jest wpływ zarażania larwami tych ostatnich? (artykuł nr 1)
- (2) Czy pokrewieństwo filogenetyczne za znanym żywicielem może być wskaźnikiem statusu żywiciela dla gatunku nowo opisanego? (artykuł nr 2)
- (3) Jaka jest dostępność ryb żywicielskich dla populacji *Unio crassus* w stanie naturalnym w czasie i przestrzeni? (artykuł nr 3)

Synteza i odpowiedzi na pytania badawcze

Trzy przedstawione badania pokazują, że sukces reprodukcyjny małży z kompleksu *Unio crassus* jest determinowany przez **złożony układ interakcji ekologicznych**, obejmujący charakterystyczny dla siedliska skład gatunkowy zespołu ryb o różnej podatności jako gospodarzy i różnej roli w przechwytywaniu glochidiów, przy słabym wpływie zróżnicowania genetycznego w obrębie jednego rodzaju. Czas uwalniania glochidiów jest dostosowany do zwiększonej dostępności ryb gospodarzy, w tym gatunków gwarantujących szerokie rozprzestrzenienie w systemach rzecznych.

Ad 1. Gatunki żywicielskie (np. *Phoxinus phoxinus*) w stanie naturalnym mają dość niski poziom infestacji w porównaniu do warunków hodowlanych, natomiast niejednokrotnie bardziej liczne gatunki nieżywiciele (np. *Gobio gobio*) osiągają podobny poziom infestacji i mogą przechwytywać znaczną część larw, nie wspierając ich metamorfozy. W efekcie powstaje efekt rozcieńczenia (*dilution effect*), kiedy glochidia umierają na nieodpowiednich gospodarzach, co zmniejsza sukces reprodukcyjny małży. Kluczowe jest to, że efekt ten jest zależny od kontekstu: najsilniej działa przy niskiej liczebności małży, a jego znaczenie rośnie wraz z dominacją gatunków nieżywicielek w zespole ryb, stanowiąc zagrożenie dla nielicznych, zamierających populacji małży, ograniczając rekrutację i zwiększając ryzyko ich wymierania.

Ad 2. Teoretycznie blisko spokrewnione gatunki ryb mogą różnić się zdolnością do pełnienia funkcji żywiciela, jednak w przypadkach ujawnionych różnic filogenetycznych między kryptycznymi gatunkami ryb (np. *Cottus microstomus* vs *Cottus gobio*) efektywność metamorfozy glochidiów jest u nowo opisanego żywiciela porównywalna z najlepszym żywicielem *Unio crassus* jakim jest *Phoxinus phoxinus*. Oznacza to, że w przypadku braku znajomości efektywności danego żywiciela, pokrewieństwo filogenetyczne może być wskazówką dla doboru ryb żywicieli w programach ochrony.

Ad 3. Rzeczywista dostępność żywicieli jest funkcją zarówno ich liczebności, jak i synchronizacji z okresem uwalniania glochidiów. Zespoły ryb w czasie uwalniania glochidiów są stosunkowo bogate (9–13 gatunków), ale tylko niewielką część stanowią potencjalni żywiele (3–5 gatunków), a efektywną rolę żywiciela pełnią zwykle 1–2 gatunki dominujące. Dostępność żywicieli jest silnie zmienna w czasie, jednak najwyższa w okresie uwalniania larw przez małże, co wskazuje na dostosowanie zachowania małży do obecności ryb. Standardowe procedury monitoringu często nie obejmują tego kluczowego okresu. Ruchliwość niektórych gatunków ryb żywicielskich (np. *Leuciscus cephalus*) wskazuje na znaczne możliwości rozprzestrzeniania gatunku małża korzystającego z tego żywiciela. Wobec między- i wewnątrz-sezonowego zróżnicowania w czasie epizodów uwalniania glochidiów i dostępności ryb efektywność przeobrażania glochidiów jest zapewne cechą zmienną i zależną od lokalnych warunków ekologicznych.

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Załączniki

Attachments

Artykuł (I)

Dolega, J., Zając, T.A., Ćmiel, A. Lipińska A., Tatoj K., Zając K. 2025. Evidence for dilution effect by *Gobio gobio*, a dead-end host in the *Unio crassus*–Cyprinidae coevolutionary system. *Sci Rep* 16, 2832 (2026). <https://doi.org/10.1038/s41598-025-32601-x>

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

Jacek Dołęga, Tadeusz A. Zając✉, Adam Ćmiel, Anna Lipińska, Krzysztof Tatoj & Katarzyna Zając

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 evolution¹. 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 loss². Ongoing large-scale changes in biodiversity and community structure may prevent many parasitic transmission stages from encountering suitable hosts³. 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 simplified⁴, 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 systems⁵, 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 individual⁸. The primary ecological role of fish hosts is thought to be the dispersal of mussel larvae over greater distances⁹. Because mussels exist as metapopulations, host-mediated dispersal enables range expansion and colonization of new habitats within river systems¹⁰.

Institute of Nature Conservation, Polish Academy of Sciences, Al. Adama Mickiewicza 33, Kraków 31-120, Poland.
✉email: tzajac@iop.krakow.pl

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 item¹¹, including the larvae themselves¹².

The number of glochidia released by adult mussels is generally consistent and size-dependent¹³, with individuals typically reaching an asymptotic size in a given habitat¹⁴. 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 surface¹¹. 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 h¹¹.

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		Change
	Species	Plot 1	Plot 2	Plot 3	Total		All plots	All plots	All plots	Total		
		(N)	(N)	(N)	(N)		%	(N)	(N)	(N)		
1	<i>Alburnus alburnus</i>	0	1	0	1	0.0	0	0	0	0	0	0.0
2	<i>Barbatula barbatula</i>	93	113	207	413	17.1	58	8	27	93	7.1	−10.0
3	<i>Carassius gibelio</i>	0	1	0	1	0	0	1	0	1	0.1	0.1
4	<i>Cottus gobio</i>	0	0	7	7	0.3	3	0	0	3	0.2	−0.1
5	<i>Eudontomyzon mariae</i> *	49	22	12	83	3.4	7	5	3	15	1.1	−2.3
6	<i>Gobio gobio</i>	92	119	250	461	19	234	92	128	454	34.6	15.6
7	<i>Leucaspius delineatus</i>	2	0	3	5	0.2	0	0	0	0	0	−0.2
8	<i>Phoxinus phoxinus</i>	422	244	638	1304	53.9	303	66	74	443	33.8	−20.1
9	<i>Rhodeus amarus</i>	32	32	24	88	3.6	102	112	40	254	19.4	15.8
10	<i>Salmo trutta</i>	19	6	12	37	1.5	28	2	14	44	3.3	1.8
11	<i>Squalius cephalus</i>	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	<i>Unio crassus</i>											
	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 water¹¹ or employing species-specific lures¹⁷ 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 hosts¹⁸. Instead, they behave like particulate matter temporarily suspended in water, with their concentration measurable in quantitative terms¹⁹. 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	<i>Gobio gobio</i>	64	19	3.1	0	12
		<i>Phoxinus phoxinus</i>	295	548	90.6	0	23
	2	<i>Gobio gobio</i>	66	54	16.8	0	42
		<i>Phoxinus phoxinus</i>	156	263	81.7	0	14
	3	<i>Gobio gobio</i>	185	222	33.8	0	57
		<i>Phoxinus phoxinus</i>	370	419	63.8	0	20
2016	1	<i>Gobio gobio</i>	28	0	0.0	0	0
		<i>Phoxinus phoxinus</i>	127	187	99.5	0	13
	2	<i>Gobio gobio</i>	53	2	2.0	0	1
		<i>Phoxinus phoxinus</i>	88	100	98.0	0	11
	3	<i>Gobio gobio</i>	65	7	3.3	0	3
		<i>Phoxinus phoxinus</i>	268	198	92.1	0	11
2015–2016	total	<i>Gobio gobio</i>	461	304	14.6	0	57
	total	<i>Phoxinus phoxinus</i>	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.

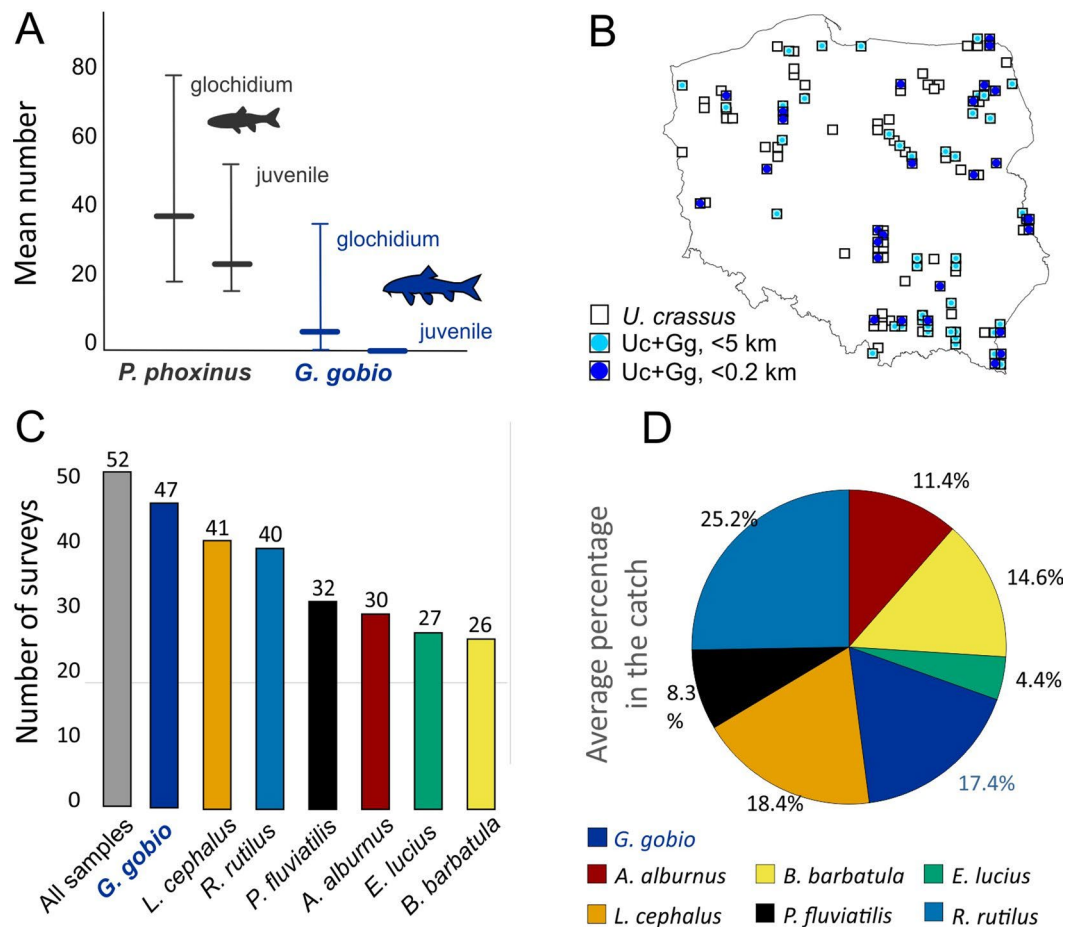


Fig. 1. The role of *G. gobio* as a decoy species. **(A)** Results of the infestation experiment: mean number of glochidia attached to fish five days after artificial infestation and mean number of successfully metamorphosed juveniles recovered over 40 days for *P. phoxinus* and *G. gobio*. Whiskers 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	<i>p</i>
Intercept	2.94	0.027	11,469	<0.00001
<i>G. gobio</i> Sum glochidia	−0.0133	0.0023	34.5	<0.00001
N Uc	−0.0002	0.0005	0.12	0.726
N Uc* <i>G. gobio</i> 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.

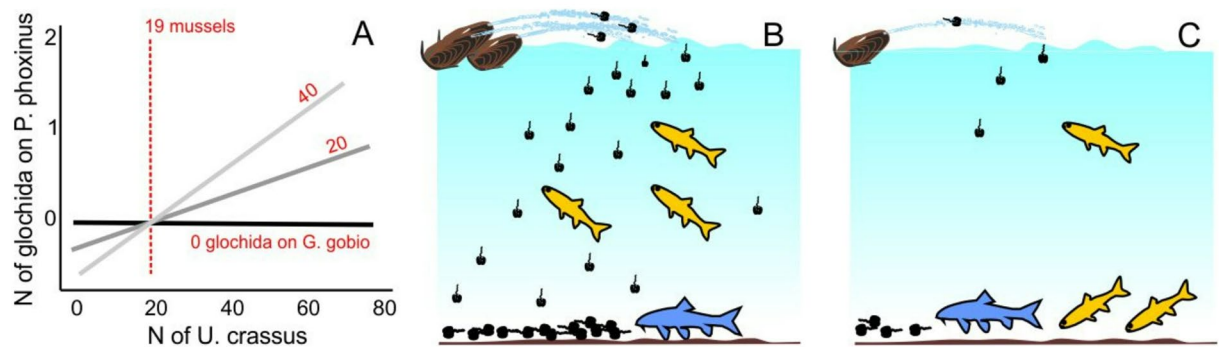


Fig. 2. Conceptual model of “foraging-based” dilution: (A) graphical representation of the interaction between *U. crassus* density and glochidia load on the two dominant fish species; (B) at high mussel densities, large quantities of glochidia are released over a limited water surface, *P. phoxinus* 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 competition²³.

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 spurring 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 effect²⁴. 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 (Zajac 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 production²⁵. 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 temperature¹¹. 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 barbels³⁰, 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 species³¹. 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 context²⁷: 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 invasions³⁴, and other anthropogenic pressures³⁵ may profoundly influence host–parasite dynamics. While declines in freshwater mussels are often attributed to reductions in host fish populations³⁶, 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 established³⁷, 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 snails³. Similarly, *Schistosoma mansoni* larvae may be diverted toward resistant or evasive snail species⁷. In terrestrial systems, the Virginia opossum (*Didelphis virginiana*) eliminates large numbers of ticks via grooming, acting as an ecological sink³⁸.

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 to³⁹. 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 to⁴⁰, 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* larvae¹². 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 dataset⁴¹, 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 glochidia³⁹. 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 conglomerates 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:

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

Rearranged:

$$N = -\beta_{\text{Gobio}}/\beta_{\text{interaction}}$$

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.

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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

Correspondence and requests for materials should be addressed to T.A.Z.

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Artykuł (II)

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Baltic sculpin *Cottus microstomus* as a host for larvae of two species belonging to *Unio crassus* complex: *Unio crassus* Philipsson, 1788 and *Unio nanus* Lamarck, 1819

Jacek A. Dołęga^{1,*} , Adam M. Ćmiel¹, Dariusz Halabowski², Katarzyna Zajac¹, Monika Mioduchowska³ and Tadeusz Zajac¹

¹ Institute of Nature Conservation of Polish Academy of Sciences, al. A. Mickiewicza 33, 31-120 Kraków, Poland

² University of Lodz, Faculty of Biology and Environmental Protection, Department of Ecology and Vertebrate Zoology, Banacha 12/16, 90-237 Łódź, Poland

³ University of Gdańsk, Faculty of Biology, Department of Evolutionary Genetics and Biosystematics, Wita Stwosza 59, 80-308 Gdańsk, Poland

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Abstract – Freshwater mussels of the family Unionidae require an obligatory parasitic larval stage on a specific fish host species to complete their ontogeny. This relationship can be highly specific to co-evolving species and geographically variable, although information regarding host suitability remains scarce. Recent advances in molecular and phylogenetic studies of fish have uncovered cryptic host species that have yet to be tested for their role in mussel development. The genus *Cottus*, particularly European bullhead *Cottus gobio*, is known to be a primary host for the threatened thick-shelled river mussel *Unio crassus* complex, a group of cryptic species of large freshwater mussels endemic to Europe. However, the recent revision of the geographical distribution of another *Cottus* species, Baltic sculpin *Cottus microstomus*, raises questions about its suitability as a host for species in *U. crassus* complex. We report that the transformation of mussel larvae from mixed population of *U. crassus* sensu stricto and *U. nanus* into juvenile mussels is successful when *C. microstomus* serves as the host, with an effectiveness comparable to that of the well-established host Eurasian minnow *Phoxinus phoxinus*. The sympatric distribution of *C. microstomus* and *U. crassus* complex is confined to several river drainages in Central and Eastern Europe, areas that are affected by anthropogenic factors, which may pose challenges to their conservation.

Keywords: parasite-host interaction / conservation / artificial infestation / glochidia / fish host

1 Introduction

Large freshwater mussels (Unionidae, hereafter referred to as naiads) require a mandatory parasitic larval phase to complete their ontogeny. Females produce tens of thousands, or even hundreds of thousands, of tiny parasitic larvae (larval length is approximately 0.05–0.45 mm), known as glochidia (Bauer, 2001). Once fully developed, glochidia are expelled by the female through the exhalant siphon into the water, where they passively float and attract a fish host to which they attach, typically to the gills or fins (Aldridge et al., 2023) where they remain encysted for several weeks in the case of *Unio crassus* complex (Lamand et al., 2016; Schneider et al., 2017a) or even up to 11 months in *Margaritifera margaritifera* (Linnaeus, 1758) (Bauer and Vogel, 1987). During this period, the larvae

metamorphose and detach from the host as juvenile mussels. This phase is crucial for naiad reproduction, and a key concern is that not all fish species are capable of successfully hosting larvae of every naiad species. Due to co-evolution, each naiad species has a specific set of host species (Bauer and Wachtler, 2001). Moreover, local co-evolution and co-diversification can further specialise this relationship (Bauer and Wachtler, 2001; Douda et al., 2017; Neemuchwala et al., 2023).

From the perspective of both biodiversity and nature conservation, coevolution is particularly important when threatened naiad species depend on sensitive fish hosts, which could pose a risk of cascading extinctions (Modesto et al., 2018). This is the case for species belonging to *U. crassus* complex, including *Unio crassus* Philipsson, 1788 and *Unio nanus* Lamarck, 1819 – a globally endangered species (Lopes-Lima et al., 2024, Lopes-Lima and Prié, 2024) endemic to Europe, and protected by both EU (Council of the European Communities, 1992) and national regulations

*Corresponding author: dolega@iop.krakow.pl

(Journal of Laws of the Republic of Poland, 2016) under the common name of *U. crassus*. One of the best-known hosts for larvae of this species complex is the European bullhead *Cottus gobio* Linnaeus, 1758 (Teleostei: Scorpaeniformes) (Douda *et al.*, 2012; Lamand *et al.*, 2016). This species is actually one of several within the *C. gobio* group (Freyhof *et al.*, 2005), a complex often referred to as *C. gobio* sensu stricto, whose members are locally considered vulnerable (VU) (Witkowski, 2009) and are similarly, legally protected under national (Journal of Laws of the Republic of Poland, 2016) and European law (Council of the European Communities, 1992). To date, this sculpin has been tested several times for its host role in artificial infestation experiments using *U. crassus* complex glochidia under controlled conditions, e.g., by Douda *et al.* (2012), Taeubert *et al.* (2012a, 2012b) and Schneider *et al.* (2017a). The geographical origin of the fish used in these experiments suggests that, in fact, only *C. gobio* sensu stricto has been tested as a host to date. However, both morphological and molecular analyses have revealed that in the Oder, Vistula (Baltic Sea catchment), and Dniester (Black Sea catchment) river drainages, the sculpin species that co-occurs with the *U. crassus* complex is not the European bullhead *C. gobio*, but the Baltic sculpin *Cottus microstomus* Heckel, 1837 (Freyhof *et al.*, 2005; Sideleva *et al.*, 2018). A more recent study by Sideleva *et al.* (2022) shows that *C. microstomus* also occurs in river systems of the eastern Baltic Sea catchment, such as the Neman and Venta rivers (including the Krasnaya River).

Given the cases of strict co-evolution between naiads and their host species, it is crucial to identify the host species of *U. crassus* complex across its entire geographical range, particularly in relation to its threatened status. A key question concerns the role of species of the genus *Cottus*, which may serve as one of the most suitable hosts. In this study, we present the results of an experiment designed to assess whether *C. microstomus* is also a primary host for *U. crassus* complex larvae, as this fish species is found in the larger, eastern portion of *U. crassus* complex range.

2 Material and methods

2.1 Animal collection and acclimation

To test the effectiveness of *C. microstomus* as a host for mussel larvae, we conducted an artificial infestation using *U. crassus* complex glochidia under controlled conditions. As a reference species, we used the Eurasian minnow *Phoxinus phoxinus* (Linnaeus, 1758), which is widely used as a host in artificial infestation experiments and is known for its ability to transform *U. crassus* complex larvae (e.g., Taeubert *et al.*, 2012a, 2012b; Douda *et al.*, 2012, 2014; Schneider *et al.*, 2017a; Geist *et al.*, 2023). Fish and mussel sampling took place in two rivers within the Nida River system, a tributary of the Vistula River in the Baltic Sea catchment. Five gravid females belonging to *U. crassus* complex and 11 specimens of *P. phoxinus* were collected from the Warkocz River near Niestachów, while 11 specimens of *C. microstomus* were collected from the Mierzawa River near Sędowice (both sites are located in the Świętokrzyskie Voivodeship, southern Poland). At the time of the experiment, only the Warkocz River supported a significant population of mussels belonging to *U. crassus* complex, allowing for their sampling without harm

to the local population. The choice of site for obtaining *C. microstomus* specimens was deliberate: not only it is located in terra typica of the species, but also, during the recent rediscovery and redescription of Heckel's holotype from year 1837, non-type specimens from this site were confirmed as *C. microstomus* (Sideleva *et al.*, 2018). Specimens of the *U. crassus* complex had never been observed in the Mierzawa River, despite intensive surveys conducted since 1997 (unpublished data), thus ensuring that the collected *C. microstomus* specimens were naive to glochidia. A map showing the locations of animal collection sites and the cited geographic ranges of the discussed species is provided in Figure 1.

Fish were collected using a backpack electrofishing device (IUP-12A, prod. Radet, Poland) and transported to the mussel breeding facility in Krzyżanowice Średnie (Świętokrzyskie Voivodeship, southern Poland) in plastic bags filled with fresh river water and oxygen. At the site of sympatric occurrence of *P. phoxinus* and *U. crassus*, fish were inspected for visible glochidia attached to their fins or gills by gentle opening of the operculum. Only specimens appearing free of larvae were selected for the experiment. Even though *C. microstomus* is the only *Cottus* species inhabiting the Vistula river drainage, some anatomic traits were checked during fish collection: e. g. smooth dorsal profile (no hump behind the head), upper jaw reaching the anterior margin of an eye, lobular genital papilla occurrence in males and distinct narrowness of caudal peduncle (Sideleva *et al.*, 2018). Total length of all fish was measured to the nearest 1 mm. Fish body weight data were not collected, however, considering the different allometry of *C. microstomus* and *P. phoxinus*, body mass of each specimen was calculated using the length–weight relationship equation with coefficients specific to the species or family, as determined by Froese *et al.* (2014). At the breeding facility, fish were placed individually in 13-litre tanks within a recirculating aquaculture system for acclimation. Each tank, equipped with a surface overflow, was constantly supplied with aerated and purified water with controlled parameters. Encystation and transformation of larvae occurred within these tanks. The entire facility was air-conditioned, maintaining a constant temperature. Following previous findings that glochidia viability and metamorphosis of larvae depend on water temperature (e.g., Zimmerman and Neves, 2002; Taeubert *et al.*, 2014; Lamand *et al.*, 2016; Benedict *et al.*, 2021), and that mussel reproduction is adapted to local thermal conditions (Schneider *et al.*, 2017b), we maintained a temperature of 16°C. This approximated natural environmental conditions while allowing efficient larval transformation and minimizing thermal stress to animals. Fish acclimation lasted for eight days.

Adult mussels (age class 5+) were located while wading in the Warkocz River, hand-collected, sexed, and inspected for fully developed, “snapping” glochidia using marsupial puncture, as described by Zajac and Zajac (2021). Five gravid females of *U. crassus* were transported in a box with fresh, aerated water and ice to the breeding facility, where they were placed in a 96-litre aerated tank. The tank was checked daily to monitor whether glochidia were released into the water, and whether the gentle opening of the valves in each specimen revealed any changes in the marsupial fill.

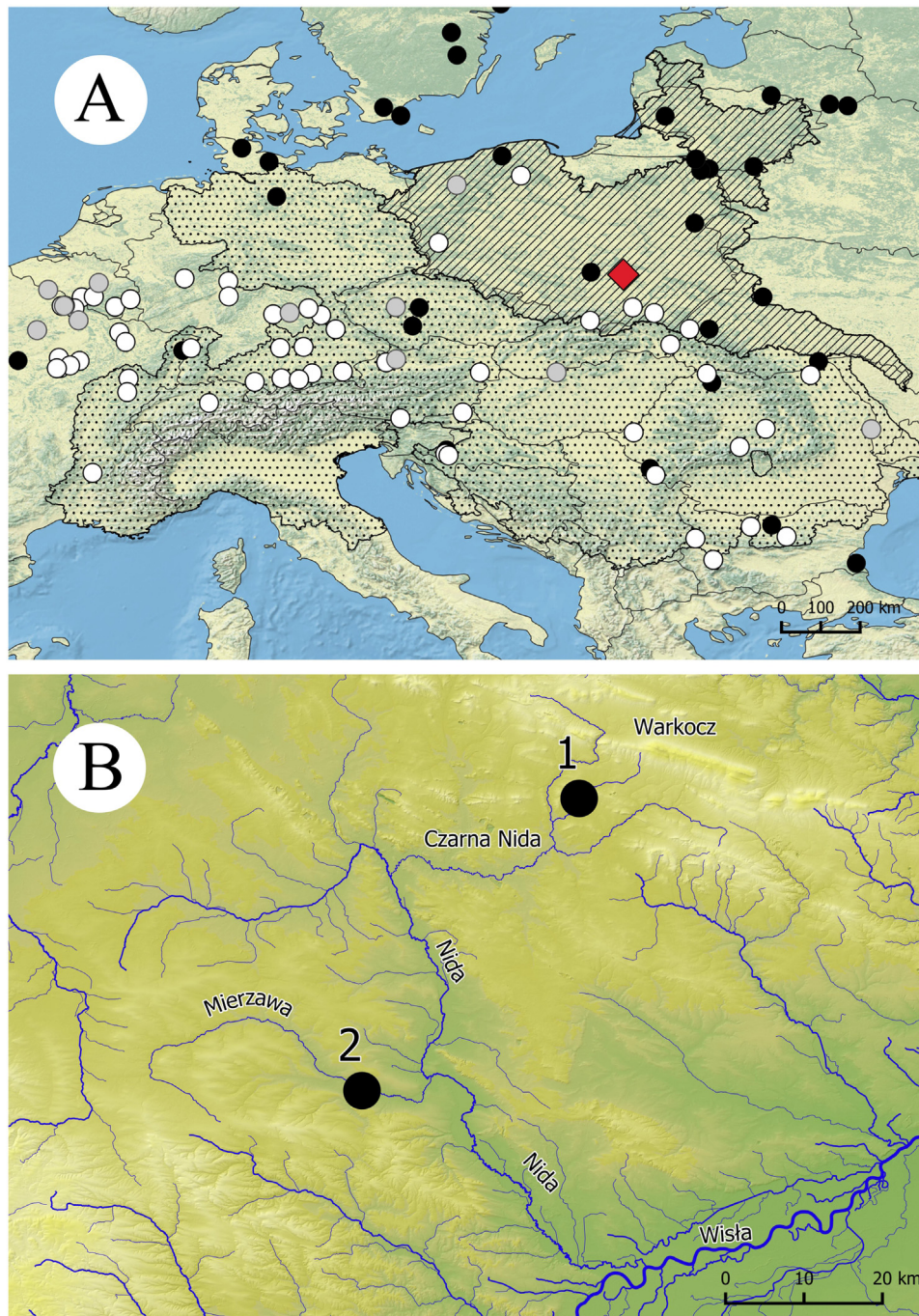


Fig. 1. A map of geographical ranges of discussed species and the study location; A) range of occurrence of *C. microstomus*: a polygon with hatched fill -spatial data was provided by IUCN (MfN Berlin, 2021b) and updated by authors to include drainages of Neman and Venta, as no available spatial data for Krasnaya river drainage was found; and *C. gobio*: a polygon with dotted fill -spatial data provided by IUCN: (MfN Berlin, 2021a); points indicate exact sites of collection of specimens used in the study by Lopes-Lima *et al.* (2024) to assess *U. crassus* complex genetic divergence (limited to the map range): black points - *U. crassus*, white points – *U. nanus*, grey points – sites of sympatric occurrence of both species; the red diamond indicates the area of this study; B) the sites of collection of animals in this study: 1- *P. Phoxinus* specimens used during artificial infestation, *U. crassus* complex specimens used in artificial infestation, *U. crassus* complex individuals used for genetic reassessment of the population inhabiting the river Warkocz; 2- *C. microstomus* specimens used during artificial infestation.

2.2 Infestation of fish and juvenile mussel collection

Once visible conglomerates of glochidia were observed in the tank and it was ensured that the females had emptied their

marsupia, the mussels were removed from the tank. The contents were gently mixed, and all fish were simultaneously placed into the glochidia suspension to ensure a uniform infestation rate across both fish species. The fish were exposed

to glochidia for 20 min. During this period, the suspension tank was continuously aerated and hand-stirred to prevent glochidia sedimentation and to stimulate fish movement. Following the exposure, each fish was carefully returned to its individual 13-litre tank within the recirculating aquaculture system. At this stage, each tank was equipped with a net (mesh size 2 mm), which was fixed horizontally approximately 3 cm above the tank bottom, to prevent the fish from foraging on juvenile mussels detaching from their gills and sinking to the bottom. Juvenile mussels were collected every 3 to 4 days. On each occasion, the individual 13-litre tank was removed from the recirculating aquaculture system, and the fish were gently transferred using a fine mesh net to a temporary holding tank. The contents of the 13-litre tank were then flushed several times through a planktonic net (mesh size 0.01 mm), which led to a small tank. The contents of this tank were subsequently passed through a sieve (mesh size 0.15 mm), and the material retained in the sieve was transferred onto a petri dish. Transformed and viable juveniles were counted using a binocular magnifier (Delta Optical, SZ-450B). After the procedure, the tank was refilled with water, and all components were restored to their original configuration to allow the procedure to be repeated.

2.3 Statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics. In order to investigate differences between glochidia transformation patterns in consecutive controls between both species of fish, a generalized mixed model with repeated measures (Log-Poisson) was constructed; dependent variable: number of transformed and vital juvenile mussels at given time post infestation of fish, grouping variable: host species, continuous predictor: fish weight, random effect: fish specimen ID. The random effect was used to account for potential differences between fish specimens, which may result from earlier acquisition of immunity to glochidia, or individual condition, infestation intensity or any other non-controlled factor resulting from testing a random group of wild fish. Differences between specific levels of variables were tested using pairwise contrasts post-hoc tests and are presented in the Supplementary Materials. Since juvenile mussels were collected every 3 or 4 days, in the descriptive statistics we refer to the “potential excystation period” and “potential parasitism period”, which reflects respectively: the maximum possible time between first and last excystment of juvenile mussel from a host, and maximum possible time from infestation to excystment of the last juvenile, both determined for each host fish specimen. The mean parasitism duration was calculated based on the time each individual glochidium spent attached to the host, from initial attachment until collection of transformed juvenile mussel - these data were then pooled for each host species.

2.4 Molecular taxonomic verification of the *U. crassus* complex species from the Warkocz River

While much of the existing literature and legal frameworks refer to “*U. crassus*” as a single species, recent findings suggest that it should be regarded as a species complex. In their

recent study, [Lopes-Lima *et al.* \(2024\)](#) identified 12 molecularly distinct species within what was previously recognised as “*U. crassus*”, two of which are likely present in our study area: *U. crassus* sensu stricto and *U. nanus*. To reassess the taxonomic identity of *Unio* specimens from the Warkocz River under the revised taxonomic framework of the *U. crassus* complex, we reanalyzed mitochondrial cytochrome c oxidase subunit I (COI) barcode sequences previously obtained by our team ([Kilikowska *et al.*, 2020](#)). At the time of the original study, all specimens were assigned to *U. crassus* according to the taxonomy then in use.

Following the integrative taxonomic revision by [Lopes-Lima *et al.* \(2024\)](#), which recognized 12 geographically structured species within the *U. crassus* complex, we conducted a comparative molecular analysis to determine the species that inhabited the Warkocz River. Two previously published COI sequences represented two haplotypes from the Warkocz population (GenBank accession numbers KJ525912 and KJ525917; [Kilikowska *et al.*, 2020](#)) were analyzed together with one representative COI sequence for each of the 12 species described by [Lopes-Lima *et al.* \(2021, 2024\)](#). All sequences have been downloaded from GenBank (accession numbers listed in supplementary materials, Tab. S3).

The COI sequences were aligned using the MUSCLE algorithm implemented in MEGA X v10.0.3 ([Kumar *et al.*, 2018](#)) with default settings. All barcode sequences were trimmed to equal length, resulting in a final alignment of 614 bp. The alignment contained no gaps or ambiguous sites. To confirm the absence of pseudogenes, sequences were translated into amino acids using the invertebrate mitochondrial genetic code, and no stop codons were detected.

Uncorrected pairwise genetic distances (p-distances) were calculated in MEGA X. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). The resulting distance matrix was used to assess genetic divergence between haplotypes of *U. crassus* species complex from Warkocz River and reference sequences representing the newly delimited species within the *U. crassus* complex. All pairwise p-distance values are provided in Table S3 in supplementary materials.

3 Results

3.1 Artificial infestation

The Baltic sculpin *C. microstomus* demonstrated the ability to successfully transform glochidia released by females originating from a mixed population of *U. crassus* sensu stricto and *U. nanus* into viable juvenile mussels. Observations on equal groups of fish kept under identical conditions revealed a higher efficiency of larval transformation in *P. phoxinus* compared to *C. microstomus*. The number of successfully transformed juveniles per specimen in *C. microstomus* ranged from 44 to 134, mean = 82.2, SD = 27.27, while in *P. phoxinus* it ranged from 26 to 211 (mean = 114.1, SD = 63.67). In total, *C. microstomus* produced 904 juvenile mussels, while *P. phoxinus* produced 1255 juvenile mussels. The results of constructed GLZ (log-poisson) model showed that the difference in number of juveniles collected during the whole excystation period between species was not statistically

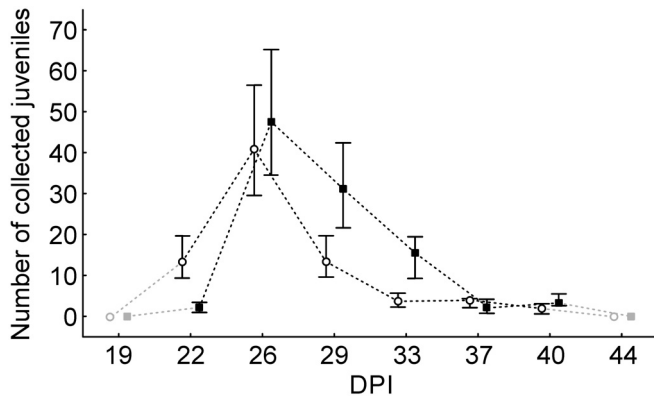


Fig. 2. The differences in number of juvenile mussels in consecutive counts between studied species: *C. microstomus* (empty circles) and *P. phoxinus* (solid squares); points: predicted average, whiskers: 95% confidence interval, DPI (Days post-infestation): days from exposition of fish to *U. crassus* glochidia; gray markers indicate last and first control in which no juveniles were detected (estimated start and finish point).

significant ($F(1, 119) = 0.46, p = 0.501$) and also, that it was not significantly influenced by the fish weight ($F(1, 119) = 0.99, p = 0.323$). The number of juvenile mussels (pooled across both host species) differed significantly between most of the collections dates ($F = 246.2, df1 = 5, df2 = 119, p < 0.001$) indicating, that juvenile mussels release showed dynamic changes over the collection dates; there was no statistically significant difference in the number of juveniles collected on the 22nd and 33rd and on the 37th and 40th days post-infestation (see Tab. S1 in the Supplementary Materials).

The model also indicated a statistically significant interaction between host species and collection date ($F = 37.9, df1 = 5, df2 = 119, p < 0.001$), showing that the number of successfully metamorphosed juvenile mussels varied between species in a collection-specific manner.

A random effect was also found to be statistically significant, although it explained less than 23% of the variation in the data ($\eta^2 = 0.223, SE = 0.088, p = 0.012$). To further illustrate the data, a figure displaying the mean percentage of juveniles collected at each time point has been included, which visually depicts the differences in excystation patterns for both host species in a cumulative diagram (Fig. 3).

Both host fish species exhibited similar maximum time frames for larval transformation. The first transformed, viable juveniles of *U. crassus* were collected from both species on the 22nd day post-infestation, and the last juvenile mussels detached from the fish by the 40th day after infestation, with a pronounced peak in juvenile collection on the 26th day (Fig. 2). No juveniles were collected during the control checks conducted on the 19th and 44th days post-infestation. The potential excystation period lasted from 18 to 21 days (mean = 20.45, SD = 1.21) in *C. microstomus* and from 11 to 21 days (mean = 18.36, SD = 3.88) in *P. phoxinus*. The potential parasitism period lasted from 37 to 40 days (mean = 39.45, SD = 1.21) in *C. microstomus* and from 33 to 40 days (mean = 37.91, SD = 2.77) in *P. phoxinus*. Mean duration of successful parasitism was 26.93 days (SD = 3.9 days) in *C. microstomus* and 28.39 days (SD = 3.5 days) in *P. phoxinus*.

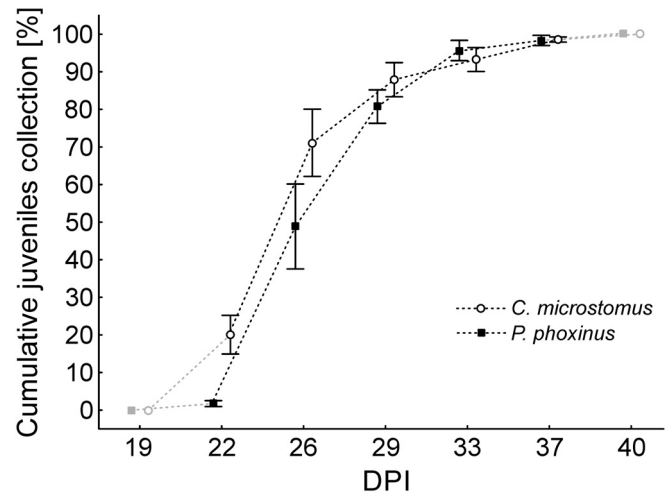


Fig. 3. Cumulative percentage of juvenile mussels collected from fish in consecutive steps: juveniles collected from *C. microstomus* (empty circles) and *P. phoxinus* (solid squares); points: predicted average, whiskers: 95% confidence interval, DPI (Days post-infestation): days from exposition of fish to *U. crassus* glochidia; grey markers indicate last and first control in which no juveniles were detected (estimated start and finish point).

The size of 11 specimens of *P. phoxinus* ranged between 48 mm and 90 mm (median: 72, SD = 11.51), while the size of 11 specimens of *C. microstomus* ranged between 63 mm and 127 mm (median: 82, SD = 17.56). The calculated weight of 11 specimens of *P. phoxinus* ranged between 0.95 g and 6.86 g (median: 3.41, SD = 1.68), while for 11 specimens of *C. microstomus* it ranged between 1.94 g and 17.66 g (median: 4.45, SD = 4.36).

3.2 Mitochondrial divergence in the Warkocz River population of the *U. crassus* species complex

The uncorrected p-distances within the *U. crassus* species complex revealed substantial genetic differentiation between the two haplotypes obtained from the Warkocz River population (KJ525912 and KJ525917, Kilikowska et al., 2020), which differed by 2.8%. The first haplotype (KJ525912) showed the lowest divergence from *U. nanus* ($p = 0.010$), followed by *U. vicarius* (0.023) and *U. bruguierianus*, *U. carneus*, and *U. crassus sensu stricto* (each 0.028). In contrast, the second haplotype (KJ525917) was nearly identical to *U. crassus sensu stricto* ($p = 0.003$) and showed low divergence from *U. gontierii* (0.010), while distances to other taxa were markedly higher (≥ 0.023). Both Warkocz haplotypes exhibited substantially greater divergence from more distantly related taxa such as *U. sesirmensis* (0.057 - 0.062) and *U. tumidiformis* (0.078 - 0.083). Overall, the observed pattern indicates pronounced mitochondrial heterogeneity within the Warkocz River population, with one haplotype closely affiliated with *U. crassus sensu stricto* and the other showing the strongest affinity to *U. nanus*, indicating the presence of two coexisting species from the *U. crassus* species complex in the Warkocz River: *U. crassus sensu stricto* and *U. nanus*.

4 Discussion

Unionid mussels (Unionida) represent one of the classic examples of tight coevolution, in which one group of organisms has effectively made its reproductive success dependent on another group (host fish). Consequently, the prospects for their conservation are closely linked to the availability of suitable host fish. In some studies on host suitability, binary tests (presence/absence) are performed to determine whether a given fish species is capable of transforming glochidia or not (reviewed in Lopes-Lima *et al.*, 2017). However, as recently demonstrated by Dołęga *et al.* (2025), the influence of fish species that reject larvae may also be indirect and depend on the probability of larval interception by dead-end hosts, as well as on the abundance of both fish and mussel species. In turn, Douda *et al.* (2014) identified differences in the ability of *U. crassus* to infest particular host fish species between nearby and recently isolated mussel populations, indicating a significant effect of even slight genetic differences among populations.

In both groups of animals, substantial progress has recently been made in phylogenetic research. Assuming after Douda *et al.* (2014) that phylogenetic differences, often not detectable without detailed genetic analyses, play an important role in shaping interactions between mussels and fish, studies incorporating recent taxonomic advances gain particular importance. Such an approach may explain many of the observed differences in infestation success and transformation efficiency among mussel and fish populations.

In the case of mussels, species identification can be challenging due to the high phenotypic plasticity of shell morphology (e.g., Zajac *et al.*, 2018, Egg *et al.*, 2025) and the lack of clear diagnostic morphological traits distinguishing recently described species (Lopes-Lima *et al.*, 2024). Furthermore, the possibility of hybridization demonstrated in our study further complicates species identification based solely on morphology. In fish, ongoing advances in phylogenetic analyses are leading to taxonomic revisions, revealing previously unrecognized diversity that may translate into differences in host suitability and transformation efficiency. There is therefore a need to effectively reinstate research programmes aimed at assessing the susceptibility of different fish lineages to glochidial infestation and transformation, incorporating recent advances in genetics.

In the present study, we tested a mussel population whose precise species identity was uncertain, as the taxonomic revision by Lopes-Lima *et al.* (2024) had not yet been available at the time of sampling. Retrospectively, we can assign these individuals to two lineages (*U. crassus* and *U. nanus*) dominating in this part of Europe, which, according to our data, can hybridize. At the same time, following the recognition of a new potential fish host species, *C. microstomus*, we demonstrated successful transformation of larvae attributed to *U. crassus* in the traditional sense. This finding is of considerable importance, as all three taxa involved (*U. crassus*, *U. nanus*, and *C. microstomus*) are widespread in this part of Europe, and conservation strategies for the *U. crassus* complex must necessarily account for their interactions. Although our experiment does not allow us to determine precisely which mussel species is responsible for successful transformation, we can state with confidence that

conservation of *C. microstomus* may contribute to the persistence of the co-occurring members of the *U. crassus* complex: *U. crassus* and *U. nanus*.

The results of the statistical model indicate, that overall transformation of *U. crassus/nanus* larvae on *C. microstomus* did not differ significantly from that observed on the well-established host species *P. phoxinus*. However, the rapid development of larvae on *C. microstomus*, with a statistically significant advancement in juvenile excystation (juvenile collection on 22nd day post infestation, Fig. 2), suggests that the ontogenetic transition is not hindered in this host, even in comparison to *P. phoxinus*. Although the overall time frames of juvenile mussel excystation and parasitism duration are similar in both hosts, a more detailed view on excystment timing—at the level of host individuals and even individual glochidia—reveals differences in the temporal patterns of transformation between the two host species. This is consistent with observations reported by other authors (Tab. S4, Supplementary Materials), where both transformation success and temporal parameters are usually lower for *Cottus* sp. than for *P. phoxinus*. The generally shorter duration of parasitism is suggested to play a minimal role for *U. crassus* complex larvae, as according to Taeubert *et al.* (2012b), glochidia of *U. crassus* doesn't grow significantly during encystation, confining benefits of parasitic stage of mussels life to dispersal rather than individual condition.

Given the diversity of methodologies and study objectives in the literature, direct comparisons remain difficult, as already noted by Douda (2013). In study by Douda *et al.* (2012) *C. gobio* and *P. phoxinus* were identified as primary hosts, each exhibiting high transformation rates (>50% of attached larvae). *C. gobio* hosted on average 365 glochidia per fish, with 57.3% successfully transforming c. 209 juveniles per fish, whereas in our study the mean number of juveniles detached from *C. microstomus* was 82.2 (SD = 27.27), however, initial infestation levels were not quantified in our study. In contrast, Taeubert *et al.* (2012a) reported that *C. gobio* lost approximately 90% of glochidia, classifying it as a relatively unsuitable host. They also noted variation in host suitability among populations of *C. gobio*, possibly reflecting obscured lineage-specific responses within the *C. gobio*–*U. crassus* interaction.

According to *in situ* observations by Schneider *et al.* (2018), during the reproductive period of *U. crassus*, *C. gobio* carried fewer glochidia per individual than *P. phoxinus*, despite both species being simultaneously active and exposed. This may suggest that *P. phoxinus* is a more effective host *in situ* possibly due to microhabitat preferences and behavioural traits that increase susceptibility to infestation (Ćmiel *et al.*, 2018). A more detailed comparison of literature data with the results of the present study is provided in Table S4 of the supplementary materials.

In the Warkocz River population, Mioduchowska *et al.* (2016) identified individuals exhibiting discordant mitochondrial lineages: *Fcox1* haplotypes corresponded to *U. crassus*, whereas *Mcox1* haplotypes were assigned to *U. nanus*. These lineages correspond to the northern and southern clades described in Poland and were later recognized as distinct species within the *U. crassus* complex by Lopes-Lima *et al.* (2024). This pattern provides clear evidence of interspecific

gene flow and hybridization within the complex. Such hybrids may result from secondary contact following historical divergence, but they may also indicate that speciation has proceeded under conditions of incomplete reproductive isolation, allowing ongoing genetic exchange. The coexistence of mitochondrial genomes from two nominal species within single individuals raises important questions regarding species boundaries and the applicability of traditional species concepts in freshwater mussels.

In this study, we demonstrated that *C. microstomus* is an effective host species for one or both species of the *U. crassus* complex occurring in Poland (*U. crassus* and/or *U. nanus*). Despite taxonomic differences and geographical separation from other *Cottus* species used in previous studies, *C. microstomus* supports successful infestation and transformation of glochidia of one or both of these two representatives of the *U. crassus* complex. Both *C. microstomus* and the *U. crassus* complex have wide distributions across diverse river habitats, yet both are highly susceptible to environmental changes, making them prone to co-extinction - a process documented for associated fish-mussel systems (Modesto *et al.*, 2018). Our findings demonstrate that uncovering cryptic host-parasite relationships is not only of academic interest but constitutes a prerequisite for effective conservation planning.

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Supplementary material

Tab. 1. p-values of pairwise contrasts post-hoc test for performed repeated Measures generalized Linear Model (log, Poisson) of the total number of juveniles collected in each control (aggregated for both species); DPI – days post infestation of fish.

Tab. 2. Results of pairwise contrasts post-hoc test for performed repeated Measures generalized Linear Model (log, Poisson) of the differences in number of juveniles collected in given control between both host species (*P. phoxinus* and *C. microstomus*); DPI – days post infestation of fish.

Tab. 3. Estimates of evolutionary divergence between COI sequences of *U. crassus* complex; references: A - Kilikowska *et al.* 2020, B - Lopes-Lima *et al.* 2021, C - Lopes-Lima *et al.* 2024; the referenced citations are included in the main text of the manuscript, in the “References” section.

Tab. 4. Reference to cited literature on host suitability for larvae of *U. crassus* complex (comparisons between *C. gobio* and *P. phoxinus*); A – results obtained from artificial infestation, N –

results obtained from natural infestation; DPI – days post infestation; *- only data from a trial performed concurrently with *C. gobio* were considered in the table; where possible, data from the present study were presented in the same format as in the cited studies for comparison; the referenced citations are included in the main text of the manuscript, in the “Literature” section.

The Supplementary Material is available at <https://www.kmae-journal.org/10.1051/kmae/2026010/olm>.

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Artykuł (III)

Dolęga, J.A., Ćmiel, A.M., Zając, K., Zając, T. 202X. Host fish availability during *Unio crassus* Philipsson, 1788 glochidia release

Maszynopis nieopublikowany w momencie składania rozprawy doktorskiej.

Host fish availability for the *Unio crassus* complex during the glochidia release period

Jacek A. DOŁĘGA^{*,1}, Adam M. ĆMIEL¹, Katarzyna ZAJĄC¹, Tadeusz ZAJĄC¹

¹-Institute of Nature Conservation, Polish Academy of Sciences, Al. A. Mickiewicza 33, 31-120 Kraków

*corresponding author: Jacek A. DOŁĘGA

telephone number: +48 792 880 354

e-mail address: dolega@iop.krakow.pl

short title (running head): Host fish availability for the *Unio crassus*

keywords: temporal patterns, parasite-host interaction, conservation, glochidia, fish host

Abstract

One of the key factors influencing recruitment success of unionid mussels is the temporal and spatial overlap between glochidia release and occurrence of suitable host fish. This relationship is difficult to assess using most data provided by fish monitoring programs, as it's often carried out outside the mussel reproductive period. Therefore, it may not reflect a true host availability. We examined host fish availability for *Unio crassus* complex during glochidia release period in five Polish rivers: four rivers were subjected to subsequent fish sampling and one was location for complementary fish movement study using PIT-tag technique. Glochidia release phenology varied among rivers and years, beginning between late March and late April and usually ending in July. During the release period, fish assemblages comprised from 9 to 13 species, however, suitable hosts represented only a limited part of the community, with 3 to 5 host species recorded per river each year. Usually only 1 or 2 host species exceeded 10% of the fish assemblage and therefore, probably reached abundance levels sufficient to perform an effective host role for *U. crassus*. During the site- and year-specific period of glochidia release, the proportion of host fish ranged from 12.5% to 57.0%, with continuous availability provided only by *Phoxinus phoxinus* in both foothill rivers and *Leuciscus cephalus* in every of the studied lowland system. Other host species were sampled in low numbers or discontinuously throughout the study period. Host fish numbers were generally lower before and after the glochidia release period, indicating that late season surveys commonly accepted for the purposes of environmental monitoring may misrepresent actual host availability. The data gathered during the PIT-tag study, showed that *L. cephalus* was near continuously present in vicinity of mussel bed and visited it from tagging points located up to 1.4 km downstream, suggesting both high infestation opportunity and dispersal potential for *U. crassus* larvae. Our findings highlight that host availability during the glochidia release period should not be overlooked as a component of conservation assessment and can be considered as potential context for interpreting declines of *U. crassus* populations.

38 Introduction

39 In their seminal paper Ehrlich & Raven's (1964) viewed co-evolution as any ecological interaction
40 (competition, mutualism or antagonism) that resulted in coadaptation among interacting species. A
41 classic example of plant-herbivore interaction shows the co-evolution mechanism: if plant develops
42 novel mechanical or chemical defence it escapes from herbivore pressure. Then herbivore may
43 circumvent plant defence, leading to new radiation both in plant and herbivore.

44 There is a large number of issues to be explained in the studies of co-evolution (Carmona *et al.* 2015),
45 however, one seems to be especially interesting from ecological point of view: whether co-evolution
46 needs not only involve independent interactions between a pair of species (pairwise co-evolution), but
47 may include multiple species that alter the outcome of pairwise co-evolutionary processes (diffuse co-
48 evolution, as in Janzen, 1980). This question is especially interesting from the nature conservation point
49 of view, whether extinction of one species will unequivocally lead to extinction of the other ("extinction
50 cascade" Strona 2015), or it is possible that extinct species role might be played by other species, finally,
51 what are the hosts "repertoire" available to parasite.

52 In the case of threatened large freshwater mussels from family Unionidae the co-evolution issue is
53 especially important because they have developed unique reproductive system: being sedentary they
54 have limited dispersal abilities, especially in running water, which throws their larvae only downstream.
55 They developed a larva which has ability to infest the best dispersal factor in freshwater, fish, enabling
56 effective dispersal on large distances. Co-evolution of fish and mussels is highly asymmetric: for mussel
57 fish infestation is a matter of all or nothing in reproduction, for fish only very severe infestation can
58 cause a real health problem (glochidiozis – Rock *et al.* 2022). Parasitism can drive adaptations among
59 interacting species, leading to evolution of highly specialized parasitic larva, glochidium (Kat 1984,
60 Ćmiel *et al.* 2021), numerous adaptations for fish manipulation aiming at their infestation (Aldridge *et*
61 *al.* 2023, Barnhart *et al.* 2008), and among fish, different levels of resistance: there are species rarely
62 infested, diluting (infesting but not transforming larvae) and hosting (infesting and transforming larvae).
63 Their relative frequency may decide about the mussels success (Dołęga *et al.* 2025).

64 Considering such a large variety of the host susceptibility, the most interesting question is about the
65 pairwise relation leading to the risk of extinction cascade vs diffuse co-evolution. The ideal fish host
66 should co-occur with the mussels in time and space: the glochidial release should happen when the most
67 of fish hosts should be available at the same time (Schneider *et al.* 2019), ideal would be host which
68 stays for longer at the mussel site, enabling receiving large glochidia number, however, without the
69 ability to move on a longer distance it loses his dispersal function.

70 There is a significant shortfall in the studying this relation: host suitability is usually tested in captivity
71 (Zale and Neves 1982, Geist *et al.* 2023), needing a priori assumed, literature based host (e.g. Schneider
72 *et al.* 2017a), whereas in vast areas of Europe standard methodologies of ichthyological studies requires
73 fish catch in the late season (Breine *et al.* 2005, Adamczyk *et al.* 2013, Sutela *et al.* 2017). This way
74 large body of fish monitoring or distribution studies do not provide information on species of fish that
75 are actually available for infestation during glochidia release periods. This is important issue, because
76 the role of fish host might be played in different way: a sedentary species of host will pay its role only
77 if co-occur with mussels in the same functional habitat, if they do, they may be effectively infested but
78 will not spread the mussels distribution, on the opposite, very movable species may spread the larvae on
79 long distance, however, will be less frequently infested than sedentary species with his functional habitat
80 constantly overlapping with mussels.

81 It has been postulated (Zajac *et al.* 2026) that freshwater mussels monitoring should be coordinated with
82 fish monitoring to supply information about the impact of fish composition on mussels population
83 perspective, however, it must be answered whether they co-occur in the period of mussels' larvae
84 release, otherwise the fish impact on mussel recruitment might not function at all and monitoring results
85 will be misleading.

86 We want to answer these questions on the basis of our study of four populations of the threatened naiad
87 lineage *Unio crassus* complex. We have studied the composition of the fish assemblages during the period
88 of glochidia release in *Unio crassus* in two mountain and two lowland rivers in order to identify host
89 fish availability in this period, and, in the case of one river, consequences of host fish distribution
90 patterns for the local *Unio crassus* spread.

92 Methods

93 1. Study sites

94 The study was conducted in central and southern Poland in five rivers: the Warkocz, the Cedron, the
95 Nida and the Czarna Włoszczowska (where consecutive fish sampling was conducted to assess fish
96 assemblage composition in relation to the *U. crassus* complex glochidia release period), and the Smuga
97 Umianowicka (where supplementary fish-movement studies were carried out using PIT-tag technology).

98 The Warkocz, located in the foothills of Świętokrzyskie Mts, is largely natural, with a meandering
99 channel and a pool-riffle sequence. At the sampling site (near Niestachów village, 259.8 m a.s.l.,
100 20.7574452 N, 50.8401446 E) the channel width is 5–7 m, and water depth ranges from shallow riffles
101 of around 0.1 m to pools reaching approximately 1 m in low flow conditions. Sandy substrate dominates
102 the riverbed, although silt, fine sediments, gravel beds occur locally. The channel is adjacent to
103 deciduous forest on the left bank, on the right is shadowed with alder curtains, adjacent to meadows.

104 The Cedron, located in Pogórze Wielickie, is an upland stream with a natural, meandering channel and
105 a pool-riffle structure. The channel is 5–8 m wide at study site (near Leńcze village, 245.9 m a.s.l.;
106 19.7323428 N, 49.8890233 E), and during low-flow periods water depth ranges from about 0.1 m in
107 riffles to 2.0 m in the deepest pools. Its bed, eroded in fine sediments, is dominated by sand, fine
108 sediment on the banks, and scarce gravel bars. The banks are bordered by curtains of riparian shrubs,
109 then adjacent to meadows.

110 The Nida is a typical lowland, meandering river, tributary of Wisła river. At study site (near Stawy
111 village; 192.9 m a.s.l., 20.4968817 N, 50.5783138 E), the channel width ranges between 20 and 30 m,
112 river has laminar flow and maximal depth of ca 1.5m. Its bed is dominated by sandy substrate, with
113 marginal vegetation composed of floating matrace of *Potamogeton* sp. and emerged *Sparganium* sp.
114 beds. The surrounding valley is largely composed of meadows and pastures.

115 The Czarna Włoszczowska, is a lowland meandering small river, mostly with natural channel and
116 laminar flow. At the sampling site (near Ciemietniki, 201.7 m a.s.l.; 19.8505679 N, 50.9473050 E) its
117 bed is dominated by sand substrate with marginal silt and scarce occurrence of gravel. The channel is

incised about 2 m into the surrounding substrate, forming a flat-bottomed channel, 7–15 m wide, during low-flow periods maximal water depth reach up to 1 m. The banks are mostly covered with herbaceous vegetation, surrounded by meadows and pastures.

A complementary part of the study, involving tracking fish movement with PIT-tag system, was conducted in the inland delta system of the Nida River, in the Smuga Umianowicka river (193.1 m a. s. l.; 20.5152669 N, 50.5831074 E). It is a side arm of the Nida river of 3-15m width, the water depth ranges between 0.3-1.2 m and the flow is laminar and slow, some parts of the channel reach even 5.0m of depth and 20-40 m of width and is connected to the system of floodplain lakes. The substrate varies in the river sections from silt to fine sand. The channel is surrounded by wetlands, remnant patches of alluvial forests, meadows and pastures. Locations of all study sites are presented in the Fig. 1.

2. Phenology of glochidia release

In each study sites, thick shelled river mussel individuals, individually marked in the preceding year, were regularly inspected since the mid-March ca every two weeks during the whole of reproductive period. Since the inspection frequency do not allow for precise identification of dates of glochidia release, the date of release was estimated on the basis of individuals for which the spawn/fertilisation date and glochidia release was known precisely (Fig. 2): the stage of larval development was identified and the date of glochidia release estimated on the basis of the development rate of glochidia elaborated for the Biała river (see Zając and Zając 2021 and Fig. 2; the data set was enlarged by observations of the known, precise dates of spawn of non-fertilized eggs or the first stage of cleavage and snapping glochidia release, recorded in this study, N=9).

3. Fish sampling in the Warkocz, Cedron, Nida and Czarna Włoszczowska

In the Cedron, the Czarna Włoszczowska and the Warkocz fish were sampled by wading electrofishing using a single upstream pass with a battery-powered backpack electrofishing unit (IUP-12, Radet, Poland). At each sampling, the entire wetted channel width was sampled within fixed sampling sections, covering a total river length of ca. 250-550m at each site. The same sampling sections, spatially overlapping with a mussel bed, were used throughout the study. Sampling sections were selected so that each upstream electrofishing pass ended below a habitat feature expected to limit further upstream fish movement, such as a riffle, chute or inflow.

In the Nida River, due to the larger channel width and depth, fish were sampled using a seine net measuring 5.5×1.20 m, with a mesh size of 8 mm. The method was appropriate for assessing the temporal occurrence of fish within shallow marginal habitats directly used by the *U. crassus* complex in the Nida river, rather than for estimating the entire fish assemblage of the main channel. Sampling was conducted at 13 fixed points (4-13 points were successfully sampled per sampling event, depending on water level), with 2 to 3 hauls performed at each point. The total sampled area was approximately 100 m² in each of the fixed points. This method allowed fish to be sampled within the functional habitats most relevant to the occurrence of the *U. crassus* complex in the Nida channel, including marginal bars, the littoral zone, shallow low-flow areas and small embayments.

Among study sites, comparisons are based on single-season observations, except for the Warkocz where three reproductive seasons of *U. crassus* were studied. Where possible, fish were caught during three general time-frames related to *U. crassus* glochidia release: before the period of glochidia release, within the period of glochidia release and after the period of glochidia release. In total, 26 fish sampling events were conducted, 15 of these took place during the glochidia-release period (Tab. 1).

Tab. 1. Fish sampling dates and number of sampling events in relation to the period of glochidia release of *Unio crassus*, determined from the inspection of gravid females

River	Year	Prior to glochidia release	During glochidia release period	After glochidia release
Warkocz	2020	18 Mar.	22 Apr.; 22 May.	21 Jul.
	2021	19Mar.; 30Mar.	20 Apr.; 20 May; 10 Jun.	28 Oct.
	2023	18 Mar.; 13 Apr.	22 May; 29 Jun.; 27Jul.	14 Sept.
Cedron	2022	-	25 May; 14 Jun.; 12 Jul.	19 Aug.
Nida	2020	25 Mar.	24 Apr.; 20 May	11, 12 Aug.
Czarna W.	2019	-	26 Jun.; 9 Jul.	-

4. Fish tracking in the Smuga Umianowicka river

The PIT-tag monitoring study was conducted in the Smuga Umianowicka river. The study design involved placing a stationary, submersible PIT-tag antenna (diameter of 0.92 m, submersible 3' Antenna System, Biomark, USA) at a *U. crassus* mussel aggregation (50m downstream, with 2m width of the channel, earlier tested distance of effective tag detection reaching 0,4m with optimal tag orientation)) and tagging fish in different parts of the Smuga Umianowicka. The antenna was exposed for a total of 80 days during two monitoring periods: 15 March 2024 to 23 April 2024 and 14

May 2024 to 22 June 2024, separated by a 20-day interruption. The antenna was able to record a tag every 2 seconds, but duplicates were recorded not more frequently than once per minute. Antenna detections were used to describe fish activity near the mussel bed short-before and during the glochidia-release period. Fish were tagged in proportions broadly reflecting the fish assemblage recorded during sampling, with an exception that no fish under the size of 110 mm of total length ensuring that PIT-tag weight did not exceed commonly accepted threshold of 2% of fish body mass (Winter 1996, Vollset *et al.* 2020, Shorgan *et al.* 2026). The area of fish capture covered the fish-passable section of the Smuga Umianowicka channel, together with local floodplain pools and intermittently connected side channels running through the inland delta of the Nida River (Fig. 3). This accessible section extended from a downstream weir near the confluence with the Nida River, which was impassable to fish, to a technical culvert located at the upstream end of the channel containing the mussel bed and the PIT-tag antenna (also impassable for fish).

Depending on habitat conditions, fish were captured using two electrofishing methods: electrofishing from a boat with a stationary petrol-powered generator unit (DEKA 5000, DEKA Geratebau GmbH, Germany), and wading electrofishing with a battery-powered backpack unit (EFGI-650, Bretschneider Spezialelektronik, Germany). Both devices were operated using non-pulsed direct current to reduce the potential risk for fish injury. Closely adjacent sampling sites, spaced approximately 100 m apart (sites 11–27; Fig. 3), were isolated during fish sampling with fine-mesh block nets to prevent fish movement between sections of the river channel. Captured fish were temporarily held in an aerated (battery pumps) 80-L tank filled with fresh river water. Whenever the number of fish in the tank reached the maximum compatible with appropriate welfare conditions, fishing was temporarily interrupted and individuals were processed for tagging. Fish were anaesthetized with tricaine methanesulfonate (MS-222; Sigma-Aldrich, Germany), and fully anaesthetized individuals were intraperitoneally implanted with 12×2 mm PIT tags using a dedicated tagging gun and sterile pre-loaded implanter needles (Biomark, USA). Following tagging, fish were transferred to aerated fresh river water with regular water exchange for recovery. Once dorso-ventral equilibrium and full mobility had been restored, individuals were released near their capture location.

5. Data analysis

The differences between proportion of host fish species (number of host fish species individuals/total number of fish individuals) within glochidia release period and proportion of host fish species individuals in both pre- and post- glochidia release periods were tested using tests between two proportions (z tests).

The differences between proportion of host fish species during the brooding period of *U. crassus* between each river and year were performed using the same procedure (Z tests). However, because of the large number of comparisons (15 in total), original P values were adjusted using Benjamini-Hochberg method for controlling the false discovery rate (FDR) in multiple comparisons (Benjamini & Hochberg, 1995).

The status of host vs non-host fish species was established on the basis of own research (unpublished data) as well as the list published by (Lopes-lima *et al.* 2017, Dołęga *et al.* 2025, Dołęga *et al.* 2026, Douda *et al.* 2014, Schneider *et al.* 2017a, Schneider *et al.* 2017b, Taeubert *et al.* 2012a, Taeubert *et al.* 2012b) assuming the host suitability as given for the Central Europe.

During field-data collection, some individuals were recorded only as “Cyprinidae fry” and were therefore not identified to species level to reduce fish handling time and to ensure that study remained non-lethal (young of the year, 10-20mm total length). Consequently, this category was excluded from the host vs. non-host classification (e.g., Tab. 4, Tab. S1, Fig. 6), however, for the purpose of characterising the whole fish assemblage, it was included in the total fish number and presented in Fig. 5 and Tab. 3 in the Results section. Statistical analysis was performed using Dell Statistica 13.0 software (Dell Inc. 2016).

221 Results

222 1. Period of glochidia release

223 In small rivers of foothil character (the Warkocz and the Cedron) the onset of glochidia release was
224 similar, around the half of April (Tab. 2), whereas in the lowland rivers, differing in size, was variable:
225 very early in the Czarna river (small, reach in nutrients), and a month later in the big lowland river, the
226 Nida, and intermediate in small side branch of the Nida river, the Smuga. The distribution of glochidia
227 releases during the breeding season is also very variable, with different maxima and skewness, even
228 within the same river in different years (e.g. see example of the Warkocz river in Fig. 4). The end of the
229 period of glochidia release is also variable, however, in the most of the cases it is the second half of July
230 (Tab. 2).

231 Tab 2. The phenology of glochidia release in *U. crassus* in each of the sites and years of the study

River	Type	Year	Glochidia release phenology			Duration of glochidia release period	N of released broods
			Start	Peak	End		
Warkocz	foothill	2020	12 Apr.	7-19 May	9 Jul.	89	128
		2021	16 Apr.	29 Apr.-12 May	17 Jul.	93	103
		2023	17 Apr.	22-28 May	28 Jul.	42	45
Cedron		2022	17 Apr.	28 Apr.-9 May	28 Jul.	103	123
Nida		2020	21 Apr	6-22 May	25 Jul.	96	37
Czarna Włoszczowska	lowland	2019	28 Mar.	13-29 Jun	15 Jul.	110	112
Smuga Umianowicka		2024	6 Apr.	31 May-14.Jun	12 Jul	113	97

232

233 2. Availability of host fish species

234 From 9 to 13 fish species per river were recorded during site- and year-specific glochidia release period

235 (see Fig. 5 for detailed information). In the fish assemblages usually dominant were non-host species:

236 *Gobio gobio* or *Rhodeus amarus*. Each of the fish assemblages in the Warkocz, Cedron, and Nida

237 included 3 species known to enable successful transformation of *U. crassus* larvae whereas in the Czarna

238 Włoszczowska river 5 host species were recorded (Tab. 3).

239

240 Tab. 3. Host fish number recorded during the period of release of *U. crassus* glochidia in all study sites,
 241 in relation to the entire fish assemblages (including Cyprinidae fry); the percentage of a given species
 242 in total catch is shown in brackets

River	Type	Year	Host fish species							Total %
			<i>Leuciscus cephalus</i>	<i>Cottus microstomus</i>	<i>Phoxinus phoxinus</i>	<i>Alburnus alburnus</i>	<i>Perca fluviatilis</i>	<i>Gymniocephalus cernua</i>	<i>Leuciscus leuciscus</i>	
Warkocz	foothill	2020	10 (0.7%)	7 (0.5%)	370 (25%)	-	-	-	-	26.2
		2021	6 (0.4%)	1 (0.1%)	190 (12%)	-	-	-	-	12.5
		2023	2 (0.3%)	1 (0.1%)	202 (28.7%)	-	-	-	-	29.1
Cedron		2022	240 (10.8%)	-	514 (23%)	1 (0.1%)	-	-	-	33.9
Nida	lowland	2020	82 (11.8%)	-	-	75 (10.8%)	1 (0.1%)	-	-	22.7
Czarna Włoszczowska		2019	12 (6%)	12 (6%)	-	-	33 (16%)	6 (3%)	1 (0.5%)	31.5
Mean			59 (5%)	5 (1.7%)	319 (22.2%)	38 (5.5%)	17 (8%)	6 (3%)	1 (0.5%)	26

243

244 The pooled proportion of host fish individuals in the total fish assemblage during the glochidia release
 245 period ranged from 12.5 to 57.0% across the studied rivers (Tab. S1). Particularly high host proportion
 246 was found in the Cedron in 2022 (57%), which differed significantly from all other samples (Benjamini–
 247 Hochberg adjusted p-values, in all comparisons $P < 0.0001$), whereas reduced host proportion was found

in the Warkocz 2021 (12.5%), which also differed significantly from all remaining samples (Benjamini–Hochberg adjusted p-values, in all comparisons $P < 0.0001$; Tab. S1).

Host fish proportions ranged from 13.8% to 23.4% before the glochidia release period and from 6.0% to 52.5% after the release period. In the four samples for which both pre-release and release-period data were available, no significant differences were detected between these two periods. Host proportions increased in three out of four cases (Tab. 4). The only decrease before vs. during the release period was observed in Warkocz in 2021.

In all five samples collected during release-period and during post-release period, host proportions decreased significantly after the release period in four out of five cases (tab. 4, Fig. 6). In Warkocz 2020, host proportion also decreased, but this difference was not significant.

Tab. 4. The differences in host fish proportions and their absolute numbers in pre-, and post-glochidia release periods compared against proportions/numbers during period of glochidia release; host proportion is shown in % of total catch; N - total number of fish sampled in given period; this dataset does not include Cyprinidae fry

Glochidia release period:	Host fish proportion			Total number of host fish		
	prior	during	after	prior	during	after
Warkocz 2020	22.8% N=636 p=0.075	26.6% N=1455	24.8% N=303 p=0.5	145	387	75
Warkocz 2021	13.8% N=682 p=0.4	12.5% N=1581	6.0% N=267 p=0.003**	94	198	16
Warkocz 2023	23.4% N=303 p=0.075	29.1% N=704	9.5% N=158 p<0.0001***	71	205	150
Nida 2020	16.7% N=12 p=0.9	22.8% N=694	10.0% N=866 p<0.0001***	2	158	87
Cedron 2022	No sampling	57.0% N=1325	52.5% N=2147 p=0.012*	No sampling	755	1128
Czarna W. 2019	No sampling	35.6% N=180	No sampling	No sampling	64	No sampling

The absolute numbers of host fish differed very much between considered periods. In all studied cases their number before period of glochidia release was 2-3 times lower than during that period, also the autumn catches were 1-5 times smaller in the Warkocz river, except the Cedron where autumn catch was 1.5 times larger.

3. Stability of host fish occurrence in time and space

In each site/year combination, at least one fish host species was present continuously in all sampling events, although not necessarily the same host species in all rivers. In the foothills rivers, *P. phoxinus* was the most consistently recorded host, occurring in every sampling event in the Warkocz and in the Cedron. In the lowland rivers, *L. cephalus* showed the highest temporal consistency, being recorded in all sampling events in both the Nida and the Czarna Włoszczowska. Some other host species were recorded discontinuously, and then their occurrence did not always coincide with the period of glochidia release (Fig. 6)

3.1 Fish movement tracked with PIT-tag system: exposure opportunity

Infestation probability should be correlated with time of exposure of fish to glochidia *in situ*, which should be the highest for resident host fish or host fish repeatedly present near the mussel bed. In the Smuga where the antenna for PIT tags reception was exposed, *L. cephalus* was the most consistently detected species, being recorded on 77 out of 80 days of antenna exposure near the mussel bed ($C = 96.25\%$) and throughout the entire glochidia release period ($C = 100.00\%$; Fig. 7). This species also generated the highest number of records and included the largest number of detected individuals among all tagged species, indicating both high temporal continuity and repeated use of the mussel bed area.

In contrast, the other known host species showed only occasional occurrence near the mussel bed. *P. fluviatilis* was detected on 11 out of 80 days of antenna exposure ($C = 13.75\%$ and during the glochidia release period $C = 8.62\%$), *L. leuciscus* was detected on seven days ($C = 8.75\%$ and during the glochidia release period $C = 8.62\%$). These records indicate additional host species were present at the mussel bed during the glochidia release period, but only occasionally.

3.2 Fish movement tracked with PIT-tag system: dispersal opportunity

In addition to the activity of individuals tagged at the study site (caught and tagged at the antenna location, see sites 1 and 2, Fig. 3) within the mussel bed area, PIT-tag detections showed that the mussel bed was also visited during the glochidia release period by fish tagged in other river sections. Among host species, this dispersal pattern was driven entirely by *L. cephalus*. Individuals of this species tagged in other areas were detected near the mussel bed on 60 out of 80 antenna-exposure days, and during all 58 days of antenna exposure, overlapping with assessed glochidia release period. Distance estimates showed that individuals recorded by antenna came from tagging locations situated 950 m (n=5), 1100 m (n=4) and 1400 m (n=1) from the mussel bed; all of these individuals were detected during the glochidia release period (Tab. 5).

304 Tab. 5. The distance from tagging site to the antenna covered by fish tagged at various sites along the
305 Smuga Umianowicka, other than vicinity of mussel bed (other than points 1 and 2 in the map in Fig. 3);
306 rate of occurrence (C, %) is expressed as the percentage of days in which tagged fish species were
307 detected by antenna in the vicinity of the mussel bed during all 80 days of antenna exposure; the same
308 parameter for the period of glochidia release (58 days) is provided in parentheses; I, % - proportion of
309 individuals detected during the glochidia release period; distance values are presented in meters;
310 asterisks indicate host species

Species body length: mean $\pm$ SD [mm]	N Tagged outside	N recorded	C, %	Distance, m			I, %
				mean	median	SD	
<i>Leuciscus cephalus</i> * 271.8 $\pm$ 49.0	119	10	75 (93)	1070	1100	137.8	100.00
<i>Rutilus rutilus</i> 172.1 $\pm$ 28.8	360	9	52 (60)	1277.8	800	821.3	88.89
<i>Leuciscus idus</i> 239.1 $\pm$ 62.3	25	2	2.5 (3.4)	650	650	0.0	100.00
<i>Perca fluviatilis</i> * 180.7 $\pm$ 28.7	20	1	7.5 (0.0)	800 (n=1)	-	-	0.00
<i>Blicca bjoerkna</i> 182.1 $\pm$ 19.7	38	5	7.5 (10.3)	1220	800	1109.4	100.00

311

Discussion

Reduced host fish availability can impede demography of naiads (Unionida) recruitment (Arvidsson *et al.* 2012, Douda *et al.* 2012, Haag & Stoeckel 2015, Stoeckl *et al.* 2015, although see Krebs *et al.* 2010). To effectively infest a fish there must be co-occurrence of fish and glochidia in time and space. From the evolutionary perspective it would be advantageous if mussels would infest the dominating species, as it was reported from the Scandinavians rivers, where Schneider *et al.* (2019) found that 99% of fish occurring during the period of female gravidity belonged to the two host species *Gobio gobio* and *Phoxinus phoxinus*. Our results are completely different: most of the most abundant fish species belong to the non-host species, whereas proportion of hosts is quite low, it ranges from 12.5 to 57 % (fry excluded), however on average it is only 30.6%, with most of the host species being only less than 5%, because only two species seem to supply the most of the host availability: *Leuciscus cephalus* and *Phoxinus phoxinus*, with the latter being related with foothills rivers; both tested by Taubert *et al.* (2012 a, b) and found the best host fish for *U. crassus* out of 8 species tested. Moreover, not only the proportions of the host fish were lower, but also their abundance: both main hosts density was equal to mean 83.5 ind./100m², which is roughly two time lower than the same species densities reported by Schneider *et al.* (2019, from 120 to 289.9 ind./100m²). Similarly broader array of available fish hosts is reported by Lamand *et al.* (2016) who sampled fish and examined glochidia load in 9 river of N-E France. Although they found also *Cottus gobio* and *Phoxinus phoxinus* as the dominant fish host like in the Sweden, however, out of 15 fish species recorded in the studied rivers, 5 species were fish hosts of *U. crassus*, providing alternative for the two main hosts. It must be also noticed, that large assembly of fish species within the river do not have to be advantageous for *U. crassus*, because not only number of host is important but also number and abundance of dead-end hosts, as was demonstrated by Dołęga *et al.* 2025. It should be also noted here that the larger fish abundance in the autumn than during glochidia release (Fig. 6 e) may have resulted from the increased presence of grown young-of-the-year fish, which had recruited in the same year and, by that time, were large enough to be captured and handled. If reproduction of *U. crassus* complex depends on single or two species, the question arise if the presence of other host species might be alternative for mussels if the main host species die-out. Their abundances

in our study sites are very low, thus anyway disappearance of the most numerous host is expected to impact adversely mussels demography. We have no data to determine whether non-host or host abundance, or proportion, influence the mussels population number. The methodologies of estimation of mussels number are not harmonised among countries (Zajac *et al.* 2026), making any comparisons difficult, however, the densities of *U. crassus* quoted by Schneider *et al.* (2019) seem to not differ much from the densities recorded in 3 of our river during state monitoring of *U. crassus*, which in the last turn of monitoring (years 2020-2021) were 2.0 individuals per square meter for the Cedron, 5.33 for the Warkocz and much lower 1.0 for the Czarna Włoszczowska (unpublished data from mussel monitoring in 2023-2025 in Poland, Chief Inspectorate of Environmental Protection). Similar situation refers to fish densities, e.g. using seine nets in lowland river, hampering comparisons. The only conclusion which can be derived that in the rivers reported in this paper, apparently lower proportion of host fish do not reduce significantly mussels densities, which is concordant with Rashleigh (2008) conclusion that occurrence of mussels depends on an array of host fish resources, combined with species' abilities to use fish hosts, because another factor at play which might be responsible for infestation effectiveness is host fish attraction (Haag & Warren 2003, Aldridge *et al.* 2023). If we assume that mechanism of host attraction described by Aldridge *et al.* (2023) is widespread in all possible habitats, than infestation might be more dependent on the predatory reaction of given fish species for tiny conglutinates spurted by mussels than on general presence in the habitat. We know little about the influence of other behavioural traits of fish on infestation, although Ćmiel *et al.* 2018 found that bottom-dwelling species forage on glochidia, e.g. *Barbus barbus*. Wengstrom *et al.* (2016) and Schenider *et al.* (2019) demonstrated that fish ventilation rates during faster swimming might positively influence infestation of trout by *Margaritifera margaritifera*.

The other factor which may compensate for lower proportion of host fish in the studied Polish river might be the length of glochidia release period. Schneider *et al.* 2019 provide only general distribution of gravid females (including all stages of glochidia development), not time of glochidia release, however, assuming 1 month of glochidia development in early spring and only 10 days for the summer (Zajac & Zajac 2021), it means that actual glochidia release period is much shorter in Sweden than in

Poland. Tab. 2 presents data of the earliest glochidia release in mid-April which implies, that gravid females appeared in mid-March, two weeks earlier than in Sweden. On the other end of time frame, the latest glochidia releases were recorded in Poland at the end of July, whereas in Sweden occurrence of gravid females (regardless of the developmental stage of larvae, could be also latest stages) effectively end with end of June. That means that glochidia release period in Poland lasts from half April to end of July (second half of April, May, June and July) whereas in Sweden only two (May, June).

The distribution of releases in time may have different shapes and in Poland it lasts for the whole period of spring and early summer, confirming a large phenotypic plasticity of breeding phenology reported for this species (Zajac & Zajac 2025). The onset of spawn is quite stable (Tab. 2) suggesting that photoperiod might be involved, however, the difference between the Czarna Włoszczowska (end of March) and Nida (21st April) equal to 24 days seems to contradict this hypothesis and makes it possible that the match between glochidia release and fish availability might be theoretically different in different rivers.

The analysis of the host fish number indicate, that shift of the reproduction to even earlier dates than reported here (Zajac and Zajac 2025), might be disadvantageous for the species demography, as in early time in the season definitely the fish availability is small in absolute numbers, sometimes dramatically decreased (see Nida 2020, e. g. Fig. 6). Also *U. crassus* cannot prolong the reproductive period towards autumn because the availability of fish hosts also substantially decreases. Proportion of fish hosts in the whole catch differs significantly in late-season from the period of glochidia release, which indicate, that postulated use of fish monitoring in assessment of the habitat suitability during mussels monitoring (Zajac *et al.* 2026), usually conducted in the autumn (Adamczyk *et al.* 2013) is not supported by our data. On the other hand, spring fish monitoring may present several methodological constraints, including elevated water levels, often reduced sampling efficiency, and lower comparability of results (e.g. Nida 2020 in this study). It may also overlap with spawning migrations and reproduction of sensitive species, potentially altering fish distribution and increasing disturbance risk (Snyder, 2003; Sutela *et al.*, 2017; Reid & Haxton, 2017). Moreover, young of the year (0+) individuals may be absent or difficult to identify reliably, limiting assessment of current-year recruitment (Hlass *et al.*, 1998). This

creates a need to develop a dedicated monitoring approach capable of assessing fish assemblage composition while minimising disturbance remain robust under the difficult and variable conditions typical for spring sampling. One possible option would be to focus sampling on selected functional habitats or mussel monitoring sites, as illustrated by Canadian mussel conservation monitoring, where seine-net fishing assessments were conducted at freshwater mussel index stations (Gáspardy *et al.*, 2021).

The probability of fish infestation depends not only on the occasional occurrence of fish hosts but on their stability of occurrence: if fish are sedentary, then if they occur at mussel bed, the infestation should be intensive. In our data both *P. phoxinus* and *L. cephalus* are doubtless the most stable species occurring at mussel beds both in space and time (Fig. 6). Other species occur discontinuously and in low numbers. This was confirmed by PIT tag marking experiment, which shows that in lowland river *L. cephalus* is all the time available for infestation (Fig. 7).

Dispersal limitation might be important factor not only influencing spatial distribution of species (Schwalb *et al.* 2015) but also their demography (Schwalb *et al.* 2011), especially considering *U. crassus* occurrence in metapopulation systems (Zajac *et al.* 2018). The permanent presence of tagged individuals of *L. cephalus* at studied mussel bed does not imply that the fish species presence means all the time the same individuals which are sedentary. Also individuals tagged in other areas of the river catchment, which were recorded at the mussel site, were thus demonstrated to disperse on the long distances, like 1km, thus can potentially spread the glochidia on large areas, for sure enabling spreading of *U. crassus* in the whole of the Smuga Umianowicka hydrological system, setting up effective metapopulation system. However, it is worth noting that actual glochidial infestation may affect host behaviour, also in the context of dispersal (Horky *et al.* 2014, Rock *et al.* 2025) and the real outcome of larvae dispersal remains unknown.

If we assume that infestation is passive and number of infested fish is proportional to their number, then extinction of *P. phoxinus* or *S. cephalus* would bring enormous decline in mussel demography, however this decline would be almost absolute in the populations studied by Schneider *et al.* (2019), whereas less severe for populations in this and Lemand *et al.* (2016) study. Thus, in continental rivers mussels would

420 have some alternatives, in Scandinavian habitats *U. crassus* has excellent host availability, however, in
421 the case of their decline, the consequences for the mussels would be disastrous, because availability of
422 alternative host species is actually close to zero. This factors were so far neglected in the conservation
423 strategies, which might be exemplified by the general discrepancy between monitoring systems of fish
424 and mussels in many of the European countries (Zajac *et al.* 2026). This needs urgent improvement,
425 leading towards unified system of freshwater species monitoring and coordinated conservation
426 activities.

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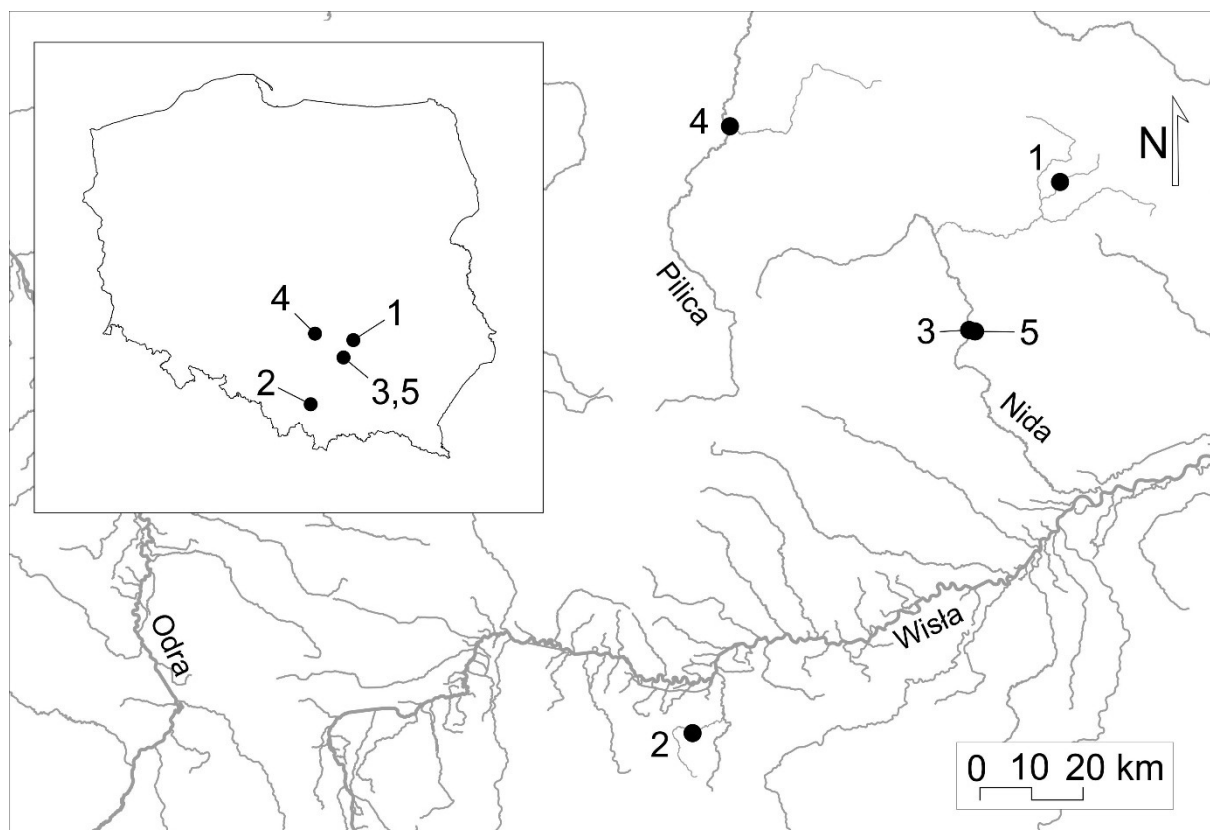
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592

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603



605
 606 Fig. 1. Map of study sites location: 1 – the Warkocz river; 2 – the Cedron river; 3 – the Nida river; 4 –
 607 the Czarna Włoszczowska river; 5 – the Smuga Umianowicka river (part of the inland delta of the Nida)

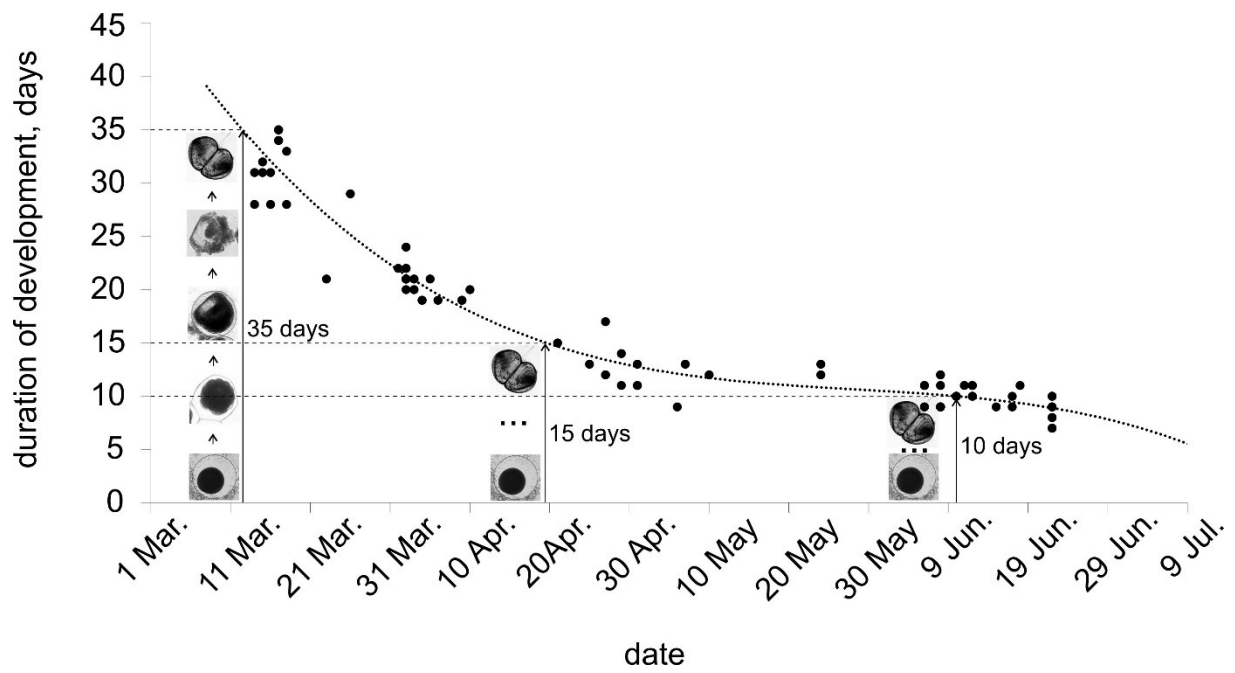
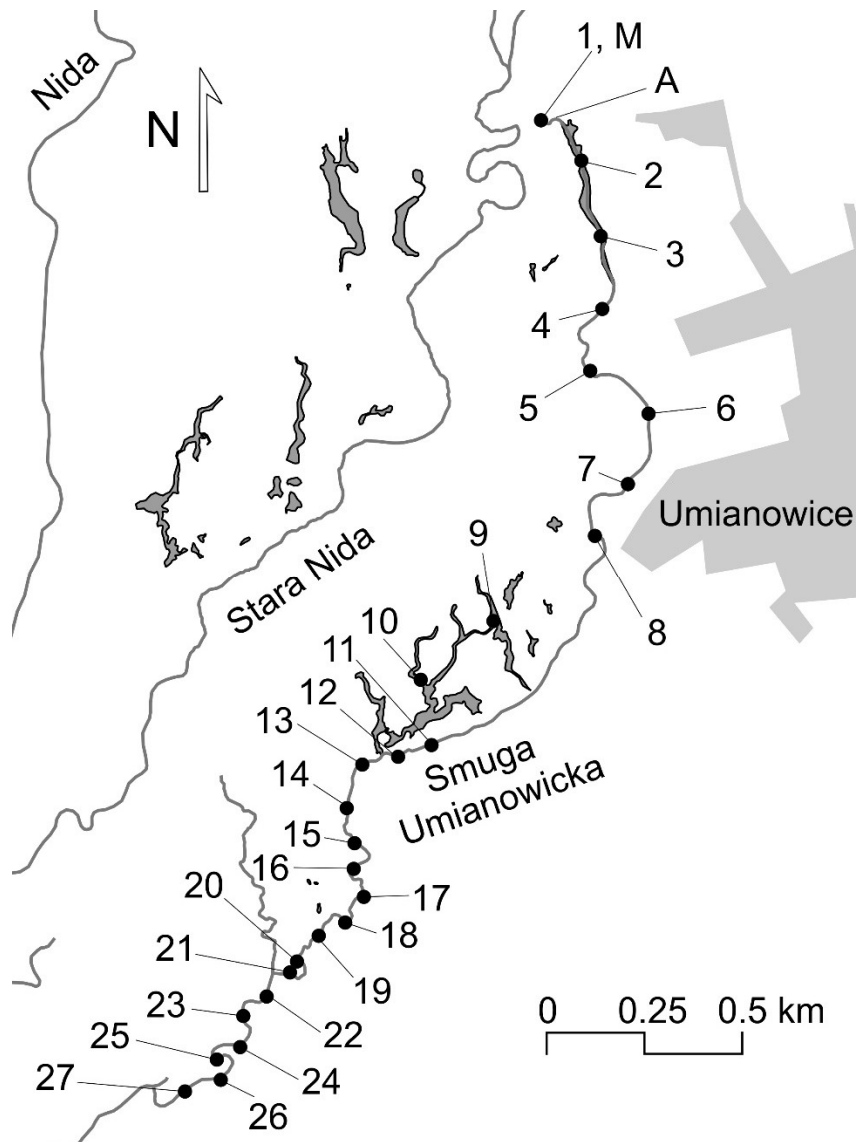


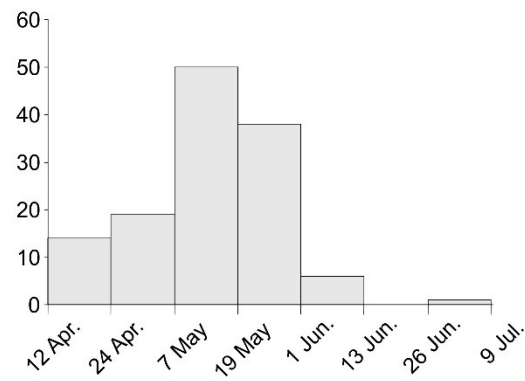
Fig. 2. The curve of the duration of glochidia development during a season, illustrated with rough estimate of the number of days needed by given developmental stage



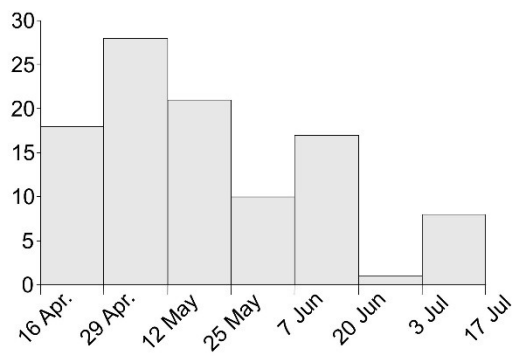
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614 Fig. 3. Fish tagging sites in the Smuga Umianowicka river; M – mussel bed location; A – location of
 615 the antenna; 1-27 – sites of fish tagging

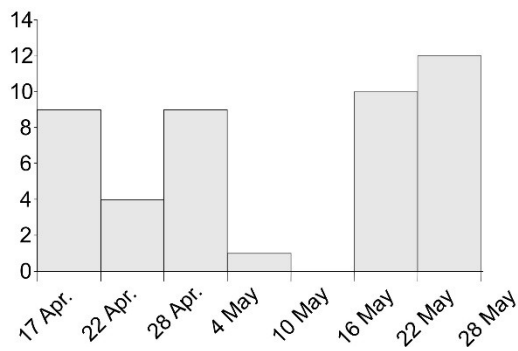
a)



b)



c)



616

617 Fig. 4. An example of the distributions of events of glochidia release in the Warkocz river in years: a)

618 2020, b) 2021 and c) 2023; the y-axis shows number of released broods from all inspected females of

619 *U. crassus*

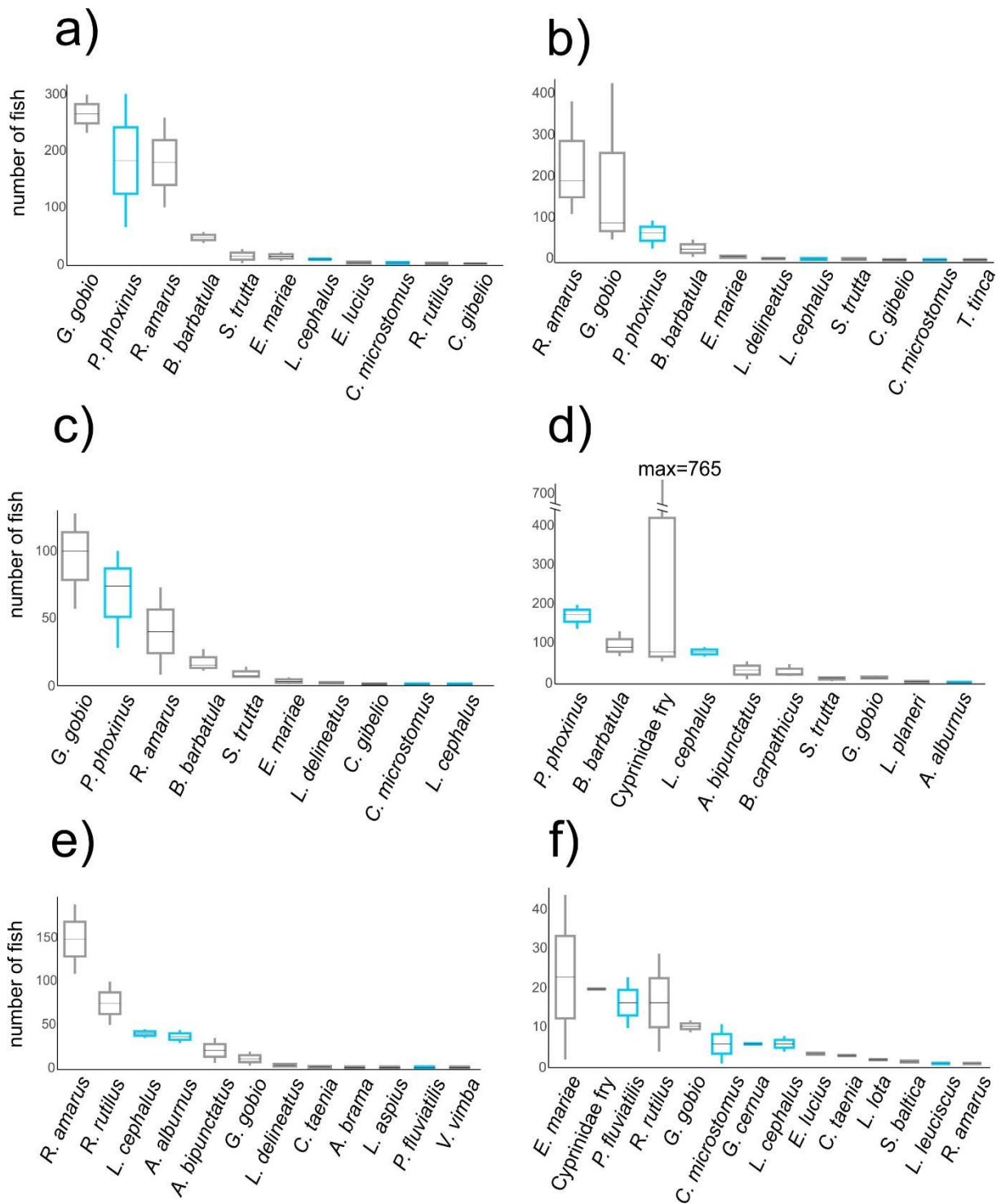
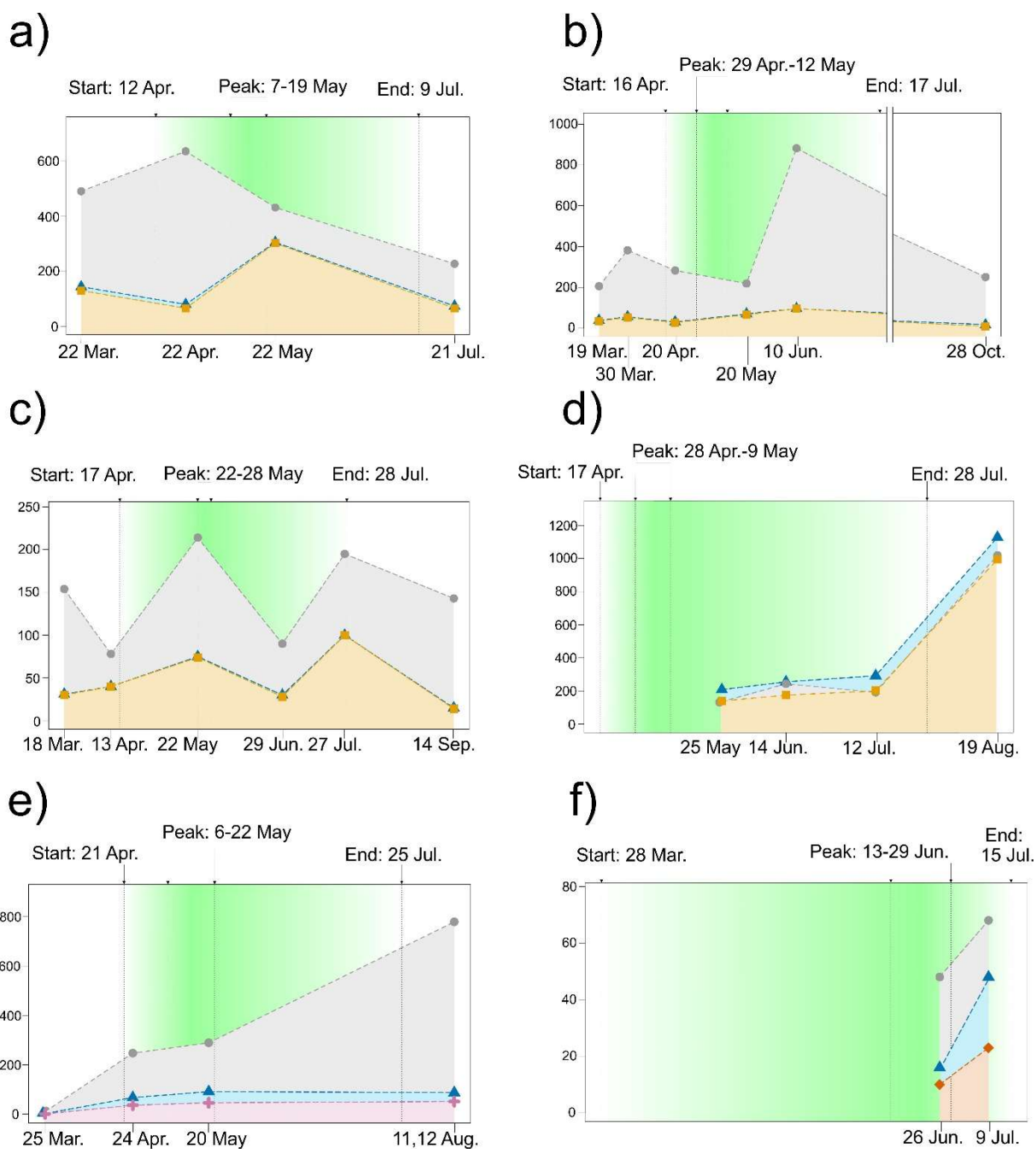


Fig. 5. Number of fish sampled during each site- and year-specific glochidia release period: a) Warkocz 2020, b) Warkocz 2021, c) Warkocz 2023, d) Cedron 2022, e) Nida 2020, f) Czarna Włoszczowska 2019; line indicates median, box indicates lower and higher quartile, whiskers indicate range of observations



Number of: —●— non-host fish, —▲— host fish, —■— *P. phoxinus*, —◆— *P. fluviatilis*, —+— *L. cephalus*

Fig. 6. Total number of non-host fish, host fish and dominant host in relation to year- and site-specific period of glochidia release (area shaded in green): a) Warkocz 2020, b) Warkocz 2021, c) Warkocz 2023, d) Cedron 2022, e) Nida 2020, f) Czarna Włoszczowska 2019; the start, peak and end of the glochidia release period are shown in the upper axis; consecutive fish sampling is referenced in the lower axis; all graphs represent the actual time scale

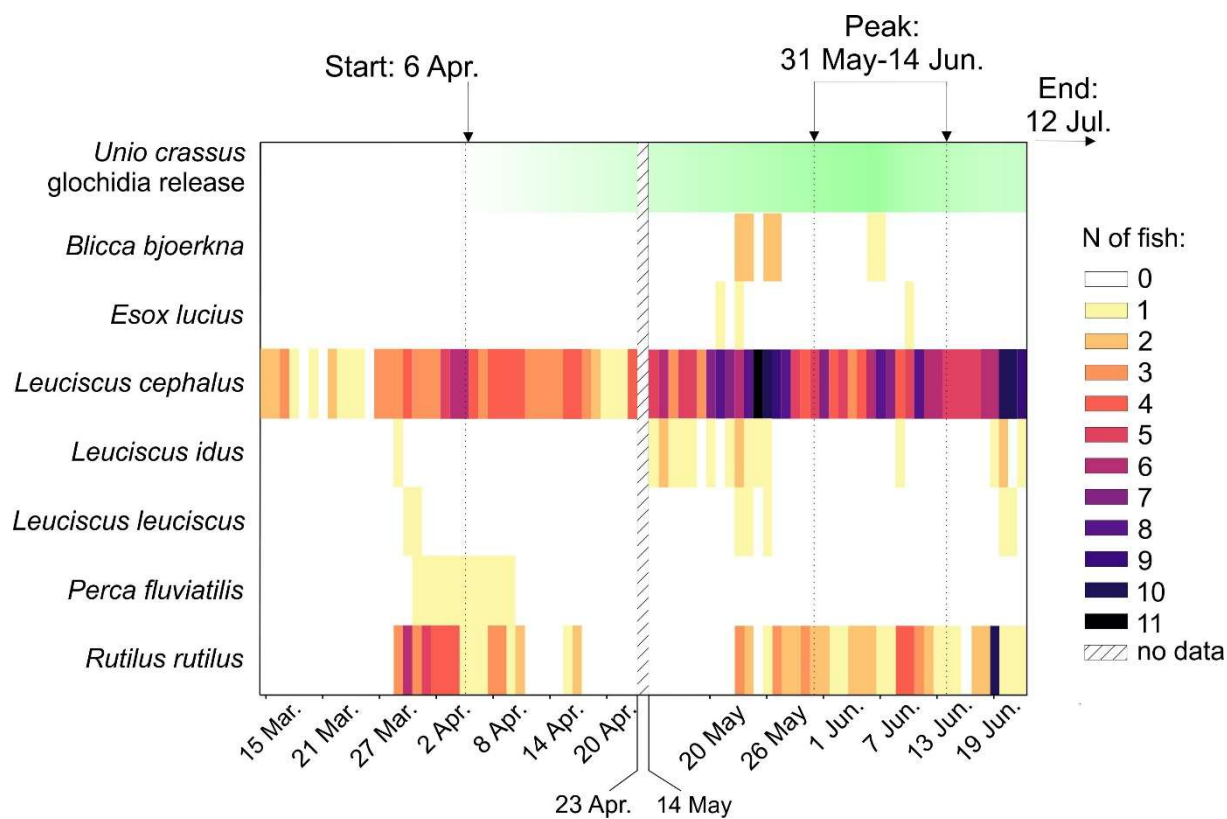


Fig. 7. Number of fish belonging to each tagged species recorded during the exposition of PIT-tag antenna located near *U. crassus* mussel bed (from 15-03-2024 to 23-04-2024 and from 14-05-2024 to 22-06-2024) in relation to the studied part of glochidia release period

635

Supplementary materials

636

Tab S1. Comparisons of host vs. non-host proportions between rivers during the period of glochidia

637

release of *U. crassus*; in the first column host proportion [%] out of the total number of fish caught

638

during a period of glochidia release in a given river

639

	Warkocz 2020	Warkocz 2021	Warkocz 2023	Cedron 2022	Nida 2020	Czarna W. 2019
Warkocz 2020 (26.6%; N=1455)	-					
Warkocz 2021 (12.5%; N=1581)	<0.0001	-				
Warkocz 2023 (29.1%; N=704)	0.2381	<0.0001	-			
Cedron 2022 (57.0%; N=1325)	<0.0001	<0.0001	<0.0001	-		
Nida 2020 (22.8%; N=694)	0.0732	<0.0001	0.0111	<0.0001	-	
Czarna W. 2019 (35.6%; N=180)	0.0179	<0.0001	0.1213	<0.0001	0.0010	-

640

641 Tab. S2. Summary of fish tagged in the Smuga Umianowicka river during this study

<i>species</i>	total length: mean $\pm$ SD, mm	total N of tagged fish	N of recorded individuals (% of tagged)
<i>Rutilus rutilus</i>	170.6 $\pm$ 29.5	392	15(3.8)
<i>Leuciscus cephalus</i>	275.0 $\pm$ 49.1	147	19(12.9)
<i>Esox lucius</i>	238.6 $\pm$ 105.1	138	1(0.7)
<i>Blicca bjoerkna</i>	183.6 $\pm$ 19.6	38	5(13.2)
<i>Leuciscus idus</i>	239.7 $\pm$ 71.2	27	4(14.8)
<i>Perca fluviatilis</i>	166.3 $\pm$ 41.1	27	2(7.4)
<i>Scardinius erythrophthalmus</i>	169.6 $\pm$ 46.1	26	0(0.0)
<i>Tinca tinca</i>	246.3 $\pm$ 37.6	13	0(0.0)
<i>Leuciscus leuciscus</i>	192.6 $\pm$ 35.6	10	1(10.0)
<i>Leuciscus aspius</i>	194.0 $\pm$ 50.9	2	0(0.0)
<i>Lota lota</i>	121.0 $\pm$ 4.2	2	0(0.0)
<i>Chondrostoma nasus</i>	247.0 $\pm$ NA	1	0(0.0)
total:		823	47(5.7)

642