

Uniwersytet Mikołaja Kopernika w Toruniu
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Rozprawa doktorska

**Wpływ wybranych czynników biologicznych
na rodzime i inwazyjne małże Unionidae**

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Feci, quod potui, faciant meliora potenses.

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Spis treści

Streszczenie	8
Abstract	10
Wstęp	12
Badania przeprowadzone w ramach pracy doktorskiej	17
Cele szczegółowe badań i hipotezy	17
Przebieg eksperymentów	19
Wyniki badań i ich omówienie	22
Podsumowanie i wnioski	26
Bibliografia	28
Publikacja 1	34
Publikacja 2	59
Manuskrypt.....	78
Podsumowanie osiągnięć naukowych	113

Streszczenie

Małże słodkowodne pełnią szereg ważnych funkcji w ekosystemie, jak przyspieszanie procesu sedymentacji, tworzenie siedlisk dla innych organizmów, kształtowanie sieci troficznych jako składnik diety niektórych drapieżników. Są to organizmy o ograniczonej mobilności, a przez to szczególnie narażone na oddziaływanie lokalnych czynników środowiskowych, w tym gatunków inwazyjnych, zarówno konkurentów jak i drapieżników. Inwazyjnym małżem, który konkuruje z rodzimymi małżami o przestrzeń życiową, pokarm oraz ryby (żywicieli dla stadiów larwalnych) jest gatunek z rodziny Unionidae – szczęźuja chińska *Sinanodonta woodiana*. Konkurencyjny wpływ na rodzime małże mogą mieć także inwazyjne, poroślowe małże z rodzaju *Dreissena*, które przyczepiając się do muszli żywych osobników mogą utrudniać im odżywianie, lokomocję i zagrzebywanie się w podłożu, a przez to narażać na ataki drapieżników lub wpływ niekorzystnych warunków środowiska. Na rodzime gatunki negatywnie mogą wpływać także inwazyjne raki sygnałowe *Pacifastacus leniusculus*, które rozszerzają swój zasięg występowania i aktywnie polują na różne bezkręgowce bentosowe, w tym małże.

Celem niniejszej rozprawy doktorskiej było określenie wpływu wybranych czynników biologicznych na preferencje siedliskowe i behavior (lokomocję i zagrzebywanie się w podłożu) rodzimych Unionidae i inwazyjnej *S. woodiana* oraz tego w jaki sposób badane czynniki potencjalnie mogą wpływać na konkurencyjną interakcję między tymi małżami. W tym celu przeprowadzono trzy zestawy eksperymentów laboratoryjnych. Wybranymi czynnikami były kolejno: (1) puste muszle i żywe osobniki *S. woodiana*, (2) poroślowe, inwazyjne małże z rodzaju *Dreissena* oraz (3) inwazyjny, drapieżny rak *P. leniusculus*.

Wyniki przeprowadzonych badań wykazały, że: (1) puste muszle i żywe osobniki *S. woodiana* w podłożu, tworzą unikane przez rodzime małże siedlisko, utrudniające im zagrzebywanie oraz stymulujące przemieszczanie, najprawdopodobniej w poszukiwaniu odpowiedniejszego siedliska, (2) poroślowe *Dreissena* spp. ograniczają zagrzebywanie się rodzimych małży ale nie wpływają istotnie na behavior inwazyjnej *S. woodiana*, która może stanowić ich rezerwuar pośrednio dający przewagę w konkurencji z gatunkami europejskimi, (3) *P. leniusculus* ograniczał zagrzebywanie się rodzimych małży, jednak to *S. woodiana* była najczęściej i najdłużej atakowana.

Przeprowadzone badania wykazały, że wybrane czynniki biologiczne w różny sposób wpływają na Unionidae w kontekście przewagi inwazyjnych gatunków małży nad rodzimymi.

Czynniki te mogą oddziaływać w sposób dający przewagę gatunkom inwazyjnym – poprzez tworzenie niesprzyjających siedlisk dla rodzimych gatunków, w postaci muszlowisk lub skupisk żywych osobników *S. woodiana* ograniczających zagrzebywanie, a także poprzez stwarzanie rezerwuaru dla inwazyjnych, poroślowych *Dreissena* spp., które ograniczają zagrzebywanie się małży rodzimych, a przez to narażają je na przykład na ataki drapieżników. Z drugiej strony to *S. woodiana* była najczęściej atakowana przez *P. leniusculus*, co może stanowić czynnik spowalniający dalszą inwazję tego małża. Uzyskane wyniki dostarczają informacji na temat wpływu różnych czynników na złożone interakcje między rodzimymi a inwazyjnymi Unionidae oraz dotyczących behawioralnych reakcji małży, które mogą mieć kluczowe znaczenie dla ich dostosowania i przetrwania w zmieniających się warunkach środowiska.

Słowa kluczowe: Bivalvia, inwazje biologiczne, konkurencja międzygatunkowa, preferencje siedliskowe, inżynierowie ekosystemu, lokomocja, zagrzebywanie się

Abstract

Freshwater mussels perform many important functions in the ecosystem, such as accelerating sedimentation, creating habitats for other organisms, and shaping food webs as a component of the diet of some predators. These organisms have limited mobility, making them particularly vulnerable to local environmental factors, including invasive species, both competitors and predators. An invasive mussel that competes with native mussels for habitat, food, and fish (hosts for larval stages) is the Chinese pond mussel *Sinanodonta woodiana*, a species of the Unionidae family. Invasive, fouling mussels of the genus *Dreissena* may also exert competitive impact on native mussels. By attaching themselves to the shells of living individuals, they can impede their feeding, movement and burrowing into the substratum, thus exposing them to predators or unfavourable environmental conditions. Native species may also be negatively affected by the invasive signal crayfish *Pacifastacus leniusculus*, which is expanding its range and actively preys on various benthic invertebrates, including mussels.

The aim of this dissertation was to determine the impact of selected biological factors on the habitat preferences and behaviour (locomotion and burrowing) of native Unionidae and the invasive *S. woodiana*, and how these factors potentially influence the competitive interaction between these mussels. For this purpose, three sets of laboratory experiments were conducted. The selected factors were: (1) empty shells and living individuals of *S. woodiana*, (2) invasive mussels of the genus *Dreissena*, and (3) the invasive, predatory crayfish *P. leniusculus*. The results of the conducted research showed that: (1) empty shells and living *S. woodiana* in the substratum create a habitat avoided by native mussels, hindering their burrowing and stimulating their movement, most likely in search of a more suitable habitat; (2) fouling of *Dreissena* spp. reduces burrowing of native mussels but do not significantly affect the behaviour of invasive *S. woodiana*, which may serve as a reservoir, providing an indirect competitive advantage against European species; (3) *P. leniusculus* limited burrowing of native mussels, but *S. woodiana* was attacked most frequently and for the longest time.

The conducted research showed that selected biological factors affect Unionidae differently in the context of the predominance of invasive mussel species over native ones. These factors may act to advantage invasive species by creating unfavourable habitats for native species, such as shell beds or aggregations of living *S. woodiana*, which limit burrowing. They also provide a reservoir for invasive, fouling *Dreissena* spp., which limit burrowing of native mussels, thus exposing them to predators. On the other hand, *S. woodiana* was most frequently

attacked by *P. leniusculus*, which may slow down further invasion of this mussel. The obtained results provide insight into the effect of various factors on the complex interactions between native and invasive unionids and the behavioural responses of mussels, which may be crucial for their adaptation and survival in changing environmental conditions.

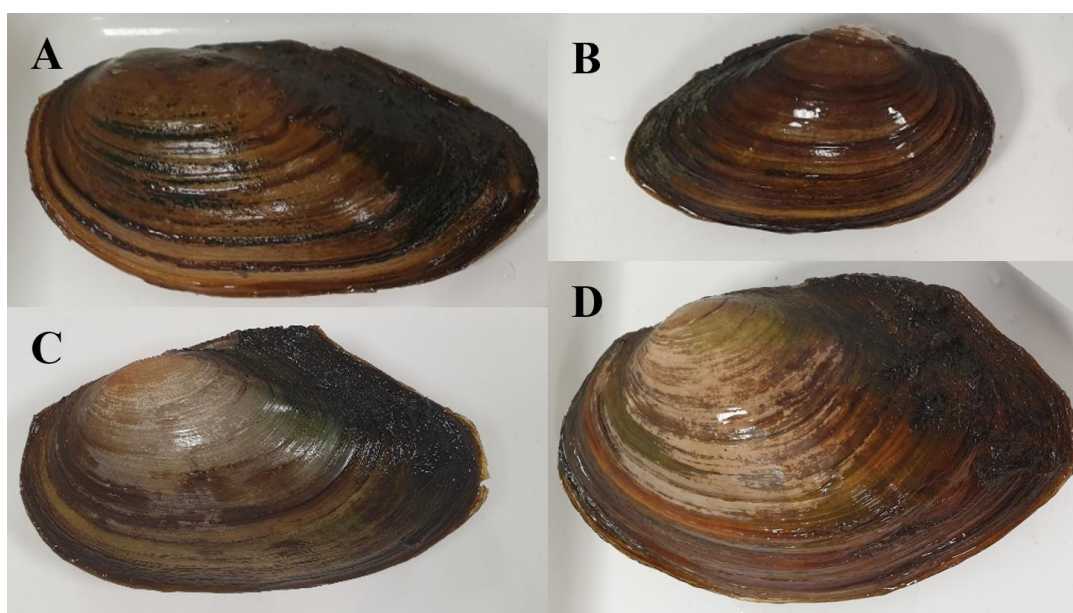
Keywords: Bivalvia, biological invasions, interspecific competition, habitat preferences, ecosystem engineers, locomotion, burrowing

Wstęp

Małże pełnią wiele ważnych funkcji w ekosystemach słodkowodnych. Są to organizmy stosunkowo mało mobilne, które filtrując wodę wychwytyją z niej drobne cząstki organiczne i organizmy w tym fitoplankton i zooplankton, którymi się odżywiają (Strayer i in. 2008; Marescaux i in. 2016). Mogą powodować tym samym poprawę przezroczystości wód, co jest szczególnie pożądane przez człowieka, ponieważ pociąga za sobą wzrost zysków z turystyki (Schröder i in. 2014). Małże wykorzystuje się również w systemach wczesnego ostrzegania o zanieczyszczeniach wody (system biomonitoringu SYMBIO) (Tomczak i Dominiak 2015), a także do oczyszczania środowiska w procesach bioremediacji rzek i jezior (Domingues i in. 2020). Ponadto małże wpływają na cykle biogeochemiczne oraz dostępność materii organicznej w mikrosiedliskach, odfiltrowując ją z toni wodnej i kierując do osadów dennych w formie fekaliów i pseudofekaliów (tj. niespożytych cząstek zawiesiny usuwanych przez syfon wpustowy) (Pigneur i in. 2014; Pouil i in. 2021). Małże mają także ważne znaczenie siedliskotwórcze. Zarówno skupiska żywych małży, jak i pozostawione po ich obumarciu puste muszle, tworzą siedliska i schronienie dla wielu organizmów bentosowych, takich jak raki (Astacidae), kielże (Amphipoda) czy larwy owadów (Insecta) (Gutiérrez i in. 2003). Natomiast żywe małże są chętnie zjadane przez drapieżniki, takie jak większe raki, ryby, ptaki i ssaki (Marescaux i in. 2016). Ze względu na pełnienie wielu funkcji i znaczący wpływ na swoje otoczenie, małże uznawane są za organizmy kluczowe dla funkcjonowania ekosystemów, a także za inżynierów ekosystemu, których obecność jest bardzo istotna w kształtowaniu warunków siedliskowych w środowiskach wodnych (Sousa i in. 2009). Wydaje się jednak, że małże należą do organizmów szczególnie narażonych na postępujące zmiany globalne. Ze względu na mało mobilny tryb życia, małże są podatne na nagłe zmiany warunków środowiska, takie jak wahania temperatury i natlenienia, pojawienie się zanieczyszczeń czy inne czynniki kształtujące jakość wody, co sprawia, że są doskonałymi bioindykatorami (Falfushynska i in. 2014). Postępujące zmiany klimatyczne, a także działalność człowieka w obrębie zbiorników i cieków wodnych oraz ich zlewni, negatywnie wpływają na populacje małży przyczyniając się do spadku ich liczebności w skali globalnej (Strayer i in. 2008). Szczególnie zauważalne jest zmniejszenie zagęszczenia wielu gatunków małży z rodziny Unionidae (skójkowate), które w wielu krajach na świecie zostały objęte ochroną w celu zapobiegania ich wyginięciu (Lopes-Lima i in. 2017).

Przykładem chronionego gatunku Unionidae jest szczeżuja wielka *Anodonta cygnea* (Linnaeus 1758) (Ryc. 1A). Jest to największy rodzimy, europejski gatunek małża, który

dorasta do 26 cm długości muszli (Feliński 1930; Piechocki i Wawrzyniak-Wydrowska 2016). W Polsce jego liczebność sukcesywnie maleje i z tego względu od 2016 jest objęty częściową ochroną (Rozporządzenie Ministra Środowiska z dnia 16 grudnia 2016 roku w sprawie ochrony gatunkowej zwierząt oraz Dz. U. 2016 poz. 2183). *Anodonta cygnea* znajduje się w Polskiej Czerwonej Księdze zwierząt zagrożonych i wymarłych ze statusem EN – gatunek bardzo wysokiego ryzyka, silnie zagrożony wyginięciem ze względu na nieliczną populację (Zając 2004). Również w innych krajach europejskich, takich jak Austria, Niemcy, Irlandia, Czechy i Norwegia, *A. cygnea* została wpisana na lokalne listy gatunków zagrożonych ze względu na postępujący spadek liczebności (Lopes-Lima 2014a). Pozostałe dwa wykorzystane gatunki małży, tj. szczeżuja pospolita *Anodonta anatina* (Linnaeus 1758) (Ryc. 1C) oraz skójka zaostrzona *Unio tumidus* Philipsson 1788 (Ryc. 1B) nie są w Polsce objęte ochroną, ale ich liczebność również maleje ze względu na zanieczyszczenie środowiska, czy presję ze strony człowieka lub gatunków inwazyjnych (Lopes-Lima i in. 2017).



Rycina 1. Morfologia muszli wykorzystanych w badaniach małży Unionidae: **A** – szczeżuja wielka *Anodonta cygnea*, **B** – skójka zaostrzona *Unio tumidus*, **C** – szczeżuja pospolita *Anodonta anatina*, **D** – szczeżuja chińska *Sinanodonta woodiana*. Skala wielkości nie została zachowana. (fot. K. Wiśniewski)

Spadająca liczebność tych gatunków spowodowała, że w takich krajach jak Austria, Niemcy, Irlandia i Rumunia, zaliczane są do bliskich zagrożenia lub zagrożonych (Van Damme 2011; Froufe i in. 2014; Lopes-Lima 2014b). Poznanie wpływu różnych czynników

biotycznych na omawiane małże, na cechy warunkujące ich przeżywalność, takie jak behavior (np. lokomocja i zagrzebywanie się), może być kluczowe w kontekście ich ochrony.

Czynnikiem biotycznym, który istotnie wpływa na liczebność małży są rozprzestrzeniające się inwazyjne gatunki obce (Strayer 2008). Są to organizmy, które zostały przypadkowo lub celowo przeniesione poza swój naturalny (rodzimy) obszar występowania, zadomowiły się w nowym środowisku i wywierają negatywny wpływ na zdrowie i/lub gospodarkę człowieka oraz rodzimą bioróżnorodność poprzez zaburzenie funkcjonowania ekosystemów (Bacher i in. 2018; Novoa i in. 2021). Przewiduje się, że tempo rozprzestrzeniania inwazyjnych gatunków będzie rosnąć, a do czynników stymulujących ten proces można zaliczyć handel drogą morską i lądową, transport różnych surowców i żywych organizmów, turystykę oraz zmiany klimatu, które mogą sprzyjać ich aklimatyzacji (Latombe i in. 2023). Inwazje biologiczne uważane są za jedno z największych zagrożeń dla rodzimych gatunków (Sala i in. 2000). Negatywny wpływ gatunków obcych na rodzime może wynikać z szeregu procesów, które prowadzą do nieodwracalnych zmian w ekosystemach (Mack i in. 2000). Są to między innymi zmiany struktury siedlisk, wprowadzanie nieznanych pasożytów i chorób, presja ze strony nowych drapieżników oraz konkurencja z gatunkami rodzimymi o zasoby pokarmowe lub przestrzeń życiową (Früh i in. 2017). Należy zaznaczyć, że inny wpływ będą wywierały inwazyjne małże, które mogą konkurować z rodzimymi małżami o przestrzeń, pokarm, czy też ryby-żywicieli dla swoich pasożytniczych larw (glochidiów), a inny wpływ będą wywierały inwazyjne drapieżniki, takie jak ryby lub raki aktywnie żerujące na małżach, które nie będą posiadały żadnych mechanizmów obrony przed nieznanym drapieżnikiem.

Jednym z inwazyjnych konkurentów rodzimych małży, osiągającym bardzo duże zagęszczenia, jest szczeżuja chińska *Sinanodonta woodiana* (Lea 1834) (Ryc. 1C) z rodziny Unionidae, której rodzimym obszarem występowania jest południowo-wschodnia Azja (Lopes-Lima i in. 2020), a obecnie rozprzestrzeniła się m.in. na obszarze Ameryki Północnej oraz Europy, w tym Polski (Douda i in. 2024). Gatunek ten rozmnaża się dwa razy w ciągu roku, w przeciwieństwie do małży rodzimych rozmnażających się tylko raz w roku (Labecka i Czarnoleski 2019). *Sinanodonta woodiana* charakteryzuje się przy tym szerszym zakresem gatunków ryb, będących żywicielami jej glochodiów i wyższymi wskaźnikami infekcji ryb (Douda i in. 2012; Huber i Geist 2019), a także wytwarza większą liczbę glochidiów niż gatunki rodzime. Ponadto, ryby-żywicieli po zakończonej inkubacji larw, indukują obronę immunologiczną przed glochidiami, co utrudnia ich ponowne zainfekowanie, w tym także

przez rodzime małże, co może uniemożliwiać im zamknięcie cyklu rozrodczego, a w konsekwencji powodować dalszy spadek ich liczebności (Rock i in. 2022). Wszystkie te czynniki przyczyniają się do szybkiego wzrostu liczebności *S. woodiana* w zajmowanym środowisku i mogą dawać przewagę konkurencyjną nad rodzimymi małżami. *Sinanodonta woodiana* charakteryzuje się znaczną plastycznością ekologiczną (Dobler i in. 2022), szybkim wzrostem i rozwojem, dużym rozmiarem, wysokimi zagęszczeniami w środowisku oraz żyje znacznie krócej niż rodzime Unionidae (Spyra i in. 2012; Urbańska i in. 2019). Konsekwencją tego jest pozostawianie po obumarciu dużych ilości muszli, które niekiedy tworzą rozległe muszlowiska znacząco zmieniające charakter podłoża w zbiorniku lub cieku wodnym (Bódis i in. 2014a). Z jednej strony puste muszle mogą stanowić dobre kryjówki dla niewielkich zwierząt bentosowych, ale z drugiej strony mogą utrudniać przepływ wody przy dnie oraz utrudniać swobodne przemieszczanie się innych małży (Gutiérrez i in. 2003; Nakano 2023).

Kolejnymi inwazyjnymi małżami, które mogą mieć istotny wpływ na populacje Unionidae są przedstawiciele rodziny Dreissenidae – racicznica zmienna *Dreissena polymorpha* (Pallas 1771) oraz *Dreissena bugensis* (Andrusov 1897), których rodzimym obszarem występowania jest region ponto-kaspijski, tj. zlewnia Morza Czarnego, Azowskiego i Kaspijskiego (Matthews i in. 2014). Obecnie gatunki te rozprzestrzeniły się w wielu miejscach w Ameryce Północnej oraz Europie (Collas i in. 2018). *Dreissena* spp. są małżami poroślowymi – przyczepiają się bisiorami (szybkoschnąca wydzielina gruczołu bisiorowego u nasady nogi tworzącego jedwabiste nici) do twardych przedmiotów zanurzonych w wodzie, tworząc obrost, który może gęsto pokrywać kamienie, zatopione pnie drzew, elementy hydrotechniczne, a także żywe organizmy, w tym małże (Ryc. 2) lub ich puste muszle (Sousa i in. 2011). Masa takiego obrostu potrafi przewyższać masę małża, do którego *Dreissena* spp. są przyczepione (Burlakova i in. 2000), powodując tym samym ograniczenie zdolności do filtrowania wody, czyli pobierania pokarmu i tlenu (Sousa i in. 2011) oraz zagrzebywania w podłożu (Van Appledorn i Bach 2007), co zaburza ich naturalny mechanizm obronny (Saloom i Duncan 2005). Konsekwencją tego może być osłabienie kondycji fizycznej małży przejawiające się spadkiem zawartości glikogenu w tkankach (Bódis i in. 2014b) oraz większa ekspozycja na zmienne lub niekorzystne warunki otoczenia i częstsze ataki ze strony drapieżników (Lymbery i in. 2021).



Rycina 2. *Sinanodonta woodiana* porośnięta przez małże *Dreissena* spp. pobrana z jeziora Balaton i wykorzystana w drugiej publikacji (fot. K. Wiśniewski)

Na behavior, kondycję i całe populacje Unionidae, wpływ mogą mieć również drapieżniki. Przykładem inwazyjnego gatunku żerującego na małżach jest rak sygnałowy *Pacifastacus leniusculus* (Dana 1852), którego rodzimym obszarem występowania jest Ameryka Północna (Bernardo i in. 2011). Rak ten został sprowadzony do Europy w XX wieku w celu zastąpienia wymierającego rodzimego raka szlachetnego *Astacus astacus* (Linnaeus 1758). Jego populacja drastycznie zmniejszyła się na skutek przełowienia, zanieczyszczenia środowiska oraz rozprzestrzeniającej się, zabójczej dla rodzimych raków, dżumy raczej wywoływanej przez lęgnowca *Aphanomyces astaci* (Schikora 1906). *Pacifastacus leniusculus* jest odporny na wspomnianą chorobę, ale jest jej nosicielem (Unestam 1973). Raki sygnałowe posiadają szeroki zakres tolerancji na warunki środowiska, takie jak wysoka trofia i niskie natlenienie (Reynolds i in. 2013, Wiśniewski i in. 2020), co sprawia, że gatunek ten ma najszerszy zasięg występowania w Europie spośród wszystkich inwazyjnych raków (Kouba i in. 2014). Raki *P. leniusculus* są wszystkożerne i aktywnie polują na różne organizmy bentosowe, w tym również na małże Unionidae (Sanders i Mills 2022). Oprócz możliwości polowania i zjadania małży, mogą także je obgryzać, powodując chowanie syfonów, a tym samym ograniczenie oddychania i odżywiania, co w przypadku częstego stresowania, obniża kondycję małży i ich rozrodczość osłabiając tym samym całe populacje małży (Ehlers i in. 2018).

Badania przeprowadzone w ramach pracy doktorskiej

Zasadniczym celem eksperymentalnych badań przeprowadzonych w ramach prezentowanej rozprawy doktorskiej było poszerzenie wiedzy na temat wpływu czynników biologicznych na małże z rodziny skójkowatych (Unionidae). Trzy rodzime gatunki małży (*A. anatina*, *A. cygnea*, *U. tumidus*) oraz jeden gatunek inwazyjny (*S. woodiana*) posłużyły jako model badawczy. Do badań wybrano czynniki biologiczne powiązane z oddziaływaniem inwazyjnych gatunków bezkręgowców na Unionidae, takie jak: (1) obecność na i w podłożu pustych muszli i żywych osobników konkurenta *S. woodiana*; (2) bezpośredni kontakt (obrost na muszlach) i obecność w pobliżu konkurenta *Dreissena* spp.; (3) bezpośredni kontakt i obecność w pobliżu drapieżnika, raka *P. leniusculus*. Ze względu na ograniczoną mobilność małży przyjęto założenie, że są to zwierzęta o ograniczonych możliwościach aktywnego, długodystansowego unikania niesprzyjających warunków środowiska, w tym oddziaływań konkurentów i/lub drapieżników. Dlatego odpowiednia lokalna, krótkodystansowa reakcja behawioralna, polegająca przede wszystkim na zagrzebaniu w podłożu, przemieszczeniu do refugium lub zamknięciu muszli, może być kluczowa dla przeżycia małży. W badaniach skoncentrowano się zatem na testowaniu takich reakcji. Porównanie reakcji behawioralnych gatunków rodzimych z inwazyjną *S. woodiana* miało na celu wskazanie potencjalnych różnic i przystosowań dających przewagę temu gatunkowi, które mogłyby promować jego dalszą ekspansję na nowe obszary.

Cele szczegółowe badań i hipotezy

Celem szczegółowym badań przedstawionych w pierwszej publikacji było określenie wpływu zanieczyszczenia podłoża pustymi muszlami (muszlowisko) i żywymi osobnikami inwazyjnej *S. woodiana* na behavior (wybór siedliska, lokomocję i zagrzebywanie się w podłożu) dwóch rodzimych gatunków małży: *A. cygnea* i *U. tumidus* oraz inwazyjnej *S. woodiana*. Sprawdzone także jak rodzime małże reagują na zanieczyszczenie podłoża muszlami innych rodzimych Unionidae i jak na takie zanieczyszczenie podłoża reaguje *S. woodiana*. Prowadzenie badań w tym kierunku jest ważne, ponieważ *S. woodiana* rozprzestrzenia się w środowisku, osiąga bardzo duże zagęszczenia, rośnie szybko, osiąga duże rozmiary, żyje krótko i pozostawia po sobie ogromne ilości pustych muszli, które zalegają na dnie i znacząco zmieniają jego charakter (Bódis i in. 2014a; Urbańska i in. 2019). Postawione zostały następujące hipotezy: **(1) Rodzime małże będą unikać podłoża zanieczyszczonego**

przez muszle i żywe osobniki *S. woodiana*. (2) Negatywny wpływ obecności muszli na preferencje siedliskowe małży będzie wynikał z utrudnionego zagrzebywania się i/lub poruszania w/na takim podłożu. Odwrotnie, wzmożona lokomocja może wskazywać na aktywne unikanie podłoża zanieczyszczonego przez *S. woodiana* i poszukiwanie odpowiedniego siedliska. (3) Puste muszle będą wpływały silniej niż żywe osobniki *S. woodiana* na rodzime małże. Może to wynikać z (i) losowego ułożenia muszli w/na podłożu w porównaniu z żywymi małżami, które żyją zagrzebane w podłożu pionowo lub (ii) obecności ostrych krawędzi muszli drażniących stopę poruszających się małży. (4) Wpływ muszli *S. woodiana* na rodzime małże będzie różnił się od wpływu muszli pochodzących od rodzimych Unionidae, co może być związane z odmienną strukturą samych muszli lub ich wielkością.

Celem szczegółowym badań przedstawionych w drugiej publikacji było określenie i porównanie wpływu obrostu i obecności inwazyjnych małży *D. polymorpha* i *D. bugensis* na lokomocję i zagrzebywanie się rodzimego *U. tumidus* i inwazyjnej *S. woodiana*. Zbadano wpływ bezpośredniego obrostu na powierzchni tych małży na ich behavior, reakcje małży na obrost obecny na innych osobnikach znajdujących się w tym samym zbiorniku eksperymentalnym oraz wpływ samych małży *Dreissena* spp. oddzielonych siatką od badanych, nieobrośniętych Unionidae. Dodatkowe obciążenie jakim jest obrost *Dreissena* spp. może znacząco wpływać na porośnięty organizm utrudniając mu jakikolwiek ruch (Van Appledorn i Bach 2007). Dlatego istotne było poznanie jak na obrost reagują małże Unionidae i czy posiadają jakieś przystosowania, dzięki którym mogą funkcjonować pomimo obecności obrostu *Dreissena* spp. lub wykazują mechanizmy pozbywania się go z powierzchni muszli. *Dreissena bugensis* jest stosunkowo nowym gatunkiem w faunie bentosowej Europy i wiedza na temat jej wpływu na rodzime małże jest ograniczona. Postawiono następujące hipotezy: (1) Obrost będzie ograniczał lokomocję i zagrzebywanie się małży w podłożu. (2) Wpływ ten może wynikać wyłącznie z mechanicznego obciążania małży (tylko w przypadku bezpośredniego obrostu na małżach) lub (3) efekt może utrzymywać się po usunięciu (zdrapaniu) obrostu z małży (z powodu długotrwałego osłabienia). (4) Unionidae będą wyczuwać i reagować na sygnały chemiczne (zapach) uwalniane przez inne obrośnięte małże lub wydzielane przez *Dreissena* spp.

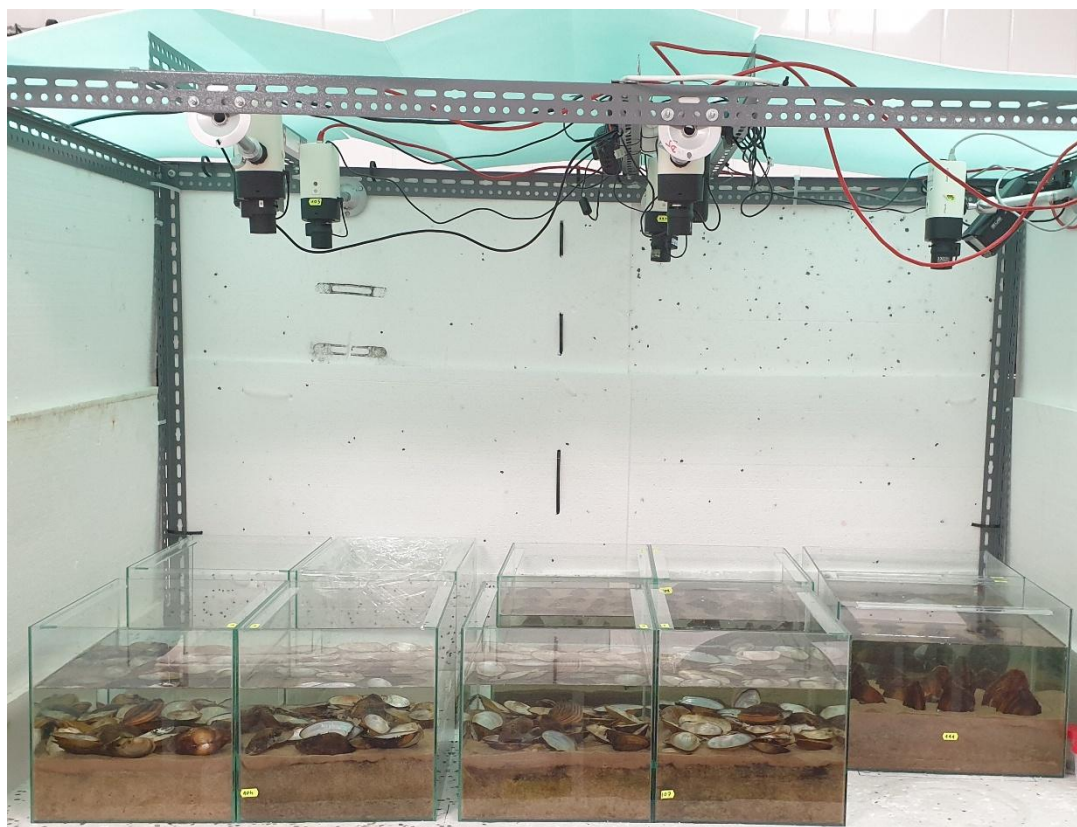
Celem szczegółowym badań zaprezentowanych w manuskrypcie, było określenie i porównanie wpływu bezpośredniej i pośredniej obecności inwazyjnego, drapieżnego raka *P. leniusculus* na lokomocję i zagrzebywanie się dwóch rodzimych gatunków małży:

U. tumidus i *A. anatina* oraz inwazyjnej *S. woodiana*. *Pacifastacus leniusculus* ma najszerszy zasięg występowania spośród inwazyjnych raków (Kouba i in. 2014), dlatego badanie jego wpływu na faunę bentosową, w tym małże Unionidae, wydaje się szczególnie ważne. Gatunek ten aktywnie poluje, a przy tym charakteryzuje się wysoką agresywnością i odwagą na tle innych raków, co sprawia, że może wywierać znaczny wpływ na zasiedlane ekosystemy (Souty-Grosset i in. 2006; Wiśniewski i in. 2020). Postawiono następujące hipotezy: **(1) Małże będą reagować na obecność raka zwiększeniem stopnia zagrzebania w podłożu oraz ograniczeniem lokomocji w celu obrony przed atakami lub ograniczeniem stopnia zagrzebania – pozostając w bezruchu na powierzchni podłoża. (2) Małże będą wyczuwały i reagowały również na zapach obecnego w zbiorniku, ale oddzielonego siatką raka-drapieżnika. (3) *Unio tumidus* będzie najrzadziej atakowany ze względu na swoją grubą muszlę i głębokie zagrzebywanie się (Poznańska-Kakareko i in. 2021). (4) *Anodonta anatina* będzie najczęściej atakowana ze względu na swoją cienką i delikatną muszlę oraz stosunkowo płytkie zagrzebywanie się w porównaniu z innymi Unionidae.**

Przebieg eksperymentów

W badaniach przedstawionych w pierwszej publikacji, związanych z zanieczyszczeniem podłoża pustymi muszlami i żywymi osobnikami *S. woodiana*, wykorzystano muszle dwóch rozmiarów (duże i małe) ze względu na duże rozmiary, jakie ten małż osiąga. Pierwszy eksperyment dotyczył testów wyboru podłoża, podczas których w akwariach znajdowały się dwa różne podłoża pokrywające równo po połowie powierzchnię dna, składające się z: piasku i piasku z dodatkiem pionowo umieszczonych w nim żywych *S. woodiana*, małych lub dużych muszli *S. woodiana* oraz muszli rodzimych małży znajdujących się na powierzchni piasku lub zagrzebanych w jego wierzchniej warstwie w różnych zagęszczeniach. Konfrontowano podłoża następująco: (i) muszle *S. woodiana* na powierzchni piasku z zagrzebanymi, (ii) żywe i częściowo zagrzebane *S. woodiana* z jej dużymi, zagrzebanymi muszlami, (iii) duże, zagrzebane muszle *S. woodiana* z małymi zagrzebanymi o zbliżonej objętości, (iv) muszle rodzimych małży z czystym piaskiem oraz (v) muszle rodzimych małży z małymi muszlami *S. woodiana*. Małże były introdukowane na środek zbiornika, w miejscu łączenia podłoży. Po 24 godzinach spisywano położenie małża (w którym podłożu się zagrzebał lub na którym leżał), następnie wyniki zostały przeanalizowane. Drugi eksperyment dotyczył analizy behawioru małży na różnych rodzajach podłoży (preferowanych i unikanych w pierwszym

eksperymentach). Małże były testowane pojedynczo i nagrywane kamerami CCTV przez 24 godziny (Ryc. 3). Następnie behavior małży został przeanalizowany pod kątem: czasu rozpoczęcia aktywności od momentu introdukcji, czasu trwania lokomocji, przebytego dystans, średniej prędkości przemieszczania, czasu i stopnia zagrzebienia w podłożu.



Rycina 3. Zestaw eksperymentalny do badania behavioru Unionidae na unikanych i preferowanych podłożach, zanieczyszczonych przez żywe *S. woodiana* oraz ich małe lub duże muszle znajdujące się na powierzchni piasku lub zagrzebane w jego wierzchniej warstwie. Zestaw wykorzystany w eksperymentach przedstawionych w pierwszej publikacji. (fot. K. Wiśniewski)

Badania ujęte w drugiej publikacji, dotyczące wpływu bezpośredniego obrotu i obecności w zbiorniku małży *Dreissena* spp. na behavior (lokomocję i zagrzebywanie się), zostały przeprowadzone w Balaton Limnological Research Institute (Tihany, Węgry) podczas odbytego przeze mnie stażu naukowego pod opieką dr Csilla Balogh. Jezioro Balaton zostało wybrane ze względu na występowanie w nim zarówno rodzimych, europejskich Unionidae, jak i inwazyjnej *S. woodiana*, a także obu gatunków *Dreissena* obrastających małże Unionidae. Podczas pierwszej części badań nagrywano kamerami CCTV od góry, przez 24 godziny, behavior (lokomocję i zagrzebywanie się) obrośniętych i nieobrośniętych przez *Dreissena* spp.

rodzimych Unionidae i inwazyjnej *S. woodiana* na piaszczystym podłożu pobranym z jeziora Balaton (Ryc. 4). Te same osobniki były testowane kilkakrotnie, przy czym w następnych powtórzeniach obrost z części osobników został usunięty. Dzięki temu sprawdzono czy behawior małży pozbawionych obrostu zmienił się oraz czy i ile małże potrzebowały czasu na regenerację kondycji. Sprawdzono także behawior osobników nieobrośniętych, ale w obecności małży posiadających obrost na swojej powierzchni. W drugiej części eksperymentów zbadano (poprzez nagrywanie przez 24 godziny) behawior rodzimych Unionidae i inwazyjnej *S. woodiana* w obecności osobników *Dreissena* spp. umieszczonych w siateczkach w akwariach zawierających to samo piaszczyste podłoże z jeziora. Dzięki temu Unionidae mogły tylko wyczuwać obecność *Dreissena* spp. w otoczeniu, ale nie miały z nimi bezpośredniego kontaktu. *Dreissena* spp. umieszczone w siateczkach również pochodziły z jeziora Balaton.



Rycina 4. Zestaw eksperymentalny do badania behawioru obrośniętych i nieobrośniętych przez *Dreissena* spp. Unionidae oraz mających pośredni kontakt z *Dreissena* spp. znajdujących się w siateczkach, wykorzystany w eksperymentach przeprowadzonych w Balaton Limnological Research Institute przedstawionych w drugiej publikacji (fot. C. Balogh)

Ostatnie badania, przedstawione w załączonym manuskrypcie, dotyczące wpływu obecności drapieżnika na Unionidae, zostały przeprowadzone w akwariach podzielonych plastikową siatką na dwie areny – dużą i małą (Ryc. 5). Zastosowano w tym przypadku dwa warianty eksperymentalne. Kontakt bezpośredni z drapieżnikiem polegał na umieszczeniu raka w dużej arenie na 24 godziny (celem aklimacji), a następnie wprowadzono jednego małża również do

dużej areny na kolejne 24 godziny. W tym momencie rozpoczynało się nagrywanie zbiornika (widok z góry). Kontakt pośredni polegał na umieszczeniu raka w małej arenie na 24 godziny, a po tym czasie umieszczano jednego małża w dużej arenie na 24 godziny i również nagrywano. Dzięki takiemu rozwiązaniu rak i małż nie miały ze sobą fizycznego kontaktu (jak w wariancie bezpośrednim) ale małż mógł wyczuwać obecność drapieżnika w otoczeniu. Wariant kontrolny polegał na umieszczeniu jednego małża do dużej areny i nagrywaniu przez 24 godziny. Po uzyskaniu nagrań przeanalizowano behavior zarówno wszystkich małży (lokomocja i zagrzebywanie) jak i raków (ilość i czas trwania ataków).



Rycina 5. Zestaw eksperymentalny do badania behavioru rodzimych Unionidae i inwazyjnej *S. woodiana* przy bezpośredniej i pośredniej obecności drapieżnego raka *P. leniusculus*, wykorzystany w badaniach przedstawionych w załączonym manuskrypcie (fot. K. Wiśniewski)

Wyniki badań i ich omówienie

Wyniki badań dotyczących wpływu zanieczyszczenia podłoża muszlami i osobnikami *S. woodiana*, które zostały przedstawione w **pierwszej publikacji** wykazały, że wszystkie badane małże, tj. *U. tumidus*, *A. cygnea* i *S. woodiana* unikały podłoża zanieczyszczonego pustymi muszlami inwazyjnej *S. woodiana*, co potwierdziło naszą pierwszą hipotezę. W przypadku rodzimych małży – porównując muszle zagrzebane z leżącymi na powierzchni podłoża oraz porównując muszle duże z małymi – wykazano, że w obu tych przypadkach

bardziej unikany był pierwszy wariant. Zgodnie z trzecią hipotezą, nie wykazano unikania podłoża zawierającego żywe, częściowo zagrzebane *S. woodiana* nawet w zagęszczeniu 133 osobników m⁻², co stanowiło dwukrotność najwyższego odnotowanego zagęszczenia w środowisku (Kraszewski i Zdanowski 2007). Analizując behavior małży, odnotowano u *U. tumidus* opóźnienie rozpoczęcia aktywności lokomotorycznej (na podłożu z żywą *S. woodiana*), wzmożone przemieszczanie się (na podłożu z żywą *S. woodiana* i z muszlami leżącymi na powierzchni) oraz zmniejszenie głębokości zagrzebywania się (na podłożu z żywą *S. woodiana* i z muszlami zarówno na powierzchni jak i zagrzebanymi w podłożu), co było zgodne z drugą hipotezą. Większa aktywność lokomotoryczna może być związana z próbą znalezienia preferowanego siedliska, czyli podłoża pozbawionego zanieczyszczenia muszlami i żywymi *S. woodiana*, które, jak wykazano, zaburzają naturalny mechanizm obronny małży (Saloom i Duncan 2005), ograniczając ich zagrzebywanie w podłożu, co może narażać je na ataki drapieżników lub zwiększoną ekspozycję na niekorzystne warunki środowiska (Block i in. 2013). Natomiast w przypadku *A. cygnea* zauważono wolniejsze przemieszczanie się (na podłożach z żywą *S. woodiana* i muszlami na powierzchni oraz zagrzebanymi), krótszy czas zagrzebywania się (na podłożu z zagrzebanymi muszlami) oraz mniejszą głębokość zagrzebywania (żywe *S. woodiana* i muszle zagrzebane w podłożu). Zanieczyszczenie podłoża muszlami rodzimych małży oraz muszlami *S. woodiana* nie było rozróżniane zarówno przez rodzime małże *U. tumidus*, jak i inwazyjną *S. woodiana*, a porównując te podłoża z czystym piaskiem – oba były unikane. Oznacza to, że muszle różnych gatunków, ale podobnej wielkości i podobnym zagęszczeniu, stanowią tak samo niekorzystne siedlisko. Jednakże, ze względu na wolniejszy wzrost i tworzenie mniejszych zagęszczeń, rodzime Unionidae nie tworzą tak rozległych muszlowisk jak *S. woodiana*, więc ich wpływ na ekosystem jest pod tym względem mniejszy (Bódis i in. 2014a).

Badania związane z wpływem poroślowych małży *Dreissena* spp. przedstawione w **drugiej publikacji** wykazały, że zgodnie z postawioną pierwszą hipotezą, obrost znajdujący się na powierzchni małży miał wpływ na ich behavior, ale tylko w przypadku *U. tumidus*, opóźniając czas od introdukcji do pierwszego ruchu i ograniczając głębokość zagrzebywania w podłożu. Podobnie jak w przypadku omawianego powyżej ograniczania zagrzebywania przez muszle *S. woodiana*, poroślowe *Dreissena* spp. swoją obecnością również mogą zaburzać mechanizm obronny małży narażając je na ataki drapieżników czy niekorzystną ekspozycję na czynniki środowiska (Saloom i Duncan 2005). Obrost występujący w dużych ilościach może zatykać syfony małży i uniemożliwiać im odżywianie oraz wymianę gazową, powodując

osłabienie organizmu (Sousa i in. 2011), które w skrajnych przypadkach może zakończyć się śmiercią małży. Po usunięciu (zdrapaniu) obrostu czas rozpoczęcia aktywności był podobny do wariantu kontrolnego, jednak głębokość zagrzebywania w podłożu wciąż była mniejsza niż w kontroli. Niemniej, głębokość zagrzebywania wróciła do poziomu kontrolnego podczas drugiego powtórzenia (dwa dni po zdjęciu obrostu). Wykazano tym samym, że obrośnięte małże były osłabione i potrzebowały czasu na regenerację, a wpływ nie był tylko chwilowy, potwierdzając tym samym naszą третią hipotezę. W kontakcie z innymi porośniętymi małżami Unionidae, *U. tumidus* ograniczał swoją lokomocję horyzontalną. Natomiast w przypadku *S. woodiana*, odnotowano tylko większą głębokość zagrzebywania się w zbiornikach zawierających *Dreissena* spp. oddzieloną siatką. Wskazuje to, że małże wyczuwały ich obecność w otoczeniu i reagowały na nią mechanizmem obronnym, w celu uniknięcia porośnięcia przez *Dreissena* spp. Tym samym czwarta hipoteza została potwierdzona. Zdolność wyczuwania przez małże obecności drapieżników (Meira i in. 2024) i pasożytów (Selbach i Mouritsen 2020), w połączeniu z rozpoznawaniem chemicznych sygnałów alarmowych wydzielanych przez zestresowane, a w szczególności uszkodzone osobniki (Leonard i in. 1999), pozwala na wcześniejszą reakcję i zwiększenie szansy na przeżycie w sytuacji zagrożenia. W przypadku naszych badań – uniknięcie obrośnięcia przez *Dreissena* spp.

Wyniki badań dotyczących wpływu inwazyjnego raka *P. leniusculus* na rodzime i inwazyjne Unionidae, przedstawione w załączonym **manuskrypcie**, wykazały, że bezpośredni kontakt z tym drapieżnikiem przyczynił się do opóźnienia rozpoczęcia aktywności wszystkich małży. Można to traktować jako mechanizm obronny polegający na przeczekaniu zagrożenia i szczelnym zamknięciu muszli oraz niewyciąganiu nogi, która mogłaby zostać zaatakowana przez raka. Takie same zachowania można zaobserwować u innych małży, jak na przykład u *D. polymorpha* w kontakcie z płocią *Rutilus rutilus* (Linnaeus 1758) (Antoń i in. 2018). Tym samym pierwsza hipoteza została potwierdzona – małże reagowały na obecność raka poprzez ograniczenie ruchu. W tym wariantcie wykazano także płytsze zagrzebywanie się *U. tumidus*, a w przypadku wariantu z rakiem oddzielonym od małża siatką (kontakt pośredni) odnotowano krótszy czas zagrzebywania się w podłożu, co potwierdziło drugą hipotezę. Wyniki te wskazują, że małże wyczuwały obecność raka w zbiorniku. Zdolność wyczuwania drapieżnika w otoczeniu występuje u wielu gatunków małży i pozwala ograniczyć ryzyko ataku lub zwiększyć szanse na jego przeżycie. Reakcje mogą być natychmiastowe lub prowadzić do utrwalenia zmian anatomicznych, jak na przykład u małży *Mytilus edulis* (Linnaeus 1758),

których muszla stała się wyraźnie grubsza u osobników współwystępujących od 15 lat z drapieżnym krabem *Hemigrapsus sanguineus* (De Haan 1853), co jest przykładem ewolucyjnego wyścigu zbrojeń między ofiarą a drapieżnikiem (Freeman i Byers 2006). Analizując natomiast behawior *P. leniusculus* można zauważyć, że zgodnie z trzecią hipotezą, *U. tumidus* był stosunkowo rzadko atakowany (podobnie było w przypadku *A. anatina*), a przy tym ani razu nie został wygrzebany z podłoża przez raki, co częściowo potwierdziło trzecią hipotezę. Zdecydowanie najczęściej atakowanym małżem była inwazyjna *S. woodiana*, a nie *A. anatina* jak założono w czwartej hipotezie. Warto zaznaczyć, że najdłuższe ataki czasem kończyły się śmiercią małża i wyjedzeniem znacznej części tkanki miękkiej z wnętrza muszli (Ryc. 6). Takie preferencje raków mogły być spowodowane faktem, że małż ten posiada cienką muszlę, którą łatwo drapieżnik może rozgryźć. Również z punktu widzenia zysku energetycznego będzie on największy, jeżeli rak zaatakuje dużą, obfitą w tkankę miękką i mającą cienką muszlę *S. woodiana*, niż małego i okrytego grubościenną muszlą *U. tumidus* (Czerniejewski i in. 2021). Podobne preferencje wykazuje krab brzegowy *Carcinus maenas* (Linnaeus 1758), który w warunkach eksperymentalnych znacznie częściej atakował i zjadał małże *Xenostrobus inconstans* (Dunker 1856) posiadające cienką i delikatną muszlę, niż *Katelysia peronii* (Lamarck 1818) o grubej i masywnej muszli (Campbell i in. 2019).



Rycina 6. *Pacifastacus leniusculus* próbujący dostać się do tkanki miękkiej *S. woodiana* podczas eksperymentów dotyczących wpływu bezpośredniej i pośredniej obecności raka na behavior Unionidae, przedstawione w załączonym manuskrypcie (fot. K. Wiśniewski)

Podsumowanie i wnioski

Wyniki badań, przeprowadzonych w ramach niniejszej rozprawy doktorskiej, dotyczących wpływu różnych czynników biologicznych na behavior rodzimych małży (szczególnie wielka *Anodonta cygnea*, szczególnie pospolita *Anodonta anatina*, skójką zaostrzona *Unio tumidus*) i inwazyjnej szczepu chińskiej *Sinanodonta woodiana* pozwoliły na wskazanie następujących obserwacji oraz sformułowanie poniższych wniosków:

1. Inwazyjna *S. woodiana* może stanowić istotny czynnik wpływający na rodzime, europejskie małże Unionidae. Puste muszle *S. woodiana* (zwłaszcza duże) znacząco zmieniają charakter podłoża, utrudniając zagrzebywanie się rodzimych małży. Najprawdopodobniej z tego powodu jest to podłoże przez rodzime małże unikane i stymulujące ich lokomocję, co świadczy o poszukiwaniu odpowiedniego, pozbawionego muszli, siedliska. Biorąc pod uwagę rozszerzający się zasięg występowania *S. woodiana*, presja wynikająca z tworzenia przez nią rozległych muszlowisk, może powodować ograniczanie zasięgu występowania rodzimych Unionidae, dalsze zmniejszanie liczebności populacji, a nawet przyspieszenie ich wymierania.
2. Inwazyjne *Dreissena* spp., porastające inne małże, mogą wpływać na interakcje między rodzimymi i inwazyjnymi małżami Unionidae poprzez wspieranie *S. woodiana*, na którą bezpośredni obrost nie wpływał (nie zmieniał behavioru małża). Porośnięty rodzimy *U. tumidus* zagrzebywał się znacznie płycej, co może narażać go na ataki drapieżników lub wpływ niekorzystnych, zmiennych warunków środowiska. Ze względu na osiągnięte rozmiary, *S. woodiana* może pomieścić na swojej powierzchni duże ilości *Dreissena* spp. i tym samym stanowić ich rezerwuar. Obrost na *S. woodiana* nie będzie zmieniał jej behavioru, ale jeśli *Dreissena* spp. przeniosą się na rodzime Unionidae, to mogą przyczynić się do spadku ich kondycji i, podobnie jak w punkcie 1., powodować ograniczanie zasięgu występowania rodzimych Unionidae, dalsze zmniejszanie liczebności populacji, a nawet przyspieszenie ich wymierania.

3. Uważane za bardzo żarłoczne, raki *P. leniusculus* są stresorem, który wpływa na behavior i przeżywalność małży. Obecność raków ograniczała aktywność i zagrzebywanie się rodzimych małży w podłożu, które szczelnie zamykały się w celu ochrony delikatnych tkanek wewnątrz muszli. Jednakże szczególnie narażona wydaje się być inwazyjna *S. woodiana*, ponieważ była najczęściej atakowana. Raki prawdopodobnie kierowały się zyskiem energetycznym – duża i łatwa w rozgryzieniu *S. woodiana* była atrakcyjniejszą ofiarą niż mniejszy, o twardszej muszli *U. tumidus*. Oznacza to, że liczebność *S. woodiana* w środowisku może być ograniczana lub utrzymywana na stałym poziomie przez drapieżnictwo *P. leniusculus*, preferującego je ponad rodzime Unionidae.

Podsumowując przeprowadzone badania można stwierdzić, że inwazyjna *S. woodiana* stanowi element słodkowodnej fauny bentosowej o dużym potencjale oddziaływania na rodzime Unionidae. Tworząc rozległe muszlowiska znacząco zmienia środowisko, w którym się pojawia i negatywnie wpływa tym samym na rodzime małże, których liczebność populacji wykazuje trend spadkowy w skali globalnej. Małże reagują behawioralnie na niesprzyjające warunki poprzez wzmożoną lokomocję czy też głębsze zagrzebywanie się w podłożu, jednak te zachowania mogą być modyfikowane przez różne czynniki biologiczne, takie jak obrastające małże z rodzaju *Dreissena* czy drapieżne raki *P. leniusculus*. Zarówno muszle *S. woodiana*, jak i obrost *Dreissena* spp. wpływają negatywnie na rodzime małże Unionidae, natomiast pojawienie się *P. leniusculus* może stanowić dla nich swoistą pomoc polegającą na redukcji liczebności *S. woodiana* (poprzez wyjadanie) i w ten sposób modulować interakcje między małżami.

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Research Article

Dead or alive: the effect of shells and living individuals of *Sinanodonta woodiana* (Lea, 1834) on habitat selection and behaviour of European unionid bivalves

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Abstract

1. Ecosystem engineering freshwater bivalves, burrowing in the substratum and accumulating shell deposits, transform bottom habitats. Especially the invasive Asian bivalve *Sinanodonta woodiana* (SW), due to its rapid growth, large size, and high fecundity, can affect benthic communities. Here, we determined its effect on habitat selection and behaviour of endangered native bivalves, *Anodonta cygnea* and *Unio tumidus*.

2. We conducted laboratory preference assays (Experiment 1: choice between two substrata) exposing the native bivalves to pure sand (control), shells (several densities on the sand surface or burrowed), or living SW. Then, we tested their locomotion and burrowing (Experiment 2) on pure sand and substrata contaminated with shells or living SW.

3. In Experiment 1, native bivalves avoided shells, but not living SW. Burrowed and larger shells were avoided compared with those on the surface and smaller ones, respectively.

4. In Experiment 2, *U. tumidus* exposed to SW delayed activity initiation (in response to living bivalves), increased locomotion (living bivalves, surface shells), and reduced burrowing depth (living bivalves, all shells). *Anodonta cygnea* exposed to SW reduced locomotion speed (living bivalves, shells), and reduced burrowing duration (burrowed shells) and depth (living bivalves, burrowed shells).

5. SW (especially shell beds) constitutes another emerging threat to native bivalves, impairing their burrowing and inducing active avoidance. As SW expands its distribution with climate warming, the range and strength of its impact is likely to increase, reducing the area available to native bivalves, exposing them to environmental dangers (due to burrowing limitation) and deteriorating physical condition (energetic resources used for excessive locomotion).

Key words: Behaviour, biological invasions, Bivalvia, ecosystem engineers, habitat selection, inter-specific interactions, species displacement, unionid mussels



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Introduction

Bivalves of the Unionidae family are freshwater bottom dwellers of limited mobility (Curley et al. 2021). Through filter-feeding, bivalves can considerably modulate the availability of resources for other organisms by transferring suspended particles to the bottom sediments (Boeker et al. 2016; Pouil et al. 2021). Both living bivalves and their empty shells, accumulating in the environment long after the animal death, constitute unique hard structures affecting community functioning (Gutiérrez et al. 2003). Therefore, bivalves are considered to be ecosystem engineers and their extinction, overpopulation or changes in their taxonomic composition lead to habitat modifications with cascading effects on the aquatic community and ecosystem services provided by these animals (Vaughn 2018).

Freshwater bivalves are threatened globally by human impact, including climate change and pressure from non-native species (Strayer 2008, Blackburn et al. 2014; Bacher et al. 2018). The number of alien bivalve species and sizes of their populations have been growing for several decades (Seebens et al. 2021; Latombe et al. 2022). Accordingly, the pressure from non-native bivalves is considered a major threat to their native counterparts (Mack et al. 2000; Pyšek et al. 2010), especially due to their ecosystem engineering properties and resulting habitat transformations (Bódis et al. 2014a; Douda et al. 2024).

The Chinese pond mussels of the genus *Sinanodonta* are unionid bivalves native to Eastern Asia, but invasive in other parts of the world. Recent genetic studies have shown that invasive lineages belong to three species: (i) *S. woodiana* (Lea, 1834), the “temperate invasive” lineage, native to southern China and invasive in Europe, as well as in western and central Asia, observed probably in Africa (finding needs genetic confirmation (Bensaâd-Bendjedid et al. 2023)); (ii) *S. pacifica* (Heude, 1878) the “tropical invasive” lineage, whose native area is Taiwan and eastern China, whereas it is invasive in North America, southern Asia and Iraq, and (iii) *S. lauta* (Martens, 1877), originating from Japan, the Korean Peninsula and eastern Russia, and invading central and southern Asia (Douda et al. 2024). In Europe, only *S. woodiana* (“temperate invasive” lineage) occurs, likely originating from a single introduction event (Konečný et al. 2018). Therefore, we will focus on this species in the current study. In colder regions (such as central and eastern Europe), its spread was initially limited to artificially heated waters (Urbańska et al. 2012), but, over the past two decades, it has accelerated and extended to habitats of natural thermal regime (Bogan et al. 2011; Bolotov et al. 2016; Lopes-Lima et al. 2017; Bepalaya et al. 2018; Kondakov et al. 2018, 2020; Konečný et al. 2018). Substratum preferences of *S. woodiana* overlap with those of the native European Unionidae (Poznańska-Kakareko et al. 2021) indicating a high risk of competitive tensions between them (Douda and Čadková 2018). This invasive bivalve exhibits a number of competitive advantages over the native Unionidae, including the higher rate of host infection by its parasitic glochidium larvae, faster development and growth rate (Douda et al. 2012; Huber and Geist 2019) and higher fecundity (Labecka and Domagała 2018; Labecka and Czarnoleski 2019).

Another potential mechanism of the impact of *S. woodiana* on native Unionidae can be the transformation of the bottom by living individuals and shell beds formed after the bivalve death (Bódis et al. 2014a; Nakano 2023). *Sinanodonta woodiana* is a large (up to 26 cm) and fast-growing species (Urbańska et al. 2019)

reaching high densities. In a Polish lake, densities of 68 ind. m⁻² and 27.9 kg m⁻² were observed (Kraszewski and Zdanowski 2007), which is the highest density of this species reported for Europe. In other European countries, the density ranges from a few ind. m⁻² in Ukraine (Yermoshyna and Pavliuchenko 2021) to c.a. 50 ind. m⁻² in Hungary and Italy (Benkő-Kiss et al. 2013; Kamburska et al. 2013). Over time, shells accumulate on the bottom surface and in the sediments, outnumbering living individuals and forming a layer significantly changing the substratum quality. More than 280 ind. m⁻² (counting both valves as one individual) were noted by Bódis et al. (2014a). Shells reduce the near-bottom current velocity, limit the access of light to the bottom, and increase microhabitat heterogeneity (Gutiérrez et al. 2003). Moreover, shells (especially large ones) can create physical barriers limiting bivalve movement and burrowing, and thus degrading the living conditions for these organisms. It is likely that the effect of shell beds formed by *S. woodiana* will be stronger than that of shells originating from the native species present in the environment before the invasion, due to the shorter lifespan of *S. woodiana*. It can live up to a maximum of 12 years (Spyra et al. 2012), compared to the maximum lifespan of 37 and 21 years exhibited by native *Anodonta cygnea* (Linnaeus, 1758) and *Unio tumidus* Philipsson, 1788, respectively (Aldridge 1999). This results in a faster accumulation rate of *S. woodiana* shells on the bottom. Moreover, its shells are larger, thus constituting larger and heavier physical obstacles in the substratum.

Knowledge of the responses of the native bivalves to the presence of *S. woodiana* will help understand the mechanisms and magnitude of its impact, as well as develop methods of dealing with this new threat. The aim of our study was to determine mechanical effects of substratum contamination with living individuals and shells of *S. woodiana* on behaviour (habitat selection, locomotion and burrowing) of two native European unionid bivalves: *A. cygnea* and *U. tumidus*. Their numbers are constantly decreasing worldwide (Lopes-Lima et al. 2017), and they are protected by law in several countries (Van Damme 2011; Lopes-Lima 2014). These species were selected due to their reported coexistence with *S. woodiana* (Lajtner and Crnčan 2011; Beran 2019) and similar habitat preferences (Poznańska-Kakareko et al. 2021). We hypothesized as follows: (1) native bivalves would avoid substrata contaminated with *S. woodiana*. (2) The adverse effect of shell beds on bivalve preferences would result from deteriorated burrowing and/or locomotion. Alternatively, increased locomotion might indicate active avoidance of the substratum contaminated by *S. woodiana*. (3) Empty shell beds would affect native bivalves to a greater extent than living *S. woodiana*. This might be due to (i) variable shell positions in the sediments (horizontally or vertically, on the surface or burrowed) compared to always vertically burrowed living bivalves (see Suppl. material 1: fig. S1), or (ii) the presence of sharp shell edges irritating the foot of moving bivalves. Options (i) or (ii) would be supported by stronger unionid responses to shells present on the sediment surface or burrowed, respectively. (4) The effect of *S. woodiana* shells on native bivalves would differ from that of native shell beds. A presumably stronger effect of the invader (compared to the shells of native bivalves) would result from either interspecific differences in shell structure (resulting in different unionid responses to shells of various species presented at the same sizes and densities) or the larger size of *S. woodiana* shells (resulting in stronger responses of unionids to larger shells).

Materials and methods

Bivalve collection in the field

Anodonta cygnea, *U. tumidus* and *S. woodiana* (shells and living individuals) were collected in early autumn from the sandy/muddy bottom (depth: 1.5–2.5 m) from the Włocławski Reservoir on the River Vistula, Central Poland (52°37'04"N, 19°19'42"E) by scuba divers. This site represents a natural thermal regime for central Europe, and has been recently invaded by *S. woodiana* (Cichy et al. 2016; Doua et al. 2024). Currently, all the study species co-exist at the location and the invader is constantly increasing its abundance and range (personal observation). We obtained *S. woodiana* shells from freshly killed individuals (on the day of collection), while native Unionidae shells were collected as fresh shells (uncorroded, undamaged) lying on the bottom of the reservoir (to avoid killing the native species). The collected bivalves were transported to the laboratory in buckets with substratum and water from the reservoir and tested after two weeks of acclimation.

Stocking conditions

Living bivalves (each species separately) and empty shells were kept in 350-L stock tanks (20–30 individuals per tank) equipped with internal filters and aeration systems, with the bottom covered by a few cm deep layer of sand taken from the collection site. The stock/experimental room was equipped with a photoperiod system (light/dark cycle: 12:12 h) imitating the natural day-night cycle, and air-conditioning which kept the water temperature in the tanks at the level similar to that observed in the reservoir during bivalve collection. We checked the water quality in the stock and experimental tanks using a multimeter Multi340i (WTW GmbH, Weilheim, Germany). The water parameters were within the following ranges: oxygen content: 7.37–7.77 mg ml⁻¹ (82.9–87.2%); temperature: 18.4–20.1 °C; pH: 8.01–8.67; conductivity: 643–827 µS cm⁻¹. The bivalves were fed twice a week with a suspension of dried *Chlorella* algae ("Chlorella super alga", Meridian company, Poland) in a concentration of 5 mg L⁻¹ (Doua and Čadková 2018).

Experiment 1: Habitat selection

Tests were conducted in 30 × 30 × 30 cm tanks divided into halves (Suppl. material 1: figs S1, S2). Each half was filled with a different substratum (see below) to a depth of 10 cm. Then, the tank was filled with conditioned (settled and aerated for at least 48 h) tap water (a 10-cm layer above the substratum surface). One bivalve individual was introduced in the central part of the tank with its ventral side down and anteroposterior axis parallel to the border between the substrata. After 24 h, the location of the tested individual was checked (choosing one of the two substrata). Each configuration of substratum types was repeated 30 times per species. We used the following substratum types:

- (1) Sand (grain diameter range: 0.2–1.4 mm; median: 0.63 mm) obtained from the bivalve collection site. This material was earlier found to be preferred by all the species tested (Poznańska-Kakareko et al. 2021). The pure sand was used as a control. The same sand type was contaminated with *S. woodiana* to create other substratum types.

- (2) Empty *S. woodiana* shells, small (mean length: 7.6 cm) or large (12.6 cm) (Table 1), composed of two valves connected naturally by the ligamentum. The shells were burrowed under the surface (covered with sand) or laid on the surface of the sandy substratum. We used these two shell position variants because a bivalve usually dies on the surface and, after some time, due to hydrodynamics and sedimentation, its shell becomes burrowed. This is especially visible in the case of mass mortalities, when large quantities of empty shells cover the surface of the bottom (Bódis et al. 2014b). Shell arrangement in/on the substratum (lying on their side or put vertically with their ventral surface down) was random (Suppl. material 1: fig. S1) to reflect their arrangement in the field. Shells were randomly put on the sand within the tank half they were assigned to (Suppl. material 1: fig. S1). Then, those assigned to the burrowed variant were gently and thoroughly covered with sand to fill all the spaces between them.
- (3) Living individuals of *S. woodiana* (mean length of 11.6 cm, corresponding to the large shells; Table 1) immobilized by adhesive tape applied to the front of the shell (to prevent their relocation) and burrowed in the sandy bottom at 75% of their length (Suppl. material 1: fig. S4). The immobilized bivalves could not extend their foot and move, which was necessary to keep them in their positions within the assigned half of the tank (Suppl. material 1: fig. S4). However, they could partially open their valves, pull out the siphons, filter water and breathe.

All bivalves and shells were thoroughly rinsed with water before use and biofilm and adhering debris were scrubbed from their surfaces. The sand was rinsed and dried in a laboratory dryer (SLW 115 STD Multiserw-Morek, Poland) at 60 °C for 6 h before use to eliminate any organisms that could potentially affect the results of the experiment. It should be noted that the size defined as large in our study is not of the maximum size of *S. woodiana* (26 cm, Urbańska et al. 2019). These, however, can be generally collected from warmer waters, whereas we used the size range commonly available at the collection site of the thermal regime natural for central Europe.

First, we checked unionid selectivity between the pure sand and various shell densities (small or large, on the surface or burrowed). We started the experiment with a density of 133 ind. m⁻² (6 shells per tank, two valves counted as one individual), i.e. twice as much as the maximum field density observed in heated waters. Then, we continued with the lower (67 ind. m⁻², 3 shells per tank) or higher (200 ind. m⁻², 9 shells per tank), depending on the presence or absence of a significant reaction to the initial density, respectively. This allowed us to determine the minimum effective density capable of influencing bivalve behaviour. We also confronted the pure sand with living *S. woodiana* at a density of 133 ind. m⁻². We did not use higher densities of living *S. woodiana*, as they would have been unrealistic given the maximum density reported in the wild (Kraszewski and Zdanowski 2007).

Moreover, we confronted the following: (i) burrowed shells vs. shells present on the sediment surface (using small shells at a density of 200 ind. m⁻²) to check if shell position makes a difference, (ii) living *S. woodiana* vs. large burrowed shells (133 ind. m⁻²) and (iii) burrowed small vs. burrowed large shells (200 vs. 133 ind. m⁻², corresponding to the same total volumes occupied by shells of the two sizes) to check whether bivalves respond differently to shell beds composed of shells of different sizes, (iv) native unionid shells vs. pure sand, (v) native unionid shells vs. small *S. woodiana* shells, to check if unionid responses to shells depend on shell origin.

Table 1. Total length of bivalves and shells [cm].

	Mean	SD	Range
<i>A. cygnea</i>	10.4	0.83	9.0-13.0
<i>U. tumidus</i>	7.2	0.60	6.5-8.5
Native bivalve shells*	7.0	0.88	5.5-9.0
<i>S. woodiana</i> living individuals	11.6	0.84	10.5-13.0
<i>S. woodiana</i> small shells	7.6	1.14	5.5-10.0
<i>S. woodiana</i> large shells	12.6	1.24	10.5-14.5

SD - standard deviation, * - *U. tumidus* with small admixture of *U. pictorum* and *A. anatina*.

Native shell beds were composed mostly of *U. tumidus* shells with a small admixture of *U. pictorum* and *A. anatina* (as they occurred in the field). They were of a size considered in the current study to be small (Table 1) and burrowed in sand (in this form they triggered stronger responses in earlier trials) at a density of 200 ind. m⁻² (effective density of small shells in earlier trials, see the Results). Treatments (iii)-(v) were conducted using only *U. tumidus*, because both native species responded similarly in earlier trials (see the Results), and we wanted to limit the use of the legally protected and endangered *A. cygnea*.

Furthermore, we tested the habitat preferences of *S. woodiana* for: (i) small burrowed conspecific shells (200 ind. m⁻²) vs. pure sand and (ii) small burrowed conspecific shells vs. shells of native unionids (200 ind. m⁻²) to check whether and how this species responds to shell beds. All the pairwise comparisons carried out within Experiment 1 are listed in Suppl. material 1: table S1.

Experiment 2: Bivalve mobility and burrowing

To test the effect of living *S. woodiana* and its empty shells on the locomotion and burrowing of *A. cygnea* and *U. tumidus*, we used tanks (40 × 30 × 35 cm) with a 10-cm layer of sand covered by the conditioned tap water (10 cm above the substratum) (Suppl. material 1: fig. S2). As substrata, we used small *S. woodiana* shells (i) burrowed or (ii) lying on the sand surface, at a density of 200 ind. m⁻², as well as (iii) living *S. woodiana* (133 ind. m⁻²) (Suppl. material 1: table S2). We used *S. woodiana* densities found to be avoided by the native unionids in Experiment 1 (see the Results). In the control treatment, bivalves were tested on (iv) pure sand without shells. A single substratum type was placed in each experimental tank. We introduced a single bivalve to the centre of the tank and recorded its behaviour using a CCTV camera (Samsung SNB-6004, South Korea) for 24 h. The tests were replicated 15 times for each substratum and species. While watching the videos, we determined the following: (i) movement initiation time (time from the bivalve introduction to the first movement), (ii) locomotion duration, (iii) locomotion distance, (iv) locomotion speed (excluding periods of immobility), (v) duration of burrowing activity, (vi) mean burrowing level [%]. Every minute, we estimated the percentage of bivalve burrowing (using a 5-level scale: 0, 25, 50, 75 and 100%) by comparing the length of the part of the shell below the substratum surface with the part of the shell protruding above the substratum (according to Poznańska-Kakareko et al. 2021). Mean burrowing level was calculated according to the following formula:

$$MB = \sum_{i=1}^4 [25\% \cdot it_i] / \sum_{i=0}^4 t_i \quad (1)$$

where: i – burrowing level: 5 steps ranging from 0 (totally exposed on the surface) to 4 (fully burrowed); t_i – time spent by the mussel at burrowing level i .

Statistical analysis

Statistical analysis was carried out using SPSS 26.0 (IBM Inc.). We checked bivalve habitat preferences in Experiment 1 using χ^2 tests of goodness of fit to compare their distribution within a given pair of habitats to a random distribution (assuming equal numbers of individuals selecting each habitat). Because of the high departures of the mobility and burrowing data in Experiment 2 from normality and homoscedasticity assumptions (tested with Shapiro-Wilk and Levene tests, respectively), we compared bivalve behaviour (each species separately) on each substratum contaminated with *S. woodiana* to their behaviour on pure sand using non-parametric Mann-Whitney U tests with a sequential Bonferroni correction for multiple comparisons.

Results

Experiment 1: Habitat selection

Both native species avoided small shells of *S. woodiana* (both burrowed and on the surface) at a density of 200 ind. m⁻² (Fig. 1a, b, Table 2) and burrowed large shells at a density of 133 ind. m⁻² (Fig. 1d, Table 2). Large shells on the surface were avoided at a density of 200 (*A. cygnea*) or 133 (*U. tumidus*) ind. m⁻² (Fig. 1c, Table 2).

Burrowed shells were avoided in favour of shells of the same size and density (200 ind. m⁻² of small shells) located on the substratum surface (Fig. 1e, Table 2). Large burrowed shells were avoided by *U. tumidus* in favour of small burrowed shells of the same total volume (Fig. 1f, Table 2).

The bivalves did not discriminate between living *S. woodiana* and pure sand (Fig. 1g, Table 2). *Unio tumidus* moved to the habitat formed by living *S. woodiana* avoiding large shells burrowed in the substratum, whereas *A. cygnea* did not discriminate significantly between these habitats (Fig. 1g, Table 2).

Unio tumidus showed a tendency to avoid shells of the native species, though it was non-significant (Fig. 2a, Table 2). Moreover, *U. tumidus* did not discriminate between shells of the native species and those of *S. woodiana*.

Sinanodonta woodiana avoided conspecific shells and did not discriminate between them and shells of the native unionids (Fig. 2b, Table 2).

Experiment 2: Bivalve mobility

Time from the introduction to the first movement of *A. cygnea* was not affected by the presence of shells and living individuals of *S. woodiana* (Fig. 3a, Table 3). *Unio tumidus* delayed the start of their activity in the presence of living *S. woodiana* (Fig. 3a, Table 3). Shells and living individuals of *S. woodiana* did not affect significantly the duration and distance of locomotion of *A. cygnea* (Fig. 3b, c, Table 3).

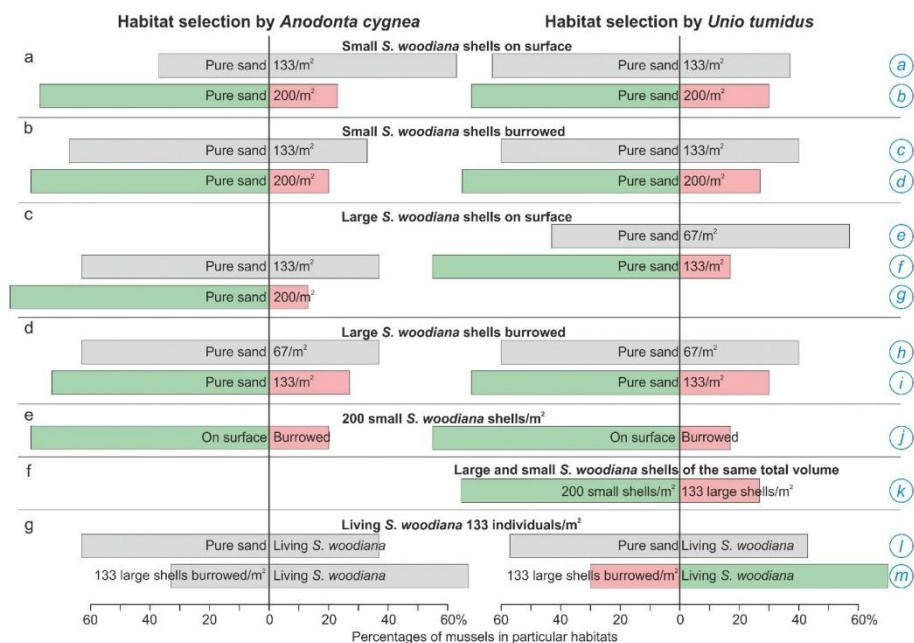


Figure 1. Habitat selection by *A. cygnea* and *U. tumidus* in the presence of substrata contaminated by *S. woodiana* in Experiment 1. Selected and avoided substrata are marked in green and red, respectively. The grey colour indicates non-significant differences. Blue letters in circles on the right refer to specific statistical tests presented in Table 2.

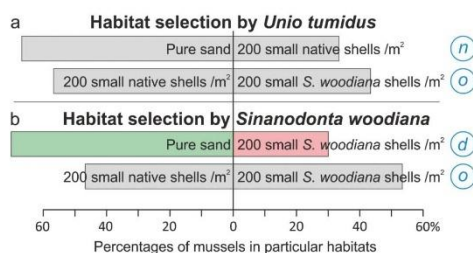


Figure 2. Habitat selection of *U. tumidus* and *S. woodiana* in the presence of burrowed shells of native and invasive bivalves in Experiment 1. Selected and avoided substrata are marked in green and red, respectively. The grey colour indicates non-significant differences. Blue letters in circles on the right refer to specific statistical tests presented in Table 2.

However, in the presence of all forms of *S. woodiana* contamination, distances travelled by *A. cygnea* were slightly shorter and movement duration longer, resulting in a significantly slower crawling speed compared to that observed in the pure sand (Fig. 3d, Table 3).

Unio tumidus increased duration and distance of their locomotion in the presence of living *S. woodiana* or its shells on the surface (Fig. 3b, c, Table 3). In the pure sand and with burrowed shells, *U. tumidus* usually did not move horizontally at all, but burrowed immediately. Due to the total lack of locomotion of *U. tumidus* in the pure sand, it was not possible to calculate their speed on this substratum.

Table 2. Statistical analysis of habitat selection by *A. cygnea*, *U. tumidus* and *S. woodiana* in Experiment 1 (χ^2 tests of goodness of fit comparing bivalve distribution within a given pair of habitats to the random distribution assuming no selection). Statistically significant differences are indicated by bold font and asterisks. χ^2 – test statistic, P – statistical significance.

			Substrata	<i>Anodonta cygnea</i>		<i>Unio tumidus</i>		<i>Sinanodonta woodiana</i>	
				χ^2	P	χ^2	P	χ^2	P
a	control (pure sand)	vs.	133 small SW shells m ⁻² on surface	2.13	0.144	2.13	0.144	–	–
b	control (pure sand)	vs.	200 small SW shells m ⁻² on surface	8.53	0.003*	4.80	0.028*	–	–
c	control (pure sand)	vs.	133 small burrowed SW shells m ⁻²	6.53	0.068	4.80	0.273	–	–
d	control (pure sand)	vs.	200 small burrowed SW shells m ⁻²	10.80	0.001*	6.53	0.011*	4.80	0.028*
e	control (pure sand)	vs.	67 large SW shells m ⁻² on surface	–	–	0.53	0.465	–	–
f	control (pure sand)	vs.	133 large SW shells m ⁻² on surface	2.13	0.144	13.33	<0.001*	–	–
g	control (pure sand)	vs.	200 large SW shells m ⁻² on surface	16.13	<0.001*	–	–	–	–
h	control (pure sand)	vs.	67 large burrowed SW shells m ⁻²	2.13	0.144	1.20	0.273	–	–
i	control (pure sand)	vs.	133 large burrowed SW shells m ⁻²	6.53	0.011*	4.80	0.028*	–	–
j	200 small SW shells m ⁻² on surface	vs.	200 small burrowed SW shells m ⁻²	10.80	0.001*	13.33	0.000*	–	–
k	200 small burrowed SW shells m ⁻²	vs.	133 large burrowed SW shells m ⁻²	–	–	6.53	0.011*	–	–
l	control (pure sand)	vs.	133 living SW m ⁻²	2.13	0.144	0.53	0.465	–	–
m	133 large burrowed SW shells m ⁻²	vs.	133 living SW m ⁻²	3.33	0.068	4.80	0.028*	–	–
n	control (pure sand)	vs.	200 small burrowed native shells m ⁻²	–	–	3.33	0.068	–	–
o	200 small burrowed native shells m ⁻²	vs.	200 small burrowed SW shells m ⁻²	–	–	0.53	0.465	0.13	0.715

Experiment 2: Bivalve burrowing

Anodonta cygnea spent a shorter time on burrowing in the substratum containing burrowed shells compared to the control sand (Fig. 4a, Table 3). There was no effect of *S. woodiana* habitats on the duration of burrowing activity of *U. tumidus*.

The mean burrowing level of *A. cygnea* was reduced in the presence of burrowed shells and living *S. woodiana* (Fig. 4b, Suppl. material 1: fig. S2, Table 3). *Unio tumidus* responded to all types of *S. woodiana* habitats by reducing its burrowing level.

Discussion

In accordance with our first hypothesis, we reported avoidance of *S. woodiana* shells by native unionids. On the other hand, living individuals of the invasive species were not avoided even at a density twice as high (133 ind. m⁻²) as the maximum densities observed so far in the field (Kraszewski and Zdanowski 2007). However, living *S. woodiana* did influence unionid behaviour: their presence delayed initiation of activity and increased horizontal locomotion of *U. tumidus*, reduced locomotion speed in *A. cygnea*, and reduced burrowing of both species. An increased locomotion was also exhibited by *U. delphinus* Spengler, 1783 in the presence of the invasive clam *Corbicula* sp. (Ferreira-Rodríguez et al. 2018). It appears that the presence of shells induced displacement of native unionids, whereas living *S. woodiana* impaired the habitat quality for the natives, which tried to counteract by changing their activity. The increase in activity may be induced by searching for a habitat free of competitors, but its side effect may be the displacement of native bivalves to suboptimal environments, where they will be exposed to increased water flow or predatory pressure (Block et al. 2013). Another consequence of increased locomotion can be the depletion of energetic resources. As

Table 3. Statistical analysis of locomotion and burrowing of *A. cygnea* and *U. tumidus* in Experiment 2. Bivalve behaviour in the presence of *S. woodiana* shells (200 ind. m⁻², on the surface or burrowed) and living *S. woodiana* (133 ind. m⁻²) was compared to the behaviour of individuals exposed on the control pure sand with pairwise Mann-Whitney U tests. Statistically significant differences are marked with asterisks and those that are still significant with the sequential Bonferroni correction are marked in bold font. Z – test statistic, P – statistical significance.

Variable	Substrata			<i>Anodonta cygnea</i>		<i>Unio tumidus</i>	
				z	P	z	P
a Movement initiation time	control (pure sand) vs.	shells on surface		2.30	0.022*	0.62	0.534
		shells burrowed		1.52	0.129	0.77	0.443
		living individuals		0.23	0.818	3.59	<0.001*
b Locomotion duration	control (pure sand) vs.	shells on surface		1.33	0.184	2.67	0.008*
		shells burrowed		1.16	0.245	1.79	0.073
		living individuals		1.05	0.293	2.40	0.017*
c Locomotion distance	control (pure sand) vs.	shells on surface		1.00	0.319	2.67	0.008*
		shells burrowed		1.22	0.223	1.79	0.073
		living individuals		0.78	0.438	2.40	0.017*
d Locomotion speed	control (pure sand) vs.	shells on surface		2.49	0.013*	–	–
		shells burrowed		2.44	0.015*	–	–
		living individuals		2.10	0.035*	–	–
e Duration of burrowing activity	control (pure sand) vs.	shells on surface		0.73	0.467	1.81	0.071
		shells burrowed		3.32	0.001*	0.56	0.575
		living individuals		1.83	0.067	1.14	0.254
f Mean burrowing level	control (pure sand) vs.	shells on surface		1.06	0.290	2.64	0.008*
		shells burrowed		3.11	0.002*	2.61	0.009*
		living individuals		2.63	0.009*	4.54	<0.001*

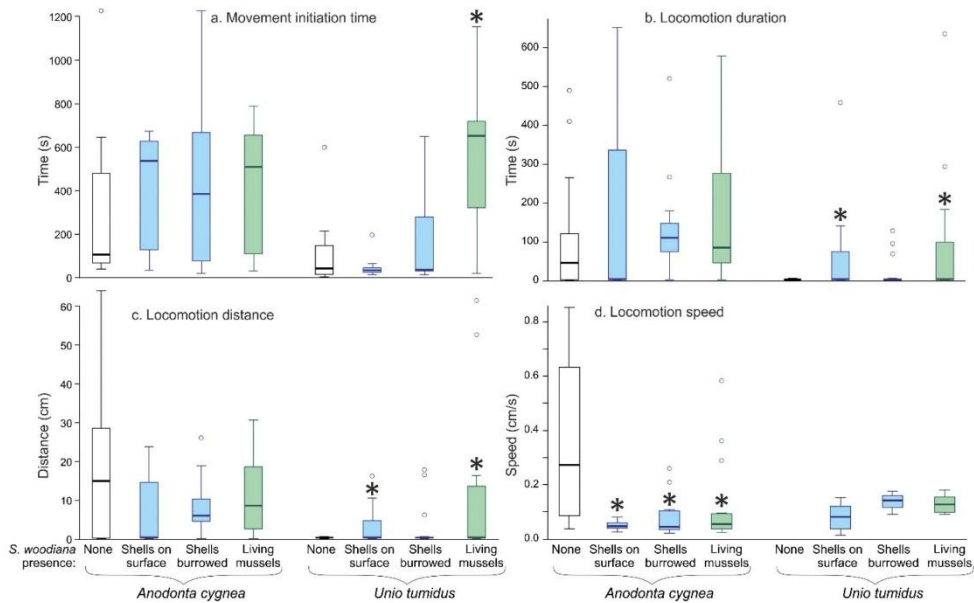


Figure 3. Mobility of *A. cygnea* and *U. tumidus* in Experiment 2: in pure sand (white bars), in the presence of *S. woodiana* shells (small shells, 200 ind. m⁻², blue bars) and in the presence of living *S. woodiana* (133 ind. m⁻², green bars) **a** movement initiation time **b** locomotion duration **c** locomotion distance and **d** locomotion speed. Asterisks indicate statistically significant differences in behaviour compared to that observed in the pure sand (see Table 3a-d for details of statistical test results). Boxplots present medians (horizontal lines), 1st and 3rd quartiles (boxes), 1.5*interquartile range (whiskers) and outliers (circles).

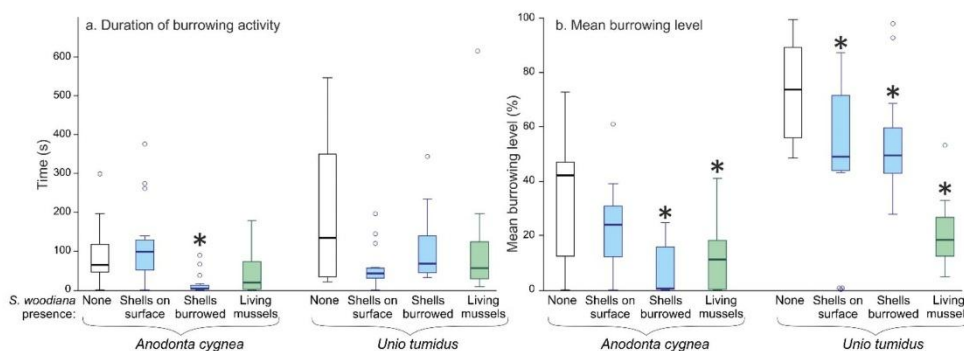


Figure 4. Burrowing of *A. cygnea* and *U. tumidus* in Experiment 2: in pure sand (white bars), in the presence of *S. woodiana* shells (small shells, 200 ind. m⁻², blue bars) and in the presence of living *S. woodiana* (133 ind. m⁻², green bars) **a** duration of burrowing activity **b** mean burrowing level (expressed as the percentage of bivalve length, see formula (1)). Asterisks indicate statistically significant differences in behaviour compared to that observed in the pure sand (see Table 3e-f for details of statistical test results). Boxplots present medians (horizontal lines), 1st and 3rd quartiles (boxes), 1.5*interquartile range (whiskers) and outliers (circles).

similar changes in behaviour took place in the presence of empty shells, they most likely resulted from mechanical properties of shells (acting as physical obstacles), rather than from infochemicals released to the water column by living *S. woodiana*.

As expected (third hypothesis), empty shells had a more aversive effect on native bivalves than living *S. woodiana*. The key result here is that the native bivalves avoided burrowed shells of *S. woodiana* to a greater extent than shells lying on the surface or living bivalves. The strongest effect of burrowed shells, immobilized in sediments, suggests that they are more difficult to push away by a moving mussel (compared to loose shells on the surface). Moreover, sharp shell edges, absent in living individuals, may irritate the foot of bivalves and discourage them from entering such a substratum. This was confirmed by the fact that *U. tumidus* did not increase their locomotion activity in the presence of burrowed shells, as they did among shells on the surface or with living *S. woodiana*. Thus, burrowed shells not only prevented mussels from entering the area, but also made it more difficult to leave a shell habitat when already present around the moving unionid. On the other hand, increased locomotion of *U. tumidus* among shells on the surface associated with their avoidance of such habitats indicates the active selection of shell-free habitats. The locomotion of *A. cygnea* in the presence of shells in or on the substratum resulted in similar distances as without shell beds, but at the cost of slower speed. This suggests a greater effort needed to obtain the same final effect, though, despite this, mussels continued to move in the presence of shells, also suggesting the active avoidance of shell beds by this species.

Avoidance of burrowed shells is related to their effect on the bivalve behaviour. We did note the negative effect of burrowed objects (empty shells and living *S. woodiana*) on the burrowing of both native species, especially *A. cygnea*. Moreover, *A. cygnea* spent less time on burrowing in shell beds, probably to avoid excessive energy expenditure. Restricted burrowing may be dangerous for unionid mussels, as being immersed in the substratum is their natural position, enabling their filtration, as well as reducing predation risk and the probability of dislodgement by water movements (Tallqvist 2001; Saloom and Duncan 2005). This indicates that the post-mortem effect of *S. woodiana* is two-faceted: it modifies the horizontal

and vertical mobility of bivalves, as predicted by our second hypothesis. This can substantially worsen the environmental conditions for native bivalves. We found that large shells are more aversive than small ones at the same volumetric quantities. This supports the idea that shells act as physical objects interfering with the movement of living mussels (a single large shell is more difficult to push away or bypass than a group of small ones). Thus, the impact of the invader is likely to be greater than that experienced by the native species before the invasion due to the much larger size of *S. woodiana* compared to native unionids, as predicted by our fourth hypothesis.

Native unionids can also create shell beds (Bódis et al. 2014a), which, as we have shown in our study, can exert a similar impact on living bivalves. However, their formation is slower than in the case of *S. woodiana*, which is capable of reaching high abundance and large body size in a relatively short time (Urbańska et al. 2019). Taking into account the thermophilicity of *S. woodiana* (Yermoshyna and Pavliuchenko 2021), it should be expected that, with progressing climate warming, mussels will reach larger sizes, higher density and biomass (Kraszewski and Zdanowski 2007; Spyra et al. 2012; Mehler et al. 2024) in temperate waters. This will increase their impact on local communities (Gutiérrez et al. 2003; Bellard et al. 2012) to an unprecedented level, not experienced so far by the native bivalves. It may be manifested by intensified competition for space, given that *S. woodiana* and the native unionids have similar substratum preferences (Poznańska-Kakareko et al. 2021), and by reduction in available area of optimum substratum due to the presence of extensive shell beds (Gutiérrez et al. 2003; Bódis et al. 2014a).

We have shown that *S. woodiana* also avoids habitats transformed by shells of its own species. This behaviour suggests that small scale spread of *S. woodiana* may be additionally stimulated by changes generated by this species in the environment, resulting in the occupation of a greater bottom area at an invaded site. Such a transformation of the environment by an ecosystem engineer associated with a negative feedback on its own living conditions is similar to the activity of cormorants (*Phalacrocorax* sp.), which pollute their surroundings (trees on which they nest in large numbers) with corrosive excrements and then move to new, not yet destroyed habitats (Ishida 1996).

Our results highlight that apart from competition for host-fish, food resources or living space, *S. woodiana* poses a further threat to native unionid bivalves by altering their horizontal and vertical movement behaviour. Whilst our tests were carried out in strictly controlled, specific conditions (i.e. stagnant water on sandy bottom), long-term negative impacts of living *S. woodiana* on native unionids can be expected to be even stronger than demonstrated in our study. We can expect that additional factors, such as water flow, different substratum or temperature would modify the relationships and behaviours observed in our study (Sullivan and Woolnough 2021). In our study, we only considered the physical effect of shells and living individuals of *S. woodiana*, showing the stronger impact of the former. In the environment, living *S. woodiana* may have additional adverse effects on native bivalves, such as competition for food or host fish, or reduction in water oxygenation. To fully understand the interactions between invasive and native Unionidae bivalves, would require to conduct longer tests, both in laboratory and in the field, examining additional potential mechanisms of impact.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

KW: Conceptualisation, Methodology, Resources, Investigation, Formal analysis, Visualisation, Writing - Original draft, Writing - Review and Editing; DS: Methodology, Resources, Investigation, Writing - Review and Editing; JK: Conceptualization, Methodology, Validation, Formal analysis, Data Curation, Visualization, Writing - Review and Editing; TK: Resources, Investigation, Writing - Review and Editing; ŁJ: Resources, Investigation, Writing - Review and Editing; MPK: Conceptualization, Methodology, Validation, Visualization, Formal analysis, Supervision, Project administration, Writing - Review and Editing

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary material

Authors: Kamil Wiśniewski, Daniel Szarmach, Jarosław Kobak, Tomasz Kakareko, Łukasz Jermacz, Małgorzata Poznańska-Kakareko

Data type: docx

Explanation note: **table S1**. Full list of pairwise comparisons conducted to check unionid habitat selection in Experiment 1. **table S2**. Full list of treatments, response variables and statistical comparisons to check changes in unionid locomotion and burrowing in response to *S. woodiana* presence in Experiment 2. **fig. S1**. Photographs of the experimental setup. Experiment 1: Habitat selection by bivalves in various configurations: (A) pure sand (control) vs. 133 ind. m⁻² of small *S. woodiana* shells on surface; (B) pure sand vs. 200 ind. m⁻² of small shells on surface; (C) pure sand vs. 133 ind. m⁻² of small burrowed shells; (D) pure sand vs. 200 ind. m⁻² of small burrowed shells; (E) pure sand vs. 67 ind. m⁻² of large shells on surface; (F) pure sand vs. 133 ind. m⁻² of large shells on surface; (G) pure sand vs. 200 ind. m⁻² of large shells on surface; (H) pure sand vs. 67 ind. m⁻² of large burrowed shells; (I) pure sand vs. 133 ind. m⁻² of large burrowed shells; (J) 200 ind. m⁻² of small shells on surface vs. burrowed; (K) pure sand vs. 133 ind. m⁻² of living *S. woodiana*; (L) 200 ind. m⁻² of burrowed shells of native bivalves vs. shells of *S. woodiana*. **fig. S2**. Schemes of experimental sets concerning habitat selection (Experiment 1) and locomotion and burrowing (Experiment 2). **fig. S3**. Percentage of time spent in different levels of burrowing (Experiment 2) by *A. cygnea* and *U. tumidus* on different substrata. Error bars – standard deviation. **fig. S4**. Photo of immobilized *Sinanodonta woodiana*.

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Supplementary materials - Appendix

Dead or alive: the effect of shells and living individuals of *Sinanodonta woodiana* (Lea, 1834) on habitat selection and behaviour of European unionid bivalves

Table of contents

Table S1. Full list of pairwise comparisons conducted to check unionid habitat selection in Experiment 1

Table S2. Full list of treatments, response variables and statistical comparisons to check changes in unionid locomotion and burrowing in response to *S. woodiana* presence in Experiment 2

Figure S1. Photographs of the experimental setup. Experiment 1: Habitat selection by bivalves in various configurations: (A) pure sand (control) vs. 133 ind. m⁻² of small *S. woodiana* shells on surface; (B) pure sand vs. 200 ind. m⁻² of small shells on surface; (C) pure sand vs. 133 ind. m⁻² of small burrowed shells; (D) pure sand vs. 200 ind. m⁻² of small burrowed shells; (E) pure sand vs. 67 ind. m⁻² of large shells on surface; (F) pure sand vs. 133 ind. m⁻² of large shells on surface; (G) pure sand vs. 200 ind. m⁻² of large shells on surface; (H) pure sand vs. 67 ind. m⁻² of large burrowed shells; (I) pure sand vs. 133 ind. m⁻² of large burrowed shells; (J) 200 ind. m⁻² of small shells on surface vs. burrowed; (K) pure sand vs. 133 ind. m⁻² of living *S. woodiana*; (L) 200 ind. m⁻² of burrowed shells of native bivalves vs. shells of *S. woodiana*

Figure S2. Schemes of experimental sets concerning habitat selection (Experiment 1) and locomotion and burrowing (Experiment 2)

Figure S3. Percentage of time spent in different levels of burrowing (Experiment 2) by *A. cygnea* and *U. tumidus* on different substrata. Error bars – standard deviation.

Figure S4. Photo of immobilized *Sinanodonta woodiana*

Table S1. Full list of pairwise comparisons conducted to check unionid habitat selection in Experiment 1

Comparison between the substrata	Species tested	Shell size	Shell position	Shell density	Purpose
Effects of Sw shell beds					
a Pure sand vs Sw shells	Ac, Ut	Small	On surface	1. The first trial: 133 shells /m ² Burrowed 2. If trial 1 non-significant: 200 Large On surface Burrowed 3. If trial 1 significant: 67 shells /m ²	1. To check NTU responses to Sw shell beds, depending on shell size, position, and density 2. To find the minimum effective shell density
b Sw shells on surface vs burrowed	Ac, Ut	Small	Burrowed	200 shells /m ²	To check the effect of shell position in the substratum on NTU responses
c Large vs small Sw shells	Ut		Burrowed	133 large & 200 small shells /m ² (the same total volumes)	To check the effect of shell size on NTU responses
Effects of living mussels					
d Pure sand vs living Sw	Ac, Ut	Large	Burrowed	133 living Sw /m ²	To check NTU responses to living Sw
e Sw shells vs living Sw	Ac, Ut	Large	Burrowed	133 shells and living Sw /m ²	To check whether the effect of living Sw on NTU differs from that of shells
Effects of native shell beds					
f Pure sand vs NTU shells	Ut	Small	Burrowed	200 shells /m ²	To check NTU responses to NTU shell beds
g NTU shells vs Sw shells	Ut	Small	Burrowed	200 shells /m ²	To check whether NTU respond differently to Sw and NTU shells
Responses of <i>S. woodiana</i>					
h Pure sand vs Sw shells	Sw	Small	Burrowed	200 shells /m ²	To check Sw responses to Sw shell beds
i NTU shells vs Sw shells	Sw	Small	Burrowed	200 shells /m ²	To check whether Sw respond differently to Sw and NTU shells
NTU – native unionids, Ac – <i>Anodonta cygnea</i> , Ut – <i>Unio tumidus</i> , Sw – <i>Sinanodonta woodiana</i> Small shells: < 10 cm, large shells: > 10 cm in length					

Table S2. Full list of treatments, response variables and statistical comparisons to check changes in unionid locomotion and burrowing in response to *S. woodiana* presence in Experiment 2

Species tested	Treatments (for each species)	Response variables (for each species and treatment)	Comparisons (for each species and response variable)
1. Ac 2. Ut	1. Pure sand	1. Movement initiation time (s)	Pure sand vs 1. Sw shells on surface 2. Sw shells burrowed 3. Living Sw
	2. Sw shells on surface 200 shells /m ²	2. Locomotion duration (s)	
	3. Sw shells burrowed 200 shells /m ²	3. Locomotion distance (cm)	
	4. Living Sw 133 ind. m ²	4. Locomotion speed (cm/s)	
		5. Duration of the burrowing activity (s)	
		6. Mean burrowing level (%)	

Ac – *Anodonta cygnea*, Ut – *Unio tumidus*, Sw – *Sinanodonta woodiana*

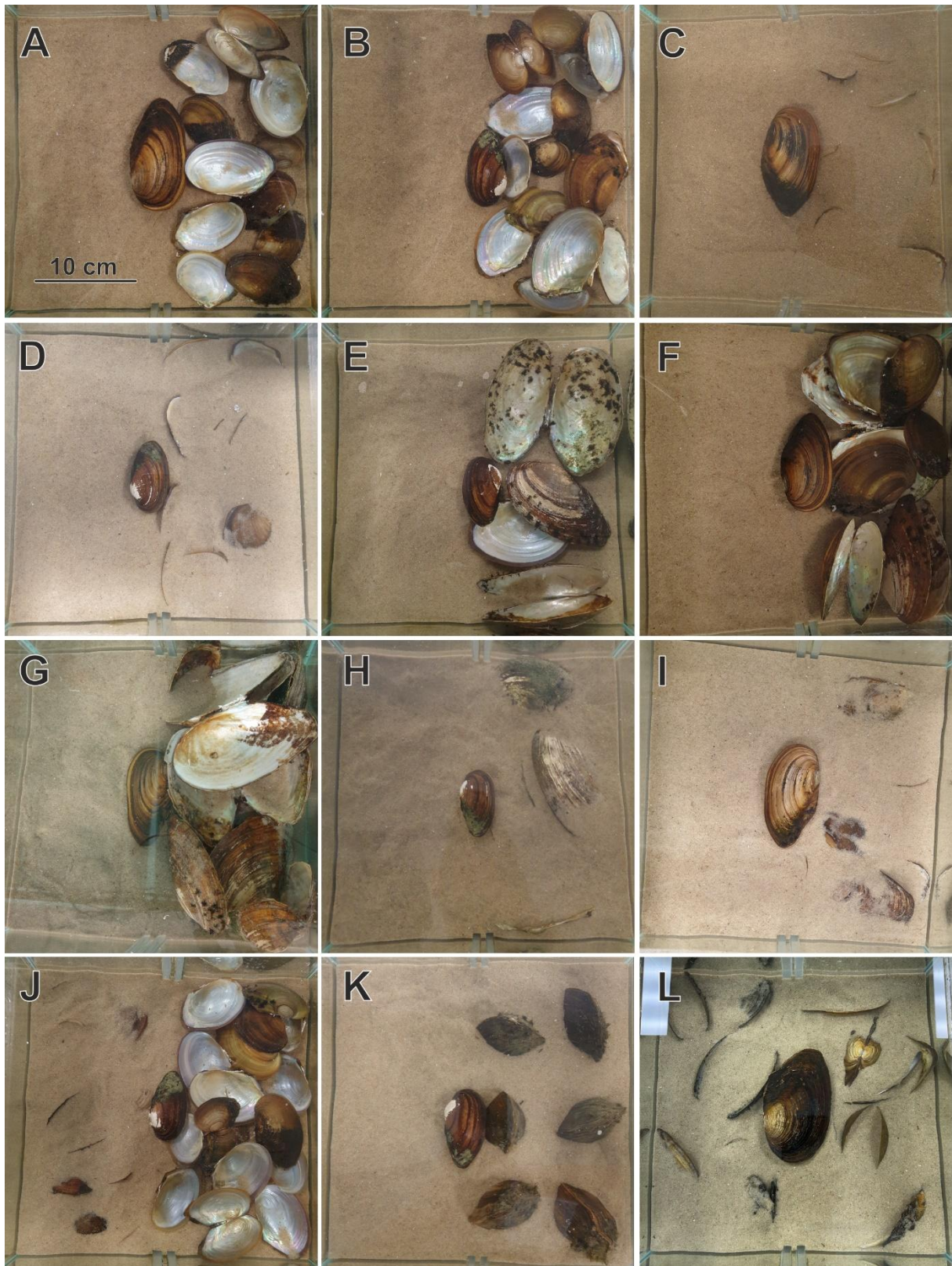
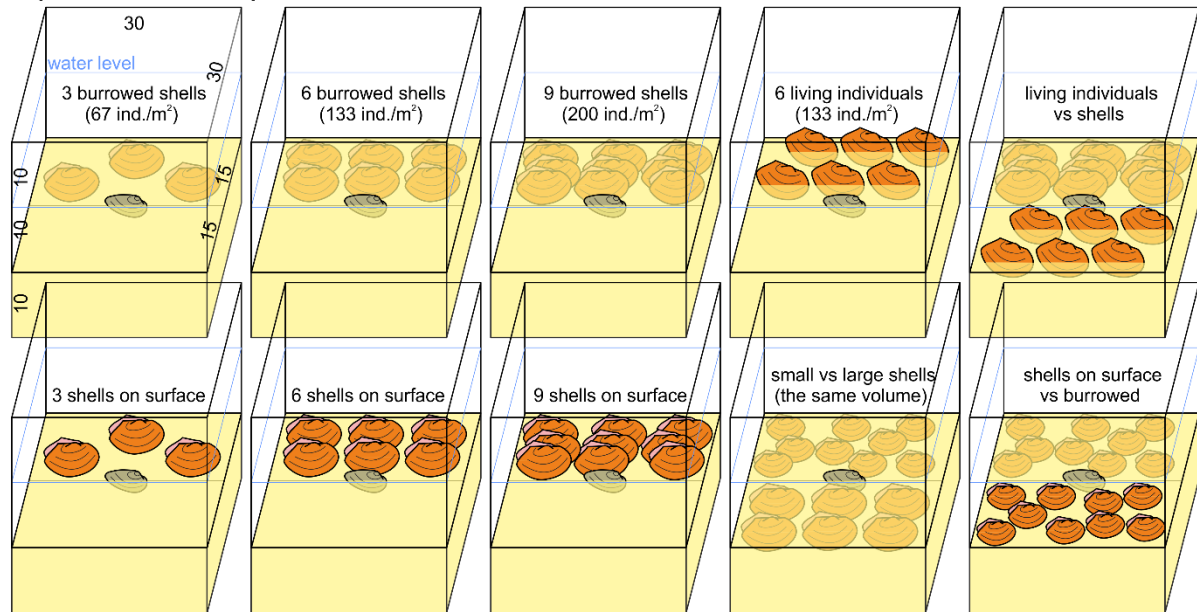


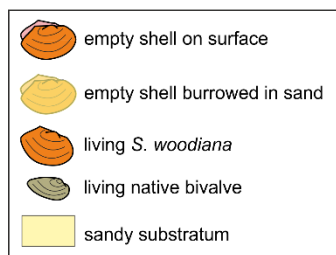
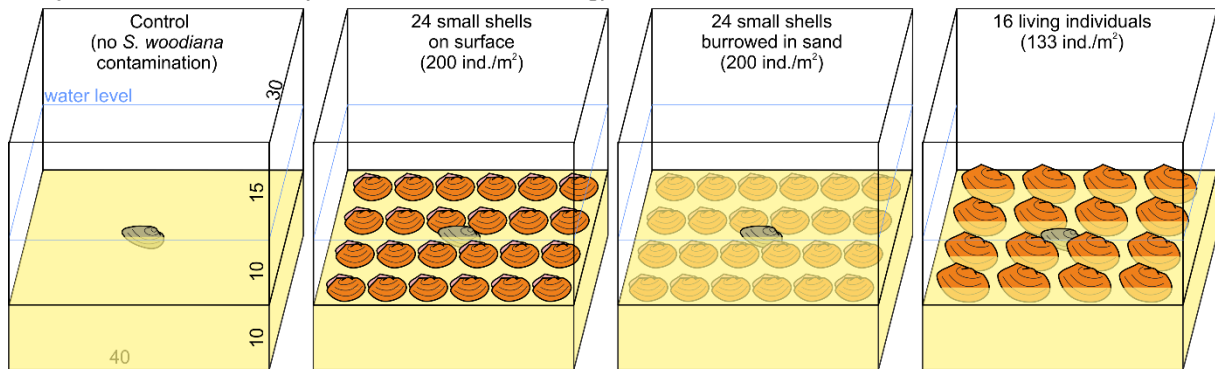
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m⁻² of small burrowed shells; (D) pure sand vs. 200 ind. m⁻² of small burrowed shells; (E) pure sand vs. 67 ind. m⁻² of large shells on surface; (F) pure sand vs. 133 ind. m⁻² of large shells on surface; (G) pure sand vs. 200 ind. m⁻² of large shells on surface; (H) pure sand vs. 67 ind. m⁻² of large burrowed shells; (I) pure sand vs. 133 ind. m⁻² of large burrowed shells; (J) 200 ind. m⁻² of small shells on surface vs. burrowed; (K) pure sand vs. 133 ind. m⁻² of living *S. woodiana*; (L) 200 ind. m⁻² of burrowed shells of native bivalves vs. shells of *S. woodiana*

Experiment 1: habitat preference



Experiment 2: behaviour (locomotion and burrowing)



all dimensions given in cm

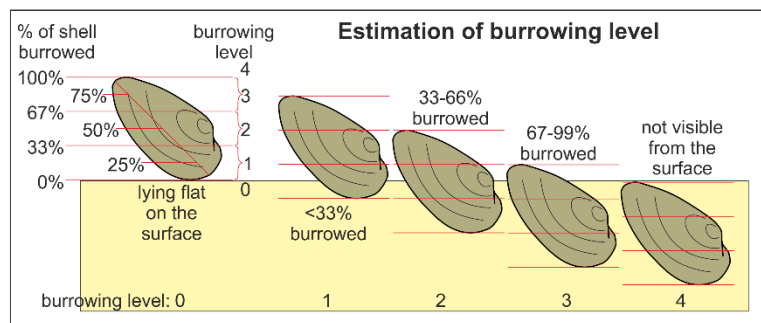


Figure S2. Schemes of experimental sets concerning habitat selection (Experiment 1) and locomotion and burrowing (Experiment 2)

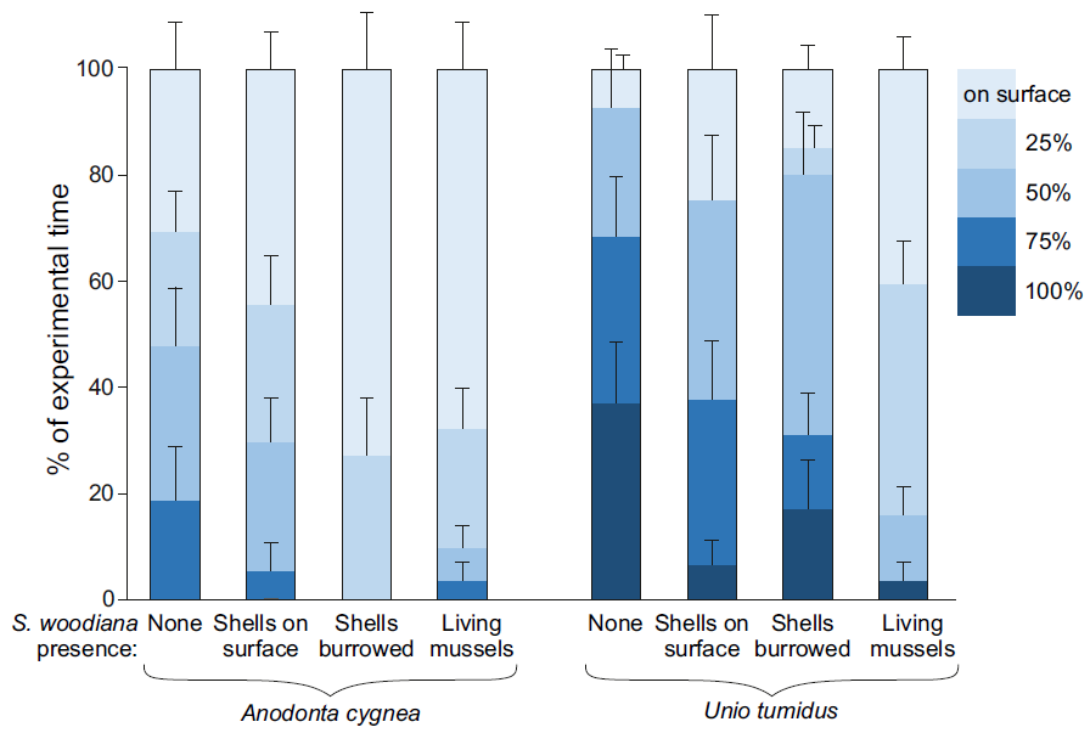


Figure S3. Percentage of time spent in different levels of burrowing (Experiment 2) by *A. cygnea* and *U. tumidus* on different substrata. Error bars – standard deviation.



Figure S4. Photo of immobilized *Sinanodonta woodiana*

Publikacja 2

Wiśniewski, K., Balogh, C., Kobak, J., Szarmach, D., Jermacz, Ł., & Poznańska-Kakareko, M. (2024). Native and non-native Unionids respond differently to the presence of fouling dreissenid mussels. *NeoBiota*, 96, 1-18. <https://doi.org/10.3897/neobiota.96.130198>

Udział autorów w powstaniu publikacji:

Imię i nazwisko współautora	Udział [%]	Pełnione funkcje
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dr Csilla Balogh	9	Koncepcja badań, opracowanie metodologii, zebranie materiału w terenie, prowadzenie eksperymentów, opracowanie wyników, napisanie pierwotnej wersji manuskryptu, poprawianie manuskryptu podczas publikowania w czasopiśmie, pozyskanie funduszy na badania
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dr hab. Małgorzata Poznańska-Kakareko, prof. UMK	13	Koncepcja badań, opracowanie metodologii, nadzór nad przebiegiem badań, zarządzanie projektem, poprawianie manuskryptu podczas publikowania w czasopiśmie

Research Article

Native and non-native unionids respond differently to the presence of fouling dreissenid mussels

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Abstract

Unionid mussels are globally threatened by several human disturbances, including the introduction of non-native species. Among these, biofouling zebra and quagga mussels of Ponto-Caspian origin are considered to be especially detrimental to unionid locomotion, filtration and physical condition. The aim of our study was to determine and compare the impact of dreissenid fouling and/or presence on locomotion and burrowing of the native *Unio tumidus* and invasive *Sinanodonta woodiana*, a novel invader expanding its range in Europe in recent decades. We tested unionids collected from Lake Balaton (central Europe) that were fouled by dreissenids (zebra and quagga mussels mixed), cleaned of fouling or non-fouled (collected without any signs of dreissenid fouling). Moreover, unionids were tested in the presence or absence of other fouled individuals and dreissenids isolated in mesh bags to determine the influence of direct fouling and presence of dreissenids in the environment on unionid behaviour. Movement initiation time, locomotion distance and burrowing level were retrieved from videos recorded for 24 hours. Direct fouling affected only the behaviour of *U. tumidus*, limiting their burrowing and delaying movements. After removal of fouling, movement timing returned back to normal, but mussels still burrowed less than the control non-fouled individuals, indicating persisting effects of fouling on physical condition. Moreover, *U. tumidus* reduced their locomotion in the presence of fouled unionids. *Sinanodonta woodiana* responded to the presence of dreissenids (especially quagga mussels) with increased burrowing. These different responses of the two unionid species to *Dreissena* spp. indicate that biofoulers may influence biotic interactions between the unionids by promoting the invasive species (less susceptible to negative effects of fouling). Moreover, *S. woodiana* may indirectly affect *U. tumidus* through apparent competition, constituting an environmental reservoir of biofoulers exerting a stronger impact on the native species.

Key words: Biofouling, biological invasions, Bivalvia, burrowing, ecosystem engineers, interspecific interactions, locomotion, *Sinanodonta woodiana*, *Unio tumidus*, Unionidae

Introduction

Bivalves are ecosystem engineers altering ecosystem structure and function by increasing water clarity and modifying the bottom quality (Strayer et al. 1999; Karatayev et al. 2002; Sousa et al. 2009). Their reported effects consist in declines in plankton abundance (Kissman et al. 2010; Karatayev et al. 2023), decreases in

young fish survival (Irwin et al. 2009), changes in fish feeding patterns (Mayer et al. 2001; Balogh et al. 2022), increases in benthic invertebrate abundance (Novais et al. 2015; Ilarri et al. 2018), and development of submerged aquatic vegetation (Chu et al. 2004; Zhu et al. 2006), resulting in a more benthic-oriented food web structure (Mills et al. 2003; Miehl et al. 2009).

Bivalve species richness and abundance decrease all over the world due to ongoing climate change, increasing water pollution and habitat destruction (Strayer and Malcom 2007). This particularly applies to freshwater mussels of the family Unionidae, with dozens of already extinct species and hundreds more heavily endangered (Aldridge et al. 2023; Sousa et al. 2023). Due to their important environmental functions and ecosystem services they provide, further unionid extinction may lead to irreversible changes and effects on the entire aquatic community (Vaughn 2018; Zieritz et al. 2022).

One of the greatest threats to native unionid mussels is the spread of invasive mussel species (Gutiérrez et al. 2014; Douđa et al. 2024), including biofouling dreissenids: the zebra mussel *Dreissena polymorpha* (Pallas, 1771) and the quagga mussel *Dreissena rostriformis bugensis* Andrusov, 1897. The dreissenids, originating from the Ponto-Caspian region (catchments of the Black, Azov and Caspian Seas and lowland parts of the inflowing rivers) have successfully invaded freshwater ecosystems in Europe and North America (Matthews et al. 2014; Collas et al. 2018). *Dreissena polymorpha* has spread in Western Europe since the beginning of the 19th century (Harzhauser and Mandić 2010), whereas *D. r. bugensis* started to spread in the 20th century (Therriault et al. 2005) and its invasion is ongoing on both continents. This has led to the co-occurrence of both species in more and more invaded locations (Zhulidov et al. 2004; Grutters et al. 2012).

Dreissena spp. rapidly develop large populations and settle on unionids in high numbers, especially when other hard substrata are scarce, and the mass of fouling dreissenids can exceed the biomass of their host (Burlakova et al. 2000). This impairs physiological condition of unionids, as *Dreissena* spp. can block their siphons preventing them from filtering and therefore breathing and feeding (Ricciardi et al. 1996; Sousa et al. 2011; Bódis et al. 2014). Dreissenids can also inhibit unionid locomotion (Van Appledorn and Bach 2007) and/or burrowing, making them vulnerable to unfavourable environmental conditions or predation (Saloom and Duncan 2005). In addition, they may compete for food with the unionids, causing severe declines in phytoplankton (Higgins and Zanden 2010). As a result, the spread of *Dreissena* species may further endanger or lead to extinction of already threatened unionid species (Ricciardi and Whoriskey 2004).

An exception in the generally endangered Unionidae family is the invasive Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834), native to Eastern Asia (Lopes-Lima et al. 2020), but in recent decades (since 1959) spreading in Europe (Douđa et al. 2024). This is a fast-growing and fast-reproducing (Douđa et al. 2021) species of high ecological plasticity, tolerant to wider ranges of environmental conditions in comparison to native European Unionidae (Kraszewski and Zdanowski 2007; Poznańska-Kakareko et al. 2021; Zieritz et al. 2021; Dobler et al. 2022). It can co-occur with native mussels and outcompete them for food, fish hosts for their larvae, and living space (Douđa and Čadková 2018).

Both native and invasive Unionidae are commonly fouled by dreissenids (Bódis et al. 2014; Balogh et al. 2024). Dreissenids can interfere with the invasion of *S. woodiana*, with the final outcome depending on the relative susceptibility of *S. woodiana*

and native unionids to fouling and its effects (Bódis et al. 2014). High vulnerability of the invasive *S. woodiana* to fouling would negatively affect its establishment in areas occupied by dreissenids, whereas its resistance would facilitate competitive exclusion of other unionid species (Sousa et al. 2011). Therefore, the aim of our study was to determine and compare the effects of fouling and/or presence of the invasive mussels *D. r. bugensis* and *D. polymorpha* (responses to own fouling, to the presence of fouled conspecifics and to the presence of dreissenids on other substratum in the environment) on the locomotion and burrowing of two unionid mussels: the native *Unio tumidus* Philipsson, 1788 and the invasive *S. woodiana*. These species often co-occur in areas invaded by *S. woodiana* in Europe (e.g. in Lake Balaton; Benkő-Kiss et al. 2013) and can interact with each other, which can be additionally modulated by biofouling. For our study, the mussels were collected from a single location in Lake Balaton (Hungary) to make sure that potential differences in local living conditions of particular species would not affect the results. We hypothesized that: (1) fouling reduces unionid locomotion and burrowing; (2) this effect can be purely mechanical (only when the fouling is directly present), or (3) can persist after fouling removal (due to worsened physiological condition of the host). Additionally, we hypothesized that (4) unionids can detect and respond by behaviour to chemical cues released by other unionids fouled by dreissenids or directly by *Dreissena* spp. individuals.

Materials and methods

Mussel collection in the field

We collected all mussels manually (randomly by hand) or using a Surber net from Lake Balaton in Keszthely, Hungary (46°46'11"N, 17°14'53"E) in summer 2022. Lake Balaton, the largest lake in Central Europe, had been an isolated water body until the opening of the Sió Canal, which created conditions for the spread of invasive species from the River Danube (Benkő-Kiss et al. 2013). This eutrophic, shallow, and mainly muddy lake provides an opportunity to collect all the mussel species at one site: *S. woodiana* (temperate lineage from Eastern Asia) (Douda et al. 2024) has been present in the lake since 2006 (Benkő-Kiss et al. 2013), zebra mussels since 1932 (Sebestyén 1938) and quagga mussels since 2008 (Balogh et al. 2018). We collected *S. woodiana* and *U. tumidus* from sandy/muddy bottom at a water depth of 0.5–1 m. Some individuals were fouled with a mixture of both *Dreissena* species, whereas others were clean (without any marks of fouling). *Dreissena polymorpha* to *D. r. bugensis* ratio on unionid mussels at the collection site is ca. 3:2 (Balogh et al. 2024). We transported the mussels in buckets (containing sand and water from the collection place) to the laboratory in the Balaton Limnological Research Institute (Tihany, Hungary), where, after two weeks of acclimation, we conducted the experiments. We did not observe any mortality during this time. After completing all the tests, the mussels were weighed and measured (Table 1). We determined the mass of the *Dreissena* spp. fouling on each unionid considering it as a whole, without separating the dreissenid species. We assumed that the total fouling mass has the greatest effect on the fouled animal, irrespective of the fouling species, individual size and numbers. The fouling to host mass ratios were similar for both unionid species (Table 1) and indicated moderate level of fouling (20% of the host mass) compared to other environments, where the total fouling mass may even exceed that of their host (Burlakova et al. 2000; Dzierżyńska-Białończyk et al. 2018a).

Table 1. Weight and length of native and invasive mussels used in experiments. Underlying data are available in Suppl. material 1.

	<i>S. woodiana</i>			<i>U. tumidus</i>		
	mean	SD	range	mean	SD	range
Wet mass (g)	149.4	46.4	52.4–285.0	41.4	8.3	18.8–64.6
Length (mm)	107.1	10.9	78–129	68.2	5.5	56–83
<i>Dreissena</i> spp. mass (g)	27.0	11.5	10.5–50.0	7.5	4.6	4.0–20.4
<i>Dreissena</i> / unionid mass ratio	0.20	0.08	0.06–0.33	0.17	0.08	0.09–0.32

SD – standard deviation.

Laboratory conditions

The laboratory with stock and experimental tanks was equipped with air conditioning to stabilise water temperature. Unionids, each species as well as fouled and non-fouled individuals separately, were stocked in aerated and filtered flow-through 250-L tanks supplied with fresh water from the lake (flow rate: 30 L/h). Water parameters in the stock and experimental tanks were measured with a multimeter Multi340i (WTW, Weilheim, Germany) (Table 2). The mussels were fed with cultured *Scenedesmus* sp. suspension at a concentration of 5 mg L⁻¹ (Douda and Čadková 2018) three times a week, also between the replicates of the experiments.

Table 2. Water parameters in the stock and experimental tanks (lake water).

	Oxygen concentration [mg/mL]	Oxygen saturation [%]	Temperature [°C]	pH	Conductivity [µS/cm]
Stock tanks					
Mean	8.90	98.33	19.26	8.60	855.67
SD	0.27	1.86	0.32	0.03	10.12
Range	8.73–9.21	96.6–100.3	18.94–19.57	8.57–8.63	844–862
Experimental tanks					
Mean	6.34	69.28	18.65	8.14	853.50
SD	0.49	5.22	0.30	0.21	21.33
Range	5.04–6.75	55.7–74.3	18.28–18.97	7.77–8.39	811–871

SD – standard deviation.

Experimental setup

Direct impact of *Dreissena* spp. fouling on unionid behaviour (Experiment 1) was tested in 30 × 30 × 30 cm tanks, containing 10 cm of sand (sifted and rinsed) covered by 10 cm layer of lake water. To each tank, we introduced 4 mussels: two individuals of *S. woodiana* and two individuals of *U. tumidus* (to mimic their co-existence in the field) (Fig. 1). The mussel behaviour was recorded for 24 h with CCTV video-cameras (Samsung SNB-6004, South Korea) equipped with infrared illumination (LIR-CS32, IRLAB, Taiwan) enabling night-time observations. We used several mussel variants depending on their fouling status and processing in the laboratory. Their full descriptions and explanations of use are presented in Table 3.

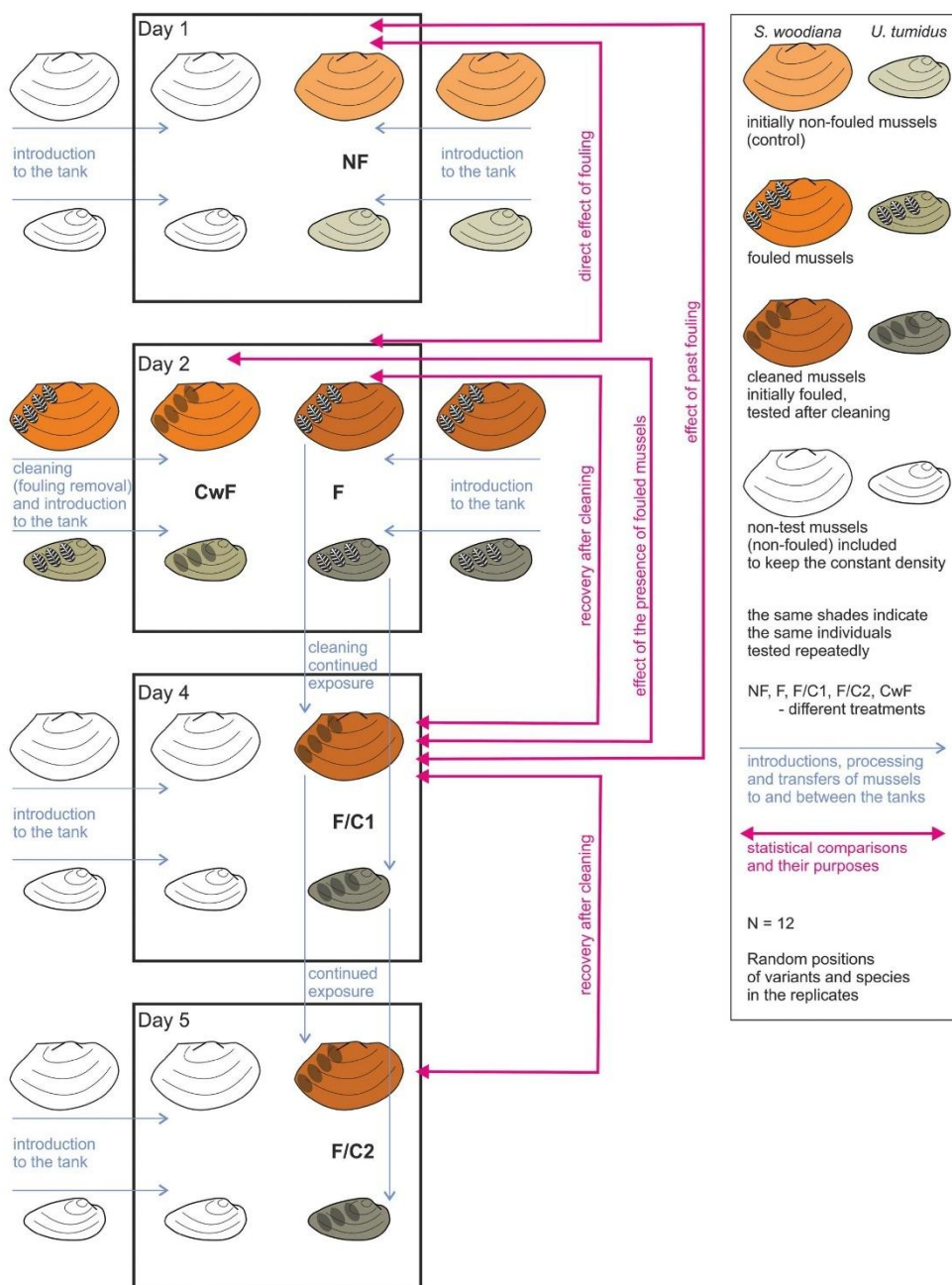


Figure 1. Mussel arrangement in the experimental tanks of Experiment 1 on the effect of *Dreissena* spp. fouling on the behaviour of *S. woodiana* and *U. tumidus*. See Table 3 for detailed explanations of variants (NF, F, F/C1, F/C2, CwF).

Table 3. Full list of variants and their comparisons in Experiment 1 with explanations of their purposes. See Fig. 1 for variant arrangements in the experimental tanks. In variants F, F/C1 and F/C2, we used the same individuals tested repeatedly.

Variants	Description	Purpose
NF (non-fouled)	Control; non-fouled unionids collected in the field without any trace of fouling on their shells	Control for the fouling variants (fouled F and cleaned F/C1)
F (fouled)	Fouled unionids tested with their fouling	To determine the direct impact of dreissenids fouling on mussel behaviour (compared to NF)
F/C1 (cleaned, time 1)	F unionids cleaned of fouling and tested for the second time two days after cleaning (with no dreissenids in the tank)	To determine whether the behaviour of mussels would change after the removal of fouling (compared to F) and whether it would return back to normal (compared to NF)
F/C2 (cleaned, time 2)	F/C1 unionids tested once again on the next day (for the third time in general, second time without fouling)	To check whether the repeated testing of mussels and passing time has an impact on their behaviour (compared to F/C1)
CwF (cleaned with fouled)	Unionids collected in the field as fouled, cleaned of fouling and tested two days after cleaning in the presence of other, fouled unionids (from variant F)	To determine whether the presence of other fouled unionids has an impact on their behaviour (compared to F/C1, exposed in the total absence of dreissenids)
Comparisons between the variants		Purpose
NF	vs F	To test the direct effect of fouling on unionid behaviour of both chemical cues and physical presence of biofoulers
NF	vs F/C1	To test the effect of past fouling on unionid behaviour (potentially deteriorated condition of mussels recently cleaned of fouling)
F	vs F/C1	To test the possibility of potential recovery from the past fouling with passing time
F/C1	vs F/C2	To test the potential effects of passing time on the responses of unionids exposed to the experimental conditions
CwF	vs F/C1	To test the unionid responses to the presence of fouled individuals in the environment (chemical cues from fouled unionids and biofoulers)

First, we tested non-fouled mussels of variant NF (one *S. woodiana* and one *U. tumidus* individual per tank) accompanied by two additional non-fouled individuals (one per species) to have the total number of four individuals per tank. Then, we tested two fouled mussels (F) accompanied by two mussels cleaned two days before the exposure (CwF). After this exposure, fouled individuals (F) were cleaned and kept in tanks for two days. Then, they were exposed again (as F/C1) in the presence of two additional non-fouled mussels. On the next day, F/C1 mussels were exposed once again (as F/C2) in the presence of two additional non-fouled mussels. Thus, each experimental tank always contained four mussels, two of each species, in different fouling conditions (Fig. 1).

Effects of the presence of *Dreissena* spp. (Experiment 2) on unionid behaviour were tested in tanks prepared similarly as for Experiment 1. We introduced two cleaned (two days before the experiment) unionids (one *S. woodiana* and one *U. tumidus*) into each tank (one individual in the centre of each half of the tank bottom). We used cleaned mussels to be sure they had some past experiences with *Dreissena* spp. fouling. In one randomly selected corner of the tank, we placed a mesh bag with 50 individuals of *D. r. bugensis* (mean wet mass: 13.5–15 g) or *D. polymorpha* (12–12.5 g). Here, the two species of *Dreissena* spp. were tested separately, as, in contrast to the direct impact of the mass of attached individuals, we expected that unionids can respond differently to the remote presence of a particular fouler species. In parallel, we carried out control trials in tanks without mesh bags with dreissenids. Our Experiment 1 showed that the fouling *Dreissena* spp. mass of the magnitude used in Experiment 2 was capable of triggering behavioural responses of Unionidae (see Results).

All configurations were recorded for 24 hours in 12 replicates.

Data analysis

After completing the experiments, we analysed the obtained videos to determine: (i) movement initiation time (time from the introduction to the first activity, i.e. initiation of locomotion or burrowing), (ii) locomotion distance and (iii) mean burrowing level [%]. Every minute, we estimated the level of bivalve burrowing (using a 5-level percentage scale: 0, 25, 50, 75 and 100%) based on the length of the part of the shell below the substratum surface to the total shell length (according to Poznańska-Kareko et al. 2021). Mean burrowing level was calculated according to the formula:

$$MB = \sum_{i=1}^4 [25\% * it_i] / \sum_{i=0}^4 t_i \quad (1)$$

where: i – burrowing level: 5 steps ranging from 0 (totally exposed on the surface) to 4 (fully burrowed); t_i – time spent by the mussel at burrowing level i.

The list of all comparisons between the variants of Experiment 1 is presented in Table 3. As the data strongly violated normality and homoscedasticity assumptions (tested with Shapiro-Wilk and Levene tests, respectively), we used non-parametric Mann-Whitney U tests to compare mussel behaviour in experimental treatments, except for the comparisons among F-F/C2 variants of Experiment 1, where the same individuals were tested several times. For those, we used Wilcoxon signed rank tests for paired samples. In Experiment 2, we compared unionid behaviour in *Dreissena* sp. presence treatments to control treatments using Mann-Whitney tests to check unionid responses to the presence of biofoulers (chemical cues from biofoulers only). We applied a sequential Bonferroni correction for multiple comparisons (within each variable and species). However, we decided to report and interpret results both with and without the correction, as it is commonly considered as overly conservative with a large number of comparisons (Moran 2003). Statistical analyses were carried out using SPSS 29.0 (IBM Inc.).

Results

Experiment 1: Unionid responses to direct *D. r. bugensis* and *D. polymorpha* fouling and presence of fouled unionids

There was no significant effect of *Dreissena* spp. fouling on *S. woodiana* movement initiation time and locomotion (Fig. 2a, e). Mean burrowing level of cleaned *S. woodiana* (variant F/C1) was reduced compared to the control individuals (NF) but increased again after a longer exposure (F/C2) (Fig. 2g, Table 4).

For *U. tumidus*, we noted differences in movement initiation time: fouled mussels (F) started to move later compared to control non-fouled individuals (NF). When the fouled *U. tumidus* (F) were cleaned (becoming F/C1), they hastened their movement initiation time up to the level exhibited by the control individuals (NF). Moreover, cleaned *U. tumidus* (CwF) exposed in the company of fouled individuals started to move later (general activity) compared to *U. tumidus* kept in the absence of dreissenids (F/C1) and did not move horizontally at all (Fig. 2b, f, Table 4). *Unio tumidus* burrowed less when fouled (F) compared to the control non-fouled individuals (NF), and this effect persisted after cleaning (F/C1) (Fig. 2h, Table 4).

Table 4. Unionid responses to direct *D. r. bugensis* and *D. polymorpha* fouling and presence of fouled unionids in Experiment 1. Variant NF: mussels collected in the field without any trace of fouling on their shells, hereafter referred to as non-fouled mussels; variant F: mussels collected as fouled by *Dreissena* spp. and tested first time with their own fouling (fouled mussels); variant F/C1: F mussels, cleaned and tested after two days in the presence of non-fouled individuals (cleaned mussels); variant F/C2: F/C1 mussels tested on the next day (for the third time); variant CwF: mussels collected as fouled, cleaned two days before testing, and tested in the presence of fouled individuals. Variants NF vs F and F vs CwF were compared with Mann-Whitney U tests; variants F-F/C2 were compared to each other using Wilcoxon signed rank tests for paired data. Statistically significant differences are marked with asterisks and those that are still significant with the sequential Bonferroni correction are marked in bold font. Z – test statistic; P – statistical significance. Underlying data are available in Suppl. material 1.

Variable	Configurations			<i>S. woodiana</i>		<i>U. tumidus</i>	
				z	P	z	P
Movement initiation time	Non-fouled (control, NF)	vs.	Fouled (F)	-0.69	0.488	-2.42	0.015*
	Non-fouled (control, NF)		Cleaned (F/C1)	-0.55	0.583	-1.21	0.225
	Fouled (F)		Cleaned (F/C1)	-0.16	0.875	-2.98	0.003*
	Cleaned (F/C1)		Cleaned (F/C2)	-0.16	0.875	-1.14	0.255
	Cleaned (CwF)		Cleaned (F/C1)	-0.40	0.686	-2.02	0.043*
Locomotion distance	Non-fouled (control, NF)	vs.	Fouled (F)	-0.79	0.429	-0.04	0.970
	Non-fouled (control, NF)		Cleaned (F/C1)	-0.41	0.684	-0.72	0.470
	Fouled (F)		Cleaned (F/C1)	-0.52	0.600	-0.34	0.735
	Cleaned (F/C1)		Cleaned (F/C2)	-0.85	0.398	-0.42	0.674
	Cleaned (CwF)		Cleaned (F/C1)	-0.41	0.684	-2.44	0.015*
Mean burrowing level	Non-fouled (control, NF)	vs.	Fouled (F)	-0.43	0.665	-3.12	0.002*
	Non-fouled (control, NF)		Cleaned (F/C1)	-2.54	0.011*	-2.94	0.003*
	Fouled (F)		Cleaned (F/C1)	-1.18	0.239	-0.63	0.530
	Cleaned (F/C1)		Cleaned (F/C2)	-2.04	0.041*	-1.88	0.060
	Cleaned (CwF)		Cleaned (F/C1)	-1.79	0.073	-0.17	0.862

Neither species showed significantly different movement initiation times or locomotion parameters exclusively due to passing time (comparison of F/C1 vs F/C2).

Experiment 2: Unionid responses to waterborne cues of *D. r. bugensis* and *D. polymorpha*

Sinanodonta woodiana showed a higher mean burrowing level in the presence of *D. r. bugensis* compared to the control (Fig. 3, Table 5). Neither the presence of *D. r. bugensis* nor *D. polymorpha* affected the locomotion and burrowing of *U. tumidus* (Fig. 3, Table 5).

Discussion

Consistently with our first hypothesis, dreissenid fouling affected the behaviour of unionid mussels. *Sinanodonta woodiana* responded only with changed burrowing level. In Experiment 1, we observed shallower burrowing of *S. woodiana* immediately after fouling removal, but, over time, burrowing returned to the level exhibited by the control, non-fouled mussels. On the other hand, in Experiment 2, *S. woodiana* burrowed deeper in the presence of quagga mussels compared to the control. This might be a defensive response of *S. woodiana* to

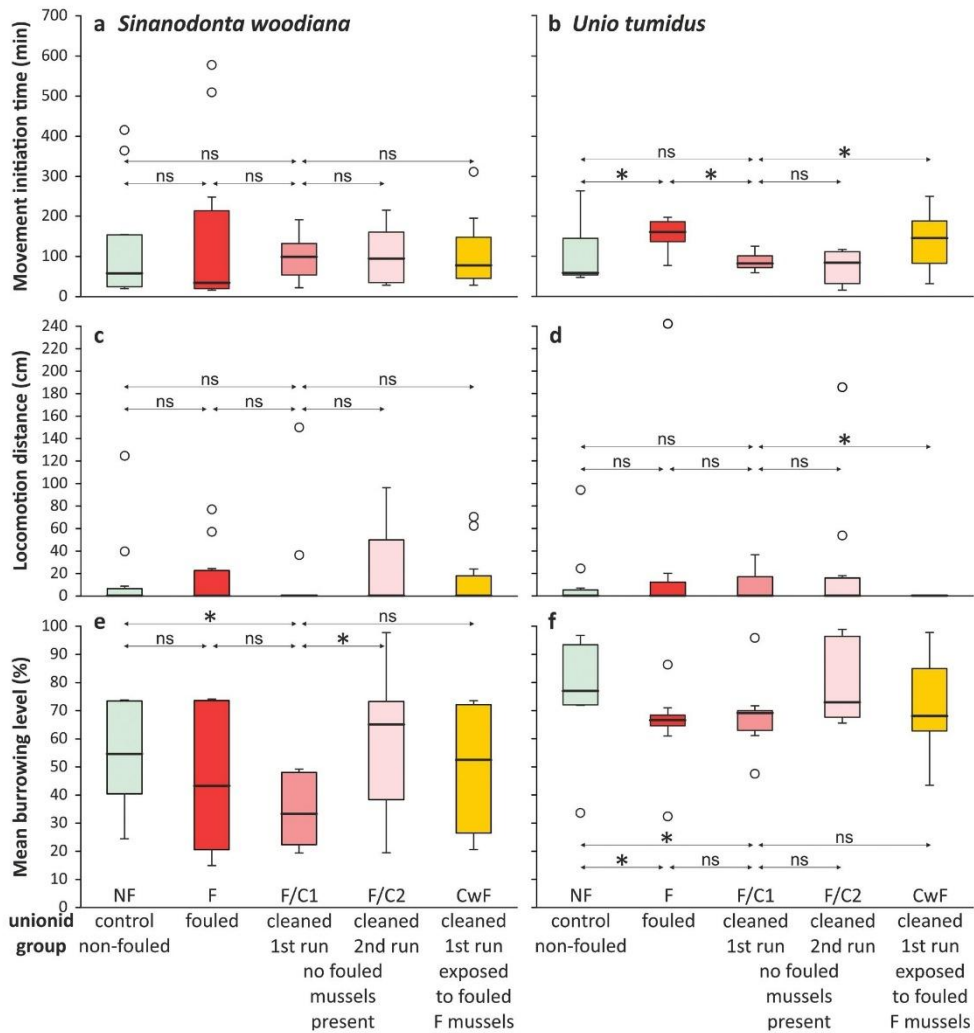


Figure 2. Unionid responses to the direct dreissenid fouling and presence of fouled unionids in Experiment 1: **a**, **b** movement initiation time [min] **c**, **d** locomotion distance [cm] **e**, **f** mean burrowing level [%]. Asterisks mark significant differences between the variants (ns – non-significant). Boxplots present medians (horizontal lines), 1st and 3rd quartiles (top and lower boxes, respectively), 1.5*interquartile range (whiskers) and outliers (circles). Variant NF: mussels collected in the field without any trace of fouling on their shells, hereafter referred to as non-fouled mussels; variant F: mussels collected as fouled by *Dreissena* spp. and tested first time with their own fouling (fouled mussels); variant F/C1: F mussels, cleaned and tested after two days in the presence of non-fouled individuals (cleaned mussels); variant F/C2: F/C1 mussels tested on the next day (for the third time); variant CwF: mussels collected as fouled, cleaned two days before testing, and tested in the presence of fouled individuals.

the presence of dreissenids, consisting in increased burrowing. It is worth noticing that a similar, though marginally non-significant tendency for increased burrowing was observed in *S. woodiana* exposed to fouled unionids (variants CwF vs F/C1, Fig. 2, Table 4).

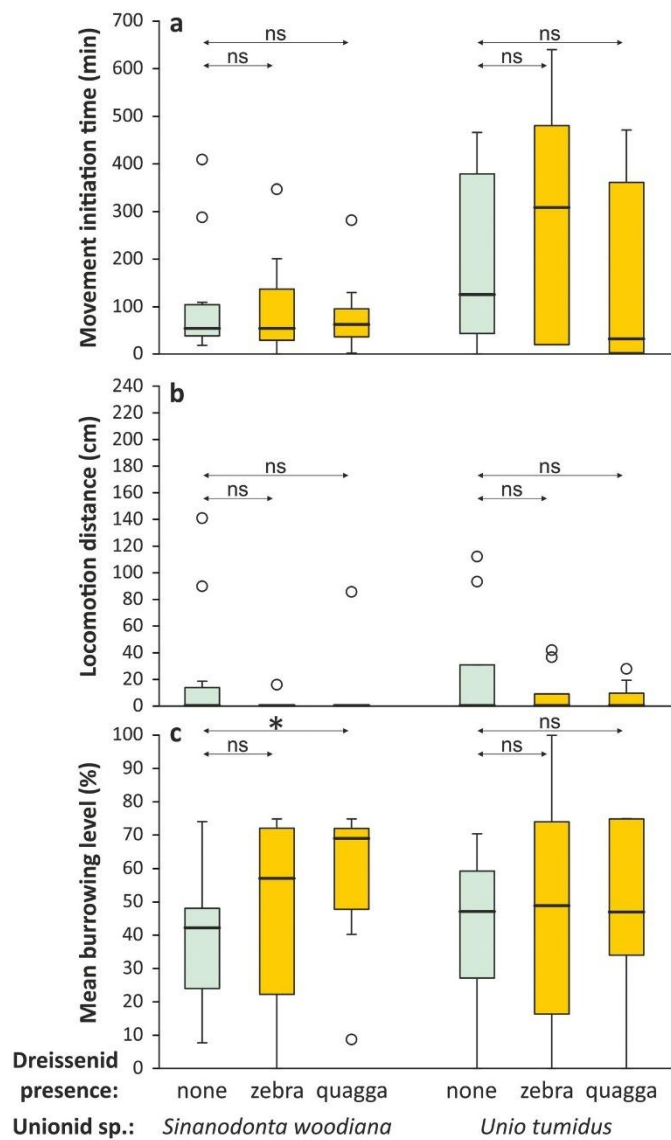


Figure 3. Unionid responses to *D. r. bugensis* and *D. polymorpha* waterborne cues in Experiment 2. Asterisks mark significant differences between the variants (ns – no significant). Boxplots present medians (horizontal lines), 1st and 3rd quartiles (boxes), 1.5*interquartile range (whiskers) and outliers (circles).

The pattern observed for *S. woodiana* in Experiment 1 can be explained by contrasting effects of fouling (mechanical obstacle to efficient burrowing) and defensive responses to *Dreissena* spp. presence (stimulating burrowing). For fouled individuals, burrowing was more difficult, but they kept trying to defend themselves from fouling, resulting in a similar level as that showed by the control mussels.

Table 5. Unionid responses to *D. r. bugensis* and *D. polymorpha* waterborne cues in Experiment 2: compared to their behaviour on the control (pairwise Mann-Whitney U test). Statistically significant differences are marked with asterisks (note that they do not pass the Bonferroni correction for multiple comparisons). z – test statistic; P – statistical significance. Underlying data are available in Suppl. material 1.

Variable	Configurations			<i>S. woodiana</i>		<i>U. tumidus</i>	
				z	P	z	P
Movement initiation time	control	vs.	<i>D. polymorpha</i> presence	-0.37	0.712	-0.60	0.545
			<i>D. r. bugensis</i> presence	-0.17	0.862	-0.90	0.369
Locomotion distance	control	vs.	<i>D. polymorpha</i> presence	-1.12	0.264	-0.60	0.551
			<i>D. r. bugensis</i> presence	-1.11	0.266	-0.47	0.636
Mean burrowing level	control	vs.	<i>D. polymorpha</i> presence	-1.10	0.273	-0.30	0.762
			<i>D. r. bugensis</i> presence	-2.14	0.033*	-0.98	0.327

Immediately after the fouling removal, with no dreissenids present in the environment (variant F/C1, no need for anti-fouling defence), they burrowed more shallowly due to recently experienced problems related to fouling (e.g. exhaustion), but, with time (the next experimental round, F/C2), their burrowing returned to the control level. Nevertheless, this effect of fouling persisting after cleaning, though short-timed, suggests that the impact of dreissenids on their hosts was not purely mechanical, but also affected their condition.

Increased burrowing is a natural defence mechanism (Saloom and Duncan 2005) and may indicate an attempt to avoid fouling mussels, which, once attached, could have a more negative impact on their host condition than when acting only indirectly by their presence nearby (Sousa et al. 2011). Mussels are known to sense the presence of predators (Reimer and Harms-Ringdahl 2001; Meira et al. 2024) and parasites (Selbach and Mouritsen 2020) from chemical cues in the environment, as well as detect alarm signals from damaged conspecifics (Leonard et al. 1999), which results in the induction of various defence mechanisms, such as increased burrowing (Griffiths and Richardson 2006). The occurrence of a defence mechanism in *S. woodiana* may give this species an advantage in unfavourable conditions, such as the presence of biofouling *Dreissena* spp. The deeper the mussel burrows, the less exposed its shell is to the fouling dreissenids, which directly affects the degree of fouling, as shown by Urbańska et al. (2019). Moreover, existing fouling can also be reduced by the burrowing of the host unionid, as dreissenids are known to detach from unsuitable (e.g. burrowed) substrata and move in search of a better attachment site (Dzierżyńska-Białończyk et al. 2018b; Balogh et al. 2024). Dzierżyńska-Białończyk et al. (2018a) and Balogh et al. (2024) have shown that burrowed *S. woodiana* (but not the native unionid species, including *U. tumidus*) were less fouled by dreissenids than individuals exposed on the surface, confirming a defensive potential of this behaviour.

In Experiment 2, *S. woodiana* responded significantly only to the presence of quagga mussels, which confirmed that unionids can detect and respond to chemical cues of other bivalves in the environment (our fourth hypothesis). However, it should be noted that a similar tendency was also apparent in the presence of zebra mussels, thus the support for the hypothesis that unionid responses to fouling depended on the fouler species remains weak. Anyway, it is possible that *S. woodiana* is more familiar with quagga mussels, which are more abundant in Lake Balaton, although this ratio may vary in different parts of the lake (Balogh et al. 2008; Balogh et al. 2024). Moreover, quagga mussels are less associated with their

substratum (D'Hont et al. 2021) and easier to detach due to their weaker adhesion strength (Balogh et al. 2019), thus burrowing can be a more effective defence mechanism against them compared to more strongly and more permanently attached zebra mussels.

In Experiment 1, fouled *U. tumidus* showed a delayed movement initiation time and shallower burrowing compared to the control, non-fouled individuals. After the fouling removal, the movement initiation time of *U. tumidus* (variant F/C1) returned to the control level, but the burrowing remained weakened. Therefore, changes in the behaviour of *U. tumidus* were driven by the mechanical effect of fouling present on their shells, as well as by the impaired condition of fouled mussels. This supported our third hypothesis, as the fouling effect persisted after the fouling removal.

We did not observe any effects of direct fouling on the locomotion, in contrast to Van Appledorn and Bach (2007) working on North American unionids *Ligumia nasuta* Say, 1817 and *Anodonta grandis* Say, 1829. However, in accordance with our fourth hypothesis, *U. tumidus* responded to the presence of other fouled individuals by stopping any horizontal movements and delayed movement initiation (here: burrowing start, as locomotion did not occur at all) (CwF vs F/C1). Theoretically, this difference might be an artefact resulting from the fact that mussels from F/C1 were tested in the experimental arena for the second time, whereas for those from CwF it was the first experimental round. However, the comparison between animals tested repeatedly in consecutive experimental rounds under the same conditions (F/C1 vs F/C2) showed no significant differences and symptoms of exhaustion or habituation due to repeated testing, thus this explanation seems unlikely. On the other hand, the observed responses of *U. tumidus* to the presence of fouled individuals may represent a general reaction to stressed (here: fouled) conspecifics secreting some infochemicals indicating stressful environmental conditions. Definitely, *U. tumidus* did not respond to direct cues from dreissenids, as shown by Experiment 2.

All changes in the behaviour of *U. tumidus* induced by fouling or nearby presence of fouled individuals seem negative, exposing them to a number of environmental threats, including predation, parasites, dislodgement by water movements, desiccation during droughts, and further fouling by dreissenids (Bowers et al. 2005; Lymbery et al. 2021).

Our study showed that the behaviour of the native *U. tumidus* in the presence of fouling *Dreissena* spp. mussels was modified to a higher extent than that of the invasive *S. woodiana*. Moreover, the responses of *S. woodiana* appeared to have defensive and preventive effects against dreissenid fouling, whereas the behaviour of *U. tumidus* seemed to be impaired compared to their normal (control) activity. Thus, despite a similar relatively moderate level of fouling of both species in our study, dreissenids presence had stronger negative effects on *U. tumidus*. Due to large body size of *S. woodiana* and its weaker burrowing (Poznańska-Kakareko et al. 2021), more dreissenids, especially zebra mussels, can attach to and persistently stay on their surface than to smaller native mussels. Therefore, *S. woodiana* can act as a reservoir for dreissenids colonizing the native species, with the native species suffering more severe negative consequences of fouling. This suggests yet another potential effect of invasive dreissenids on native communities: their presence can influence interactions between native and invasive species of Unionidae, promoting the latter. On the other hand, *S. woodiana* can provide suitable substratum for dreissenid development. Moreover, we have shown that, beside parasites (McElwain 2019) and

predators (Meira et al. 2024), dreissenids can constitute another driver of apparent competition between invasive and native species, when the invader poses a threat to the native organism by increasing the abundance and availability of biofoulers. This effect is likely to be most pronounced in locations where shells of unionid mussels constitute the primary source of hard substratum available to dreissenids, such as soft lake bottoms, or dam reservoirs and river pools with limited flows and accumulated sediments, missing rocks and artificial embankments (Dzierżyńska-Białończyk et al., 2018a; Balogh et al. 2024). Nevertheless, such places are common in the environment and are frequently used by unionids. Therefore, dreissenid mussels can extend their distribution ranges as they are able to use their shells as attachment sites.

It should be noted that our research was conducted using mussels collected from a single location in Lake Balaton. This allowed us to eliminate potential confounding effects related to different conditions experienced by experimental animals before their sampling. We checked the situation taking place within the same natural community, living under the same conditions. However, we must admit that conducting the same research using mussels from other regions of the world may result in different results. To better understand the influence of dreissenids on Unionidae, it would be useful to conduct similar comparative studies with mussels from other lakes or rivers.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

KW: Conceptualisation, Methodology, Resources, Investigation, Formal analysis, Visualisation, Writing – Original draft, Writing – Review and Editing; CB: Conceptualisation, Methodology, Resources, Visualisation, Investigation, Writing – Review and Editing, Funding Acquisition; JK: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Data Curation, Visualization, Writing – Review and Editing; DS: Methodology, Resources, Investigation, Writing – Review and Editing; LJ: Resources, Investigation, Writing – Review and Editing; MPK: Conceptualization, Methodology, Investigation, Validation, Visualization, Formal analysis, Supervision, Project administration, Writing – Review and Editing.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Dataset

Authors: Kamil Wiśniewski, Csilla Balogh, Jarosław Kobak, Daniel Szarmach, Łukasz Jermacz, Małgorzata Poznańska-Kakareko

Data type: xlsx

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Imię i nazwisko współautora	Udział [%]	Pełnione funkcje
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dr hab. Małgorzata Poznańska-Kakareko, prof. UMK	13	Koncepcja badań, opracowanie metodologii, zebranie materiału w terenie, nadzór nad przebiegiem badań, zarządzanie projektem, poprawianie manuskryptu

Invasive signal crayfish differently affects invasive and native unionid mussels

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Abstract

Freshwater unionid mussel populations are declining worldwide because of human impact, climate change, and invasive species. One such species is the North American signal crayfish, *Pacifastacus leniusculus*, which can feed on bivalves. We intended to determine how the presence of crayfish affects the behavior of native European unionids: *Anodonta anatina* and *Unio tumidus*, as well as their non-native, Eastern-Asian competitor, *Sinanodonta woodiana*. During the tests, the crayfish had direct access to the mussels or were separated from them by a mesh (indirect contact), so that the mussels could only sense its presence. We analyzed changes in mussel behavior (locomotion and burrowing) in the presence of predator, and the crayfish responses to various mussel species (frequency and duration of attacks). Direct contact

with crayfish resulted in delayed mussel activity. The presence of crayfish (direct and indirect) reduced the degree of burrowing of *U. tumidus*, but not of the other mussel species. *Unio tumidus* was the least often attacked species by crayfish, which were unable to dig out them from the sediments. This suggests that *U. tumidus* was capable of effective defense against crayfish: it recognized the danger and responded with reduced activity, decreasing the likelihood of detection, while remaining relatively safe against attacks. On the other hand, *S. woodiana* was the most often attacked species, with no specific defensive responses to crayfish presence. This suggests a complex interplay among invaders and native mussels, with crayfish potentially mitigating negative effects of *S. woodiana*, especially in the case of *U. tumidus*.

Keywords: biological invasions, predation, predator-prey interactions, behavioral responses, locomotion, Unionidae

1. Introduction

Sessile and sedentary organisms are unable to actively escape or change locations quickly when sense predators. Therefore, they react to their presence with a set of specific anti-predatory defenses, quite different than those shown typically by mobile organisms. These include burrowing into the substratum (Griffiths and Richardson 2005; Saloom and Duncan 2005), non-locomotor activity reduction as a means of detection avoidance (Czarńoński et al. 2011; Antoń et al. 2018; Dzierżyńska-Białończyk et al. 2019), aggregation forming (Naddafi and Rudstam 2013), and attachment to the substratum (Kobak and Kakareko 2009). In turn, these behaviors demand specific predation strategies from organisms feeding on this type of prey. They do not need to chase their prey, but must focus on locating them, removing from a shelter, and overcoming their structural defenses.

Common representatives of sedentary organisms in aquatic ecosystems are bivalves, including mussels and clams. Mussels of the Unionidae family are a highly diverse group of organisms with over 900 species found in freshwaters on all continents except Antarctica (Lopes-Lima et al. 2018). Mussels can dominate the benthic biomass of rivers (Newton et al. 2011) and their filtration of water, coupled with biodeposition, play a key role in transferring suspended material from the water column to the bottom, thus influencing water clarity, primary and secondary production, biogeochemical cycles, and sedimentation rates (Vaughn 2018). Their shells provide substratum for epiphytes, refuge for macrozoobenthic taxa (Ilarri et al. 2018) and significantly change the character of the substratum, which may also affect other mussels (Szarmach et al. 2024; Wiśniewski et al. 2024a).

In the past few decades, there has been a significant decline in unionid populations (Karatayev et al. 2012; Aldridge et al. 2023). There are 16 unionid mussel species recorded in Europe, most of which are threatened (Lopes-Lima et al. 2017). In extreme cases, mass mortality events occur, which can decimate populations and compromise key ecosystem functions (Fey et al. 2019). These events can be caused by chemical pollutants, hypoxia, heat waves (Cossey et al. 2025) or habitat transformations (Strayer et al. 2004; Cope et al. 2008; Downing et al. 2010). Yet another major threat to local mussel populations is the spread of invasive alien species, including bivalves competing with native species, and crayfish posing a direct predatory threat to mussels (Lopes-Lima et al. 2017; Sanders and Mills 2022).

Large Unionidae do not have many enemies due to their size, hard shell and burrowing ability. A few predators that can successfully feed on them include large fish, water birds, mammals, such as muskrat (Owen et al. 2011; Moore et al. 2018), as well as large invertebrates, such as crayfish (Sanders and Mills 2022). An invasive predator that may affect mussel assemblage is the signal crayfish *Pacifastacus leniusculus* (Dana 1852) from North America (Bernardo et al. 2011). This species was initially introduced to Sweden in 1969 (Froufe et al.

2015) and has since been gradually expanding its distribution across Europe, either naturally or through intentional introductions and aquaculture practices (Ackefors 2000). At present, it ranks among the most prevalent invasive crayfish species in Europe, with its distribution ranging from Spain through central Europe to western Russia, also including Scandinavia (Kouba et al. 2014). Signal crayfish inhabit both small and large lowland rivers, as well as lakes and ponds (Holdich et al. 1997). Large size of *P. leniusculus*, along with its high voracity (Souty-Grosset et al. 2006), contributes to its considerable impact on aquatic ecosystems, including its ability to attack and consume large bivalves (Sanders and Mills 2022).

Sinanodonta woodiana (Lea 1834), originating from southern China, is one of the invasive mussel species currently spreading in Europe (Kondakov et al. 2018; Konečný et al. 2018). This species exhibits a broad tolerance to various environmental conditions (Kraszewski and Zdanowski 2007; Poznańska-Kakareko et al. 2021; Zieritz et al. 2021; Dobler et al. 2022) and is noted for its high reproductive capacity and rapid growth rate (Douda et al. 2021). Additionally, it ranks as the largest freshwater mussel currently found in Europe (Urbańska et al. 2019). It can coexist with native mussels while outcompeting them for resources such as food, fish hosts for their larvae, and living space (Douda and Čadková 2018). It is also possible that *S. woodiana* differs from native mussels in the quality and/or efficiency of anti-predatory behavior, which can be another factor affecting their interactions and drive displacement. Specifically, unequal predator effects on mussel species may shape the outcome of their interspecific competition. This could manifest as protection for native species if *S. woodiana* is preferably consumed, but also as apparent competition, if *S. woodiana* provides additional food source and sustains predators subsequently preying on the natives.

The aim of our study was to determine and compare the effect of direct and indirect presence of signal crayfish on the behavior of native and invasive mussels. We selected *Unio tumidus* Philippon 1788 and *Anodonta anatina* (Linnaeus 1758) as native species that are still

relatively abundant in many areas, but their numbers are gradually decreasing in Europe, and in some countries, such as in Germany, Portugal or Switzerland, they are already threatened with extinction (Lopes-Lima and Prié 2024a; 2024b). We compared their responses to those of *S. woodiana*, a unionid invader currently spreading in Europe and potentially posing a threat to related native species (Wiśniewski et al. 2024b). We hypothesized that: (1) Mussels will react to crayfish presence by increased burrowing and locomotion reduction to defend themselves from crayfish attacks. Alternatively, we might also expect reduced burrowing as a consequence of the total activity reduction, decreasing the probability of detection by a predator. (2) Mussels will detect and respond not only to direct crayfish presence, but also to their scents, which would indicate their ability to recognize crayfish as a potential danger. (3) *Unio tumidus* will be the least attacked and handled species due to its thick and wide shell, and deep burrowing (Schwalb and Pusch 2007). On the other hand, *A. anatina* will be the most attacked and handled species due to its thin and narrow shell, and shallow burrowing (Poznańska-Kakareko et al. 2021). The choice of *S. woodiana* for our study was motivated by the fact that its greater or lower susceptibility to predator attacks compared to the particular native mussel species may shape their competitive relationships in the environment after the appearance of the crayfish.

2. Material and methods

2.1. Animal collection in the field

Mussels were collected in May 2024 from sandy-muddy bottom (depth: 2 – 2.5 m) from the Włocławski Reservoir on the Vistula River, Central Poland (52°37'04" N, 19°19'42" E) by scuba diving. We selected this site due to the occurrence of all three mussel species in relatively high densities. *Sinanodonta woodiana* as an invasive species has occurred at this Reservoir since at least 2016 (Dzierżyńska-Białończyk et al. 2018). Crayfish were collected in May 2024

from the Lutynia River, Central Poland (51°48'39" N, 17°41'44" E) on sandy-muddy bottom or from macrophytes (depth: 0.2 – 0.5 m) with a Surber net. Collected mussels and crayfish were transported to the laboratory and tested after two weeks of acclimation. Mussels and crayfish were randomly assigned to experimental treatments. Animal sizes are presented in Table 1.

Table 1. Total length of mussels and crayfish [cm]. Crayfish were measured from the rostrum to the telson.

	Mean	SD	Range
<i>A. anatina</i>	7.6	0.53	7.0-8.5
<i>U. tumidus</i>	7.3	0.56	6.5-8.5
<i>S. woodiana</i>	10.1	1.03	9.0-12.5
<i>P. leniusculus</i>	11.1	1.16	9.0-12.5

SD – standard deviation

2.2. Laboratory conditions

Mussels and crayfish were kept in single-species 350-L stock tanks (20 mussels per tank and 10 crayfish per tank) containing a 10 cm layer of sandy substratum from the collection site (grain diameter range: 0.2 – 1.4 mm; median: 0.63 mm), internal filters, and aeration systems. The stocking and experimental room was equipped with a photoperiod system (light/dark 12:12 h) and air conditioning, maintaining the constant water temperature in the tanks. Mussels were fed twice a week with a suspension of dried *Chlorella* algae ("*Chlorella* super alga", Meridian company, Poland) at a concentration of 5 mg L⁻¹ (Douda and Čadková 2018). Crayfish were fed three times a week with frozen Chironomidae larvae (thawed before serving).

We regularly checked the water quality in the stock and experimental tanks using a multimeter Multi340i (WTW GmbH, Weilheim, Germany). The parameters (min – max) were as follows: oxygen content: 5.06 – 6.01 mg ml⁻¹ and 55.1 – 71.0 %; temperature: 19.2 – 21.5

°C (similar to that measured at the collection sites); pH: 8.29 – 8.53; conductivity: 491 – 636 $\mu\text{S cm}^{-1}$.

2.3. Experimental set-up

Tests were conducted in tanks (bottom: 40 x 30 cm, height: 30 cm) containing 10 cm of sand from the mussel collection site (see above) and 10 cm of conditioned tap water (settled, filtered and aerated for at least 48 h). Before use, the sand was sifted, rinsed and dried in a laboratory dryer (SLW 115 STD Multiserw-Morek, Poland) at 60 °C for 6 h to eliminate any organisms that could affect the results. Tanks were divided into two parts by a plastic mesh barrier (mesh size: 1.5 x 1.5 mm): a larger part with a bottom area of 30 x 30 cm and a smaller part with a bottom area of 10 x 30 cm.

Mussels were exposed in three experimental treatments. The direct presence of the crayfish (treatment 1) was tested by introducing a single crayfish to the larger part of the tank. After 24 h, a single mussel was added to the crayfish part of the tank. Thus, the mussel could sense the presence of the crayfish and the crayfish had physical access to the mussel. To investigate the effect of the indirect presence of predator cues on mussel behavior (treatment 2), we placed a single crayfish into the smaller part of the tank, and, after 24 h, introduced a single mussel to the larger part. This allowed the mussel to sense the presence (chemical cues) of the crayfish, but the crayfish had no physical access to the mussel. The crayfish were not additionally fed during the experiments (the last feeding the day before introducing the crayfish into the tank). In the control treatment (treatment 3), a single mussel was introduced to the larger part of the tank and no crayfish were added. In all the treatments, animals were recorded for 24 h with CCTV video cameras (Samsung SNB-6004, South Korea) equipped with infrared emitters (LIR-CS32, IRLAB, Taiwan) enabling observation at night. Tests began in the

daytime, continued through the night, and ended again in the daytime. Each treatment was replicated 15 times per mussel species (using new individuals).

2.4. Data analysis

The following behavioral variables were measured and calculated (see Supplementary Material Table S1):

(1) Mussel movement start time (either locomotion or burrowing) (latency to move).

(2) Mean burrowing of mussels: $MB = 100\% * \sum_{i=1}^4 \frac{0.25it_i}{\sum_{i=0}^4(t_i)}$

where: i – burrowing level (i = 0, 1, 2, 3, 4 for 0, 25, 50, 75, and 100% of the burrowed length of the mussels shell, respectively), t_i – time spent at burrowing level i.

(3) Time spent by mussels on burrowing (duration of burrowing activity), expressed as the percentage of the experimental time.

(4) Mussel locomotion distance (initially, locomotion time was also measured, but the results mirrored those obtained for the distances).

(5) Time spent by mussels exposed on the surface after being dug out from the sediments by the crayfish (percentage of the experimental time).

(6) Number of crayfish attacks on the mussel (grabbing the mussel with claws and/or biting with mandibles).

(7) Mussel handling time by crayfish (percentage of the total experimental time).

Mussel movement start time (variable 1) and time spent on the surface after being dug out by the crayfish (variable 5) were excluded from the experimental time used for calculation of mean burrowing (variable 2) and percentage time spent on burrowing (variable 3). Variable

1 (time when mussels remained inactive on the surface before they started to move) was excluded to avoid redundancy between the variables 1 vs 2 and 1 vs 3 (otherwise, variables 2 and 3 would have also included hidden variable 1). Excluding variable 5 allowed to analyze true mussel activity (rather than position changes caused by crayfish actions). Mussels remaining inactive for the total experimental time were assigned a value of 0 for variables 2 and 3 (to avoid division by 0).

Mussel movement start time (variable 1) was also excluded from the experimental time used for determination of percentage time spent by mussels on the surface after being dug out by the crayfish (variable 5). Moreover, initial visual inspection of the data revealed that totally inactive mussels (no locomotion or burrowing) throughout the entire experiment (there were only few such cases) were treated differently by crayfish (see Results). They were included in the analysis of mussel behavior (variables 1-4) but excluded (though visualized in figures) from the analysis of direct interactions between crayfish and mussels (variables 5-7). This was because the total inactivity of these individuals was not caused by direct handling by crayfish. For instance, there was no time spent exposed on the surface by those mussels after being dug out by the crayfish, as no digging out could take place. Also, staying on the surface, they were more exposed to crayfish actions, which could bias the results.

Mussel movement start time was analyzed with a Cox proportional hazards regression including mussel species and treatment (direct or indirect crayfish presence, or no crayfish) as factors, as well as their interaction.

Mean burrowing and locomotion distance, due to strong violations of normality and homoscedasticity assumptions, were analyzed with non-parametric Kruskal-Wallis tests. First, mussel behavior without crayfish (control) was compared among different species to establish basic interspecific differences. Then, different treatments were compared within each species

separately to check for crayfish impact. Significant Kruskal-Wallis test results were further disentangled with pairwise Mann-Whitney tests.

Mussel burrowing time was analyzed with a General Linear Model including mussel species, treatment, and their interaction.

Effects of physical interactions between crayfish and mussels (the treatment with direct crayfish presence) were compared among mussel species: time spent by mussels on surface after being dug out by crayfish, number of crayfish attacks, and mussel handling time by crayfish were analyzed with a Kruskal-Wallis test, Generalized Linear Model (Poisson distribution, log link function), and General Linear Model, respectively.

The models were simplified by removing non-significant interactions. As needed, pairwise contrasts were used to disentangle significant effects involving more than two groups. All analyses were run using SPSS 29.0 (IBM Corporation).

3. Results

Mussel movement start (Fig. 1) was delayed in the direct presence of crayfish (Table 2A). Moreover, *U. tumidus* started its activity later than other species.

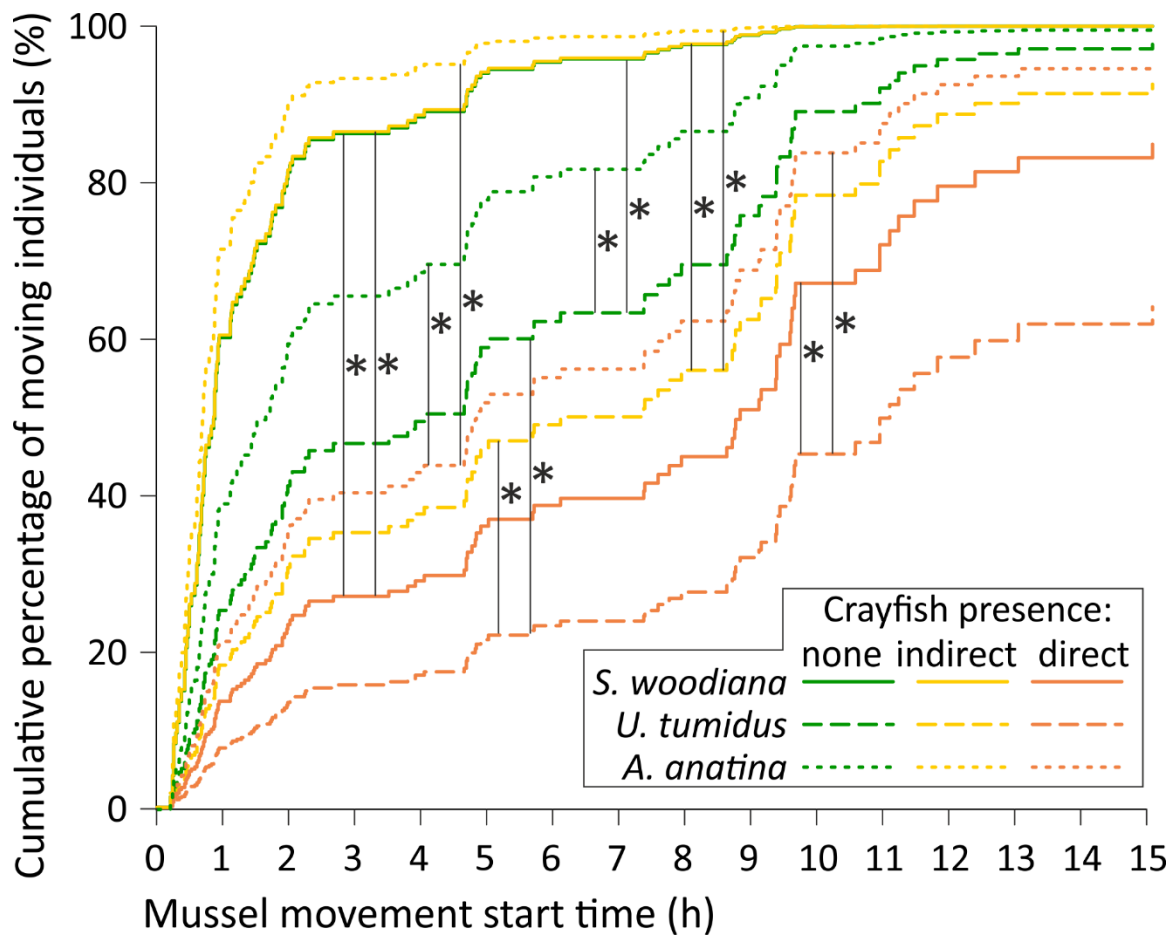


Figure 1. Cox proportional hazards regression models of mussel movement start time depending on the presence of crayfish (direct: crayfish walking freely around the mussel, indirect: crayfish behind the mesh barrier, none: no crayfish – control). Asterisks indicate significant differences between the species and experimental treatments.

Without crayfish, *U. tumidus* was significantly more burrowed than the other species (median burrowing level of 75%, compared to 43 and 24% shown by *A. anatina* and *S. woodiana*, respectively) (Fig. 2A). Burrowing of *U. tumidus* was significantly reduced in both crayfish treatments, reaching a median of 50% in the direct crayfish presence (Table 2B). The other species did show a similar tendency (Fig. 2A), but here, the differences among the treatments were non-significant.

Time spent on burrowing (Fig. 2B) was similar for all the species (always below 10% of the total experimental time) and depended on treatment (Table 2C): in direct confrontation with crayfish, it was longer than when only chemical cues were present (indirect presence).

In control conditions, *U. tumidus* did not move at all (Fig. 2C), which differed significantly from the distance covered by *S. woodiana* (median: 21 cm) and *A. anatina* (4 cm) (Table 2D). No significant effects of crayfish presence were observed (Fig. 2C).

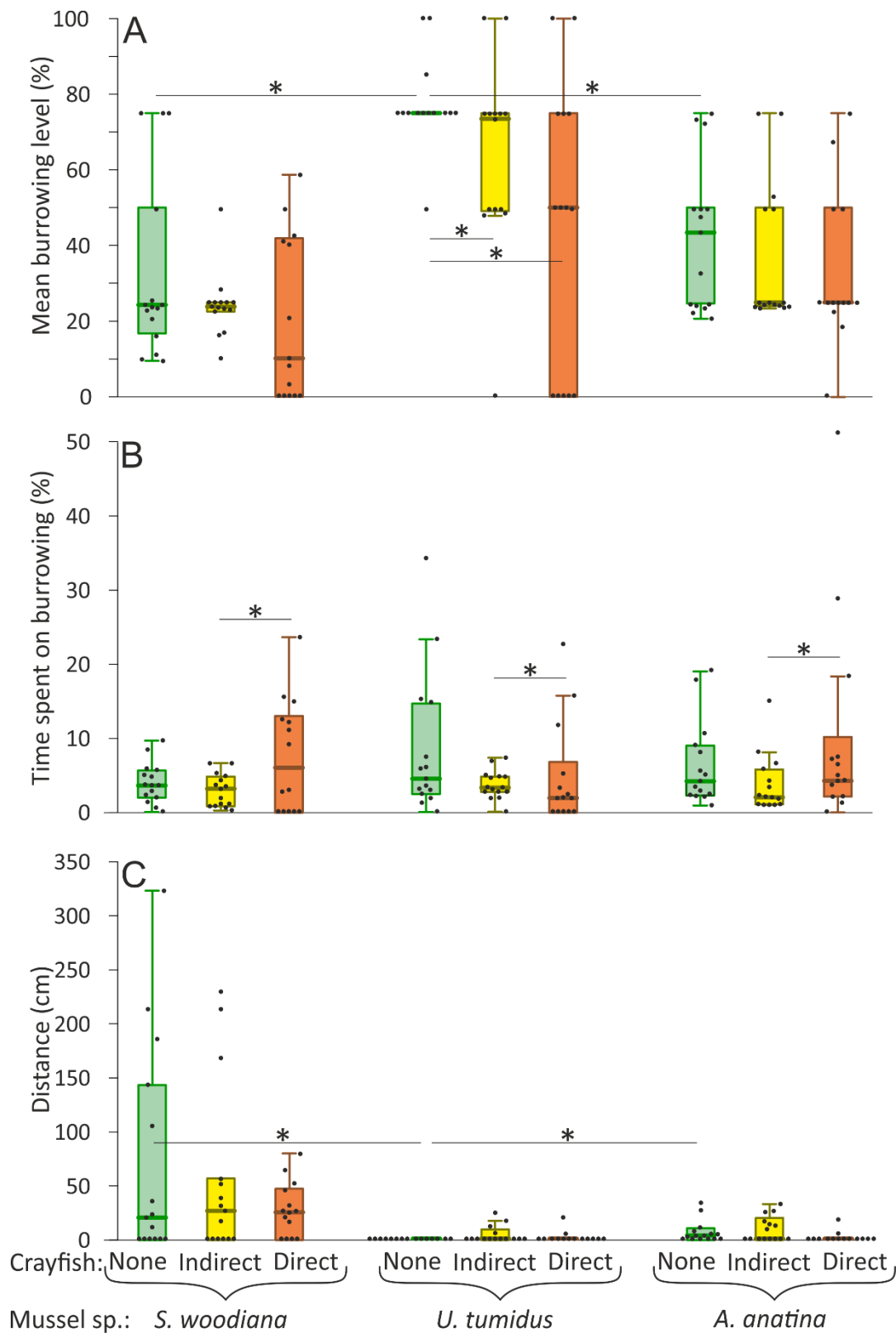


Figure 2. Mussel behavior in the presence of crayfish (direct: crayfish walking freely around the mussel, indirect: crayfish behind the mesh barrier, none: no crayfish – control): (A) mean burrowing level; (B) time spent on burrowing (percentage of the total experimental time); (C)

distance travelled. Horizontal lines, bars, whiskers, and dots indicate medians, 1-3 quartiles, 1.5*interquartile distance, and raw data points, respectively. Asterisks indicate significant differences between the species and experimental treatments.

Unio tumidus were never dug out from the substratum by crayfish, in contrast to *S. woodiana* (9 individuals handled by crayfish) and *A. anatina* (6 individuals). Consequently, time spent on the sediment surface by *S. woodiana* and *A. anatina* dug out by crayfish was significantly longer than in the case of *U. tumidus* (Fig. 3A, Table 2E). *Sinanodonta woodiana* was attacked by crayfish significantly more often (about 13 times) than the other species (less than 5 times) (Fig. 3B, Table 2F). Time spent by crayfish on mussel handling did not depend on mussel species (always below 5% of the total experimental time on average) (Fig. 3C, Table 2G). Inactive mussels, exposed on the surface for the total experimental time, tended to be attacked more often and handled for a longer time than other, burrowed individuals (Fig. 3B-C).

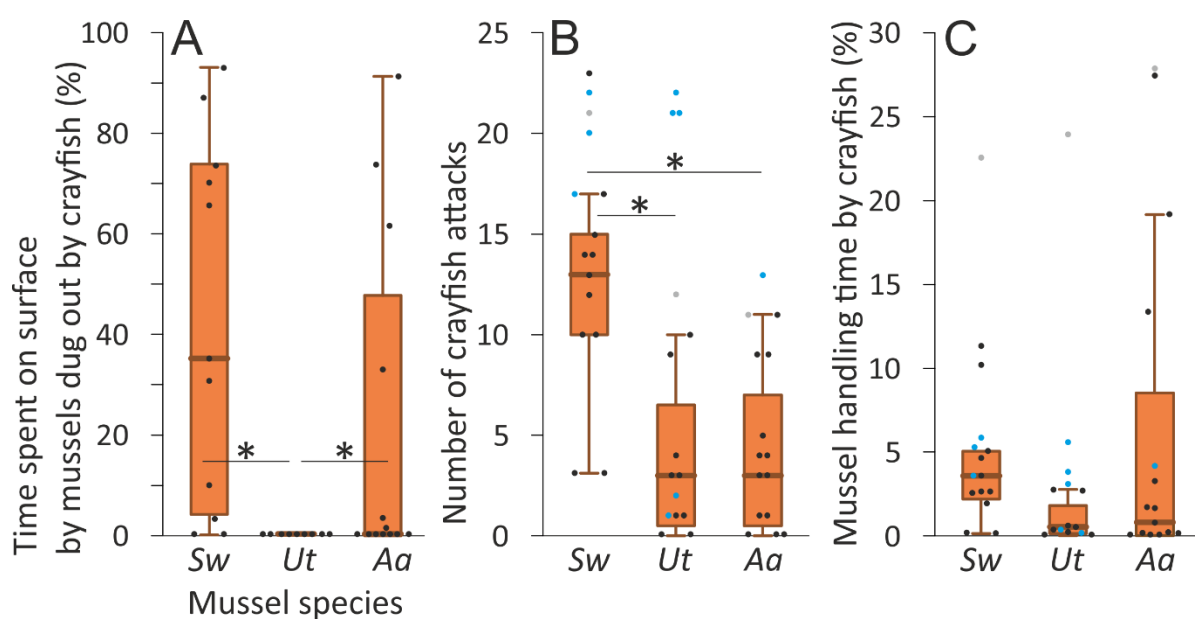


Figure 3. Direct interactions between crayfish and mussels (the treatment with direct crayfish presence only): (A) time spent on surface by mussels dug out by crayfish; (B) number of crayfish attacks on mussels; (C) mussel handling times by crayfish. Horizontal lines, bars, whiskers, and dots indicate medians, 1-3 quartiles, 1.5*interquartile distance, and raw data points, respectively. Blue and grey dots indicate mussels that did not move at all and remained on the surface during the entire exposure and those that died shortly after the beginning of the exposure, respectively. They are presented in the figures, but were excluded from the data analyses. Asterisks indicate significant differences between the species.

Table 2. Statistical analyses of behavioral variables measured in the study

Variable	Effect	df	Test statistic	P	Pairwise comparisons		
A. Mussel movement start ¹	Species	2	Wald $\chi^2=24.4$	<0.001*	Sw	Ut	
					Ut	<0.001*	
					Aa	0.989	<0.001*
	Treatment	2	Wald $\chi^2=37.6$	<0.001*	NC	IC	
					IC	0.370	
					DC	<0.001*	<0.001*
B. Mean mussel burrowing ²	Species (control treatments)	2	$\chi^2=23.1$	<0.001*	Sw	Ut	
					Ut	<0.001*	
					Aa	0.163	<0.001*
	Treatment (each species separately)	Sw	$\chi^2=3.4$	0.185			
		Ut	$\chi^2=8.2$	0.017*	NC	IC	
					IC	0.025*	
					DC	0.009*	0.316
		Aa	$\chi^2=0.9$	0.647			
C. Time spent by mussels on burrowing ³	Species	2, 127	F=0.8	0.461	NC	IC	
	Treatment	2, 127	F=3.5	0.033*	IC	0.459	
					DC	0.066	0.012*
D. Mussel locomotion distance ²	Species (control treatments)	2	$\chi^2=16.4$	<0.001*	Sw	Ut	
					Ut	<0.001*	
					Aa	0.151	<0.001*
		Sw	$\chi^2<0.1$	0.983			
		Ut	$\chi^2=4.5$	0.106			

	Treatment (each species separately)	Aa	2	$\chi^2=5.4$	0.068		
E. Time on surface by mussels dug out by crayfish ²	Species		2	$\chi^2=12.2$	0.002*	Sw	Ut
						Ut	0.001*
						Aa	0.079
							0.022*
F. Number of crayfish attacks ⁴	Species		2	Wald $\chi^2=72.3$	<0.001*	Sw	Ut
						Ut	<0.001*
						Aa	<0.001*
							0.626
G. Mussel handling time by crayfish ³	Species		2, 30	F=1.5	0.242		

278 ¹Cox proportional hazards regression

279 ²Kruskal-Wallis test

280 ³General Linear Model

281 ⁴Generalized Linear Model (Poisson distribution)

282 Sw – *Sinanodonta woodiana*, Ut – *Unio tumidus*, Aa – *Anodonta anatina*

283 NC – no crayfish (control), IC – indirect crayfish presence, DC – direct crayfish presence

284 Asterisks indicate significant effects and differences

4. Discussion

Only *U. tumidus* responded significantly to the crayfish presence by reduced burrowing. We expected the opposite response, as burrowing is a natural defense mechanism of mussels (Saloom and Duncan 2005). Remaining on the substratum surface, mussels are exposed to detection by predators and their attacks (Lymbery et al. 2021). Our result is also in contradiction with that by Griffiths and Richardson (2005), who found that the Baltic clam *Limecola balthica* (Linnaeus 1758) was induced to burrow deeper by chemical signals of the crab *Carcinus maenas* (Linnaeus 1758). On the other hand, deep burrowing may limit respiration and food intake, which reduce mussel growth and contribute to increased mortality (de Goeij and Luttikhuisen 1998). Reduced burrowing shown by *U. tumidus* in our study can be explained either as a maladaptive response to an unknown predator, co-existing with mussels for a relatively short time, or as an attempt to reduce chances of detection by a predator through a total reduction of activity (Czarneński et al. 2010; Antoń et al. 2018). As crayfish turned out unable to remove even those relatively shallowly burrowed individuals of *U. tumidus* from the sediments, and attacked them less often than *S. woodiana*, the latter option seems more likely. It should be noted that *U. tumidus* burrows in the sediments more deeply and more often than the other species studied (Poznańska-Kakareko et al. 2021), which was also shown by the current study, even for the individuals responding to crayfish presence. Thus, it is likely that *U. tumidus* reduced its burrowing activity in the presence of a potential predator to avoid detection, while even this reduced burrowing acted as a relatively efficient defense against crayfish attacks. Efficiency of activity reduction as an anti-crayfish measure is also supported by the fact that the most attacked species in our study, *S. woodiana*, was also the most active one, moving the longest distances and burrowing shallowly.

Adaptive character of the responses of *U. tumidus* to crayfish has also been supported by its ability to detect and respond to crayfish scent, in accordance with our hypothesis 2.

Mussels are able to sense the presence of predators in the environment and react accordingly (Smee and Weissburg 2006; Kobak and Kakareko 2009; Dzierżyńska-Białończyk et al. 2019). Moreover, the ability to detect a predator and respond accordingly to its presence can evolve quickly, within several generations, facilitating survival of invasive species in novel environment (Jermacz et al. 2017), but also allowing native prey to thrive in the face of novel predators, like in system consisting of *U. tumidus* and *P. leniusculus*. An example is the blue mussel *Mytilus edulis* (Linnaeus 1758), which was found to be able to respond to chemical cues of the crab *Hemigrapsus sanguineus* (De Haan 1853) with shell thickening after only 15 years of their co-existence (Freeman and Byers 2006).

Our third hypothesis was partly supported, because, in contrast to the other species, *U. tumidus* was not excavated by crayfish from the sediments, and it was less often attacked than *S. woodiana*. *Unio tumidus* was also found to be avoided by *P. leniusculus* in favor of other bivalves, such as *Dreissena polymorpha* (Pallas 1771), by Sanders and Mills (2022). *Unio tumidus* has a thick shell (Czerniejewski et al. 2021), the crushing of which can be energetically expensive for predators. Similarly, the crab *Portunus trituberculatus* (Miers 1876) preferred to feed on clam species with softer shells: *Ruditapes philippinarum* (Adams and Reeve 1850) vs. *Scapharca subcrenata* (Lischke 1896) or *Meretrix meretrix* (Linnaeus 1758) (Sun et al. 2017). Thus, several non-exclusive mechanisms can be responsible for relative safety of *U. tumidus* against crayfish predation: (i) active avoidance by predators due to its hard shell, (ii) more difficult detection due to fast and deep burrowing, (iii) difficulty in excavating *U. tumidus* from the sediments due to its shell shape (more rounded in cross-section than *A. anatina* and missing the flat wing on the dorsal edge, used as a handle for the crayfish attacking *A. anatina* and *S. woodiana*), and (iv) *U. tumidus* ability to respond (reduce activity) in the presence of predators.

The other species did not respond to the presence of crayfish. Increased burrowing time shown by individuals exposed to the direct crayfish presence was probably forced by crayfish

activity, digging mussels out from the sediments and making them repeatedly burrow again. We expected that, due to its relatively shallow burrowing, low locomotion, and thin shell, *A. anatina* will be attractive prey for crayfish, as was the case with raccoons *Procyon lotor* (Linnaeus 1758), which, feeding on mussels, included mainly the thin-shelled *Anodonta cygnea* (Linnaeus 1758) and *A. anatina* in its diet (Ważna et al. 2025). Meira et al. (2019) showed that *A. anatina* was attacked by two crayfish species, *P. leniusculus* and *Procambarus clarkii* (Girard 1852), more often than *Potomida littoralis* (Cuvier 1798), *Unio delphinus* (Splengler 1793), and *Corbicula fluminea* (O. F. Müller 1774). However, contrary to the second part of our third hypothesis, the species most frequently attacked was *S. woodiana*, which was the least burrowed and most mobile, and thus the most vulnerable to attacks by crayfish. Individuals of *S. woodiana* were larger and heavier than other mussels used in our study (following natural differences between the species in the field). Killing such a large prey will provide the predator with the richest food source and is the most profitable energetically (Prejs et al. 1990; Wong and Barbeau 2005). Opposite results regarding the preferences of *P. leniusculus* were obtained by Dobler and Geist (2021), who showed that the most frequently attacked and most damaged mussel was the thick-shelled *U. pictorum* (Linnaeus 1758), rather than *S. woodiana* and *A. cygnea*, with clearly thinner shells (own observations). This discrepancy can point to the effect of size in our study (Dobler and Geist, 2021, used mussels of equal sizes). Moreover, in our study, we used sandy substratum, which is preferred by mussels and facilitates their burrowing (Poznańska-Kakareko et al. 2021). On the other hand, Dobler and Geist (2021) used more coarse gravel, which may impair unionid burrowing and makes it easier for crayfish to extract mussels from such loose material.

Anyway, according to our results, the invasive *S. woodiana* seems to be the most vulnerable to crayfish predation. This high vulnerability of *S. woodiana* to crayfish predation can be good news for native unionid mussels, as the ongoing invasion of *P. leniusculus* (Kouba

et al. 2014) could potentially reduce *S. woodiana* populations, if crayfish attacked them more often and more willingly than the native ones. This might reduce the pressure of the invasive mussel, which is currently outcompeting native Unionidae in Europe (Douda and Čadková 2018; Yermoshyna and Pavliuchenko 2021; Geist et al. 2025). This implies that the alien predatory species exerts differential influence on competing prey species in a novel area. Such a relationship was demonstrated by Meira et al. (2019), where *P. leniusculus* and *P. clarkii* preyed more readily on native unionid mussels than on the thick-shelled *Corbicula* sp., which in this case gives the invasive species an advantage by reducing the abundance of its native competitors. Another example is the saltwater Asian date mussel *Arcuatula senhousia* (Benson 1842), invasive in North America, whose presence attracted native predators, causing apparent competition by increasing predation on the native mussels *Chione undatella* (G. B. Sowerby I 1835) and *Laevicardium substriatum* (Conrad 1837), preferred by these predators (Castorani and Hovel 2015). In aquatic ecosystems, native and invasive species can coexist in a stable equilibrium for extended periods, until the increased pressure on the one side will contribute to the other's success. This was the case with the Nile perch *Lates niloticus* (Linnaeus 1758), invasive in African Lake Victoria, and native cichlids, which existed in equilibrium for 25 years until adult cichlids began to be harvested by humans. This resulted in a decline in the population of large cichlids that previously consumed small Nile perch, contributing to a rapid increase in the invasive species and accelerated decline of the native cichlids (Goudswaard et al. 2008; van de Wolfshaar et al. 2014).

Yet another mechanism of indirect crayfish impact on unionid populations may be predation on dreissenid mussels fouling the shells of larger bivalves (Zu Ermgassen and Aldridge 2011). *Sinanodonta woodiana* was found to be protected better than native unionids against fouling: in contrast to the native species, the behavior of fouled *S. woodiana* was not impaired, and this bivalve was able to detect and to some extent respond to the risk of fouling

in the environment (Dzierżyńska-Białończyk et al. 2018; Wiśniewski et al. 2024b). Therefore, crayfish predation on dreissenids can reduce the competitive advantage of *S. woodiana*. This shows that the complex multispecies interplay among various invaders may affect relationships between unionid species in the environment, shifting competitive advantage towards the invasive or native species.

Usually, crayfish do not easily kill large mussels, but by mechanically damaging them and forcing them to hide their siphons frequently, they can contribute to their long-term weakening resulting from limited nutrition (Naddafi et al. 2007). The presence and frequent attacks of a predator can negatively affect prey reproduction, as shown by Ehlers et al. (2018) analyzing the impact of the predatory snail *Nucella lapillus* (Linnaeus 1758) on the mussels *M. edulis* and *M. trossulus* (Gould 1850). In our study, we did not check the long-term mussel condition, but a slower growth, increased mortality and/or impaired reproduction might be expected as a long-term consequence of crayfish encounters. This may affect the condition of entire mussel populations, which may be even more important in the context of their extinction on a global scale than direct killing and consumption (Nakaoka 2000).

To better understand interactions between invasive crayfish and native unionids, as well as how *S. woodiana* is involved in this interaction, it would be necessary to conduct multi-choice experiments, in which predators would have simultaneous access to several mussel species. *Sinanodonta woodiana* was attacked most frequently in our experiment, potentially indicating a dilution effect. However, in our experiment, the crayfish had no alternative prey. Its addition to the experimental design would allow us to obtain precise information about crayfish food preferences and thus the dilution effect or apparent competition. Moreover, it would be useful to check crayfish predation on juvenile mussels and their responses to crayfish presence. They are more vulnerable to predation due to their smaller size and weaker shell, but,

on the other hand, burrow faster and deeper (Annie et al. 2013), which may give them an advantage over adults.

Statements and Declarations

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Author contributions

KW: Conceptualisation, Methodology, Resources, Investigation, Formal analysis, Visualisation, Writing - Original draft, Writing - Review and Editing, funding acquisition. JK: Conceptualization, Methodology, Validation, Formal analysis, Data Curation, Visualization, Writing - Review and Editing. DS: Methodology, Resources, Investigation, Writing - Review and Editing. MA: Methodology, Resources, Investigation, Writing - Review and Editing. TK: Resources, Investigation, Writing - Review and Editing. MPK: Conceptualization,

Methodology, Validation, Visualization, Formal analysis, Supervision, Project administration,
Writing - Review and Editing. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no conflict of interest.

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Supplementary material

Invasive signal crayfish differently affects invasive and native unionid mussels

Table of contents

Table S1. Full list of measured parameters related to the behaviour of mussels and crayfish along with their explanation and units

Table S1. Full list of measured parameters related to the behaviour of mussels and crayfish along with their explanation and units

Var	Measured value	Calculation ¹	Final variable	Remarks	Analysis
Mussel behaviour					
1	Mussel movement start time (min)		Latency to move (min)	Time before the mussel started to burrow or relocate	Cox proportional hazards regression
2	Times t_i (min) spent by mussels at various burrowing levels $i = 0-4$	$\sum_{i=1}^4 (i * t_i) / 4(T_{tot} - Var1 - Var5)$	Mean burrowing level (%)	T_{tot} reduced by: (i) Var1 to exclude time before mussels started to move; (ii) Var5 to analyse true mussel activity (not position changes due to crayfish actions) If $T_{tot} = Var1 + Var5$, then 0 assigned (for totally inactive mussels)	Kruskal-Wallis test
3	Time spent by mussels on burrowing (min)	Time spent on burrowing / $(T_{tot} - Var1 - Var5)$	Time spent on burrowing (%)		General Linear Model (GLM)
4	Mussel locomotion distance (cm)		Distance (cm)		Kruskal-Wallis test
Interactions between crayfish and mussels					
5	Time spent on the surface by mussels dug out by crayfish (min)	Time spent on the surface by mussels dug out by crayfish / $(T_{tot} - Var1)$	Time spent on the surface by mussels dug out by crayfish (%)	T_{tot} reduced by Var1 to exclude time before mussels started to move (crayfish had no chance to dig mussels out) If $T_{tot} = Var1$ then the data point excluded (totally inactive mussels)	Kruskal-Wallis test
6	Number of crayfish attacks on the mussel		Number of attacks	If $T_{tot} = Var1$ then the data point excluded (totally inactive mussels)	Generalized LM (Poisson distr.)
7	Mussel handling time by crayfish (min)	Handling time / T_{tot}	Handling time (%)	If $T_{tot} = Var1$ then the data point excluded (totally inactive mussels)	GLM

¹ T_{tot} –total experimental time (24 h = 1440 min)

Var – variable

Podsumowanie osiągnięć naukowych

Publikacje niewłączone w rozprawę doktorską

- Kobak, J., **Wiśniewski, K.**, Bomba, A., Szarmach, D., Balogh, C., Jermacz, Ł., Poznańska-Kakareko, M. (w recenzji w czasopiśmie Integrative Zoology). If it's not one thing, it's another: Survival and behavioural responses of dreissenid mussels to drought and siltation. (Q1 Zoology; IF 2024 2-letni: 3,7)
- Szarmach, D., Lichocka, K., Kobak, J., Bomba, A., Krzeszowiak, A., Terebiński, S., **Wiśniewski, K.**, Poznańska-Kakareko, M. (w recenzji w czasopiśmie NeoBiota). Clams running dry: Hydrological drought and survival strategies of European native *Sphaerium rivicola* and Asian non-native *Corbicula* complex. (Q1 Biodiversity Conservation; IF 2024 2-letni: 3,0)
- Stanicka, A., Kobak, J., Hildebrand, J., Cichy, A., **Wiśniewski, K.**, Szarmach, D., Lesiak, K., Urbaniak, J., Augustyniak, M., Dlouhy, Z., Graczyk, S., Grzeczka, A., Kowaleska, Z., Lewalska, M., Lichocka, K., Terebiński, S., Wiśniewska, A., Żbikowska, Ż, Poznańska-Kakareko M. (2025). Many Evils Are Worse Than One: An Interplay Between Invasive Biofoulers and Parasites Helps Predict the Future of an Endangered Freshwater Fingernail Clam. *Freshwater Biology*, 70(11), e70125. <https://doi.org/10.1111/fwb.70125> (Q1 Marine & Freshwater Biology; IF 2024 2-letni: 2,7)
- Szarmach, D., **Wiśniewski, K.**, Kobak, J., Lichocka, K., Kakareko, T., Sousa, R., & Poznańska-Kakareko, M. (2024). Impact of habitat engineering by invasive *Corbicula* clams on native European unionid mussels. *Science of The Total Environment*, 948, 174764. <https://doi.org/10.1016/j.scitotenv.2024.174764> (Q1 Environmental Sciences; IF 2024 2-letni: 8,0)
- Szarmach, D., **Wiśniewski, K.**, Kobak, J., Kakareko, T., Labecka, A. M., Sousa, R., & Poznańska-Kakareko, M. (2023). Differences in substratum preferences and behaviour within the invasive *Corbicula* species complex. *Freshwater biology*, 68(9), 1489-1502. <https://doi.org/10.1111/fwb.14142> (Q1 Marine & Freshwater Biology; IF 2023 2-letni: 2,8)
- Poznańska-Kakareko, M., **Wiśniewski, K.**, Szarmach, D., Witkowska, A., Kakareko, T., & Kobak, J. (2021). Importance of substratum quality for potential competitive niche overlap between native and invasive unionid mussels in Europe. *Science of The Total Environment*,

799, 149345. <https://doi.org/10.1016/j.scitotenv.2021.149345> (Q1 Environmental Sciences; IF 2021 2-letni: 10,75)

- **Wiśniewski, K.**, Szarmach, D., & Poznańska-Kakareko, M. (2020). The role of abiotic and biotic factors in interspecific competition of Polish crayfish – comprehensive literature review. *Oceanological and Hydrobiological Studies*, 49(4), 428-441. <https://doi.org/10.1515/ohs-2020-0038> (Q4 Oceanography; IF 2020 2-letni: 0,82)

Udział w konferencjach zagranicznych

- Współautor referatu: 1
- Autor plakatu: 1
- Współautor plakatu: 1

Udział w konferencjach krajowych

- Autor referatu: 7
- Współautor referatu: 9
- Współautor plakatu: 1

Granty

- 2023 – Grant zespołowy (kierownik) pt.: "Behavioural response of selected Unionidae bivalves to the direct and indirect presence of invasive crayfish-predators" w VII edycji konkursu Grants4NCUStudents organizowanego w ramach programu Inicjatywa Doskonałości – Uczelnia Badawcza (IDUB) UMK w Toruniu.
- 2022-2023 – Grant zespołowy (członek zespołu) pt.: „Wpływ inwazyjnych małży z rodzaju *Corbicula* na rodzime europejskie małże z rodziny Unionidae” w ramach programu „Studenckie koła naukowe tworzą innowacje” Ministerstwa Nauki i Szkolnictwa Wyższego.

Staże, szkolenia i kursy

- 2023 – miesięczny staż w Balaton Limnological Research Institute (Tihany, Węgry) pod opieką dr Csilla Balogh i zrealizowanie projektu: „The ability of the invasive mussels *Dreissena polymorpha* and *D. r. bugensis* to burrow from various bottom sediments”.
- 2022 – tygodniowy staż w Instytucie Nauk o Środowisku Uniwersytetu Jagiellońskiego pod opieką dr Anny Marii Łabęckiej dotyczący morfologicznej identyfikacji inwazyjnych małży z rodzaju *Corbicula*.
- 2022 – dwutygodniowy staż w Balaton Limnological Research Institute (Tihany, Węgry) pod opieką dr Csilla Balogh i zrealizowanie trzech projektów dotyczących preferencji małży *Dreissena* spp. i wpływu ich obrotu na rodzime i inwazyjne małże Unionidae oraz małże *Corbicula*.
- 2019 – półroczne praktyki w zakresie opieki nad wodnymi zwierzętami laboratoryjnymi (ryby) w Zwierzętarni Wydziału Nauk Biologicznych i Weterynaryjnych Uniwersytetu Mikołaja Kopernika w Toruniu.
- 2019 – Szkolenie dla osób uczestniczących i wykonujących procedury z wykorzystaniem zwierząt oraz osób sprawujących opiekę nad zwierzętami doświadczalnymi.
- 2019 – Kurs barkodingu DNA. Przygotowanie materiału badawczego w laboratorium (izolacja, amplifikacja DNA) i analiza wyników.
- 2019 – Kurs nurkowania i środowiskowych eksploracji podwodnych (KDP/CMAS P1).
- 2018 – Kurs „Ichtiologia Morza” zorganizowany przez Stację Morską im. Profesora Krzysztofa Skóry w Helu Instytutu Oceanografii Uniwersytetu Gdańskiego.

Nagrody i wyróżnienia

- Zespołowa nagroda Rektora UMK I stopnia za osiągnięcia naukowe w roku 2024.
- Pierwsze miejsce (ex aequo) w konkursie na najlepsze wystąpienie dla Młodych Malakologów na XXXIX Krajowym Seminarium Malakologicznym w Krobi w 2025 roku.
- Drugie miejsce w konkursie na najlepsze wystąpienie podczas XXVIII Ogólnopolskich Warsztatów Bentologicznych w Sycowej Hucie w 2024 roku.

- Trzecie miejsce w konkursie im. Prof. Mariana Gieyszтора organizowanym przez Polskie Towarzystwo Hydrobiologiczne na najlepszą badawczą pracę magisterską w latach 2022-2023.
- Drugie miejsce w konkursie na najlepszą pracę magisterską na Wydziale Nauk Biologicznych i Weterynaryjnych UMK w Toruniu w roku akademickim 2020/2021.
- Trzecie miejsce w konkursie na najlepszą pracę licencjacką na Wydziale Biologii i Ochrony Środowiska UMK w Toruniu w roku akademickim 2018/2019.
- Czterokrotne Stypendium Rektora dla najlepszych studentów w latach akademickich: 2017/2018, 2018/2019, 2019/2020, 2020/2021.

Pozostała aktywność

- Nauczyciel biologii w Technikum Menedżerskim w Toruniu (Szkoły Sokrates) od 2024 roku.
- Członek Polskiego Towarzystwa Hydrobiologicznego i Stowarzyszenia Malakologów Polskich od 2022 roku.
- Prezes Zarządu Studenckiego Koła Naukowego Biologów w latach 2021-2023 roku.
- Członek Sekcji Hydrobiologii i Ichtiologii Studenckiego Koła Naukowego Biologów od 2016 roku.