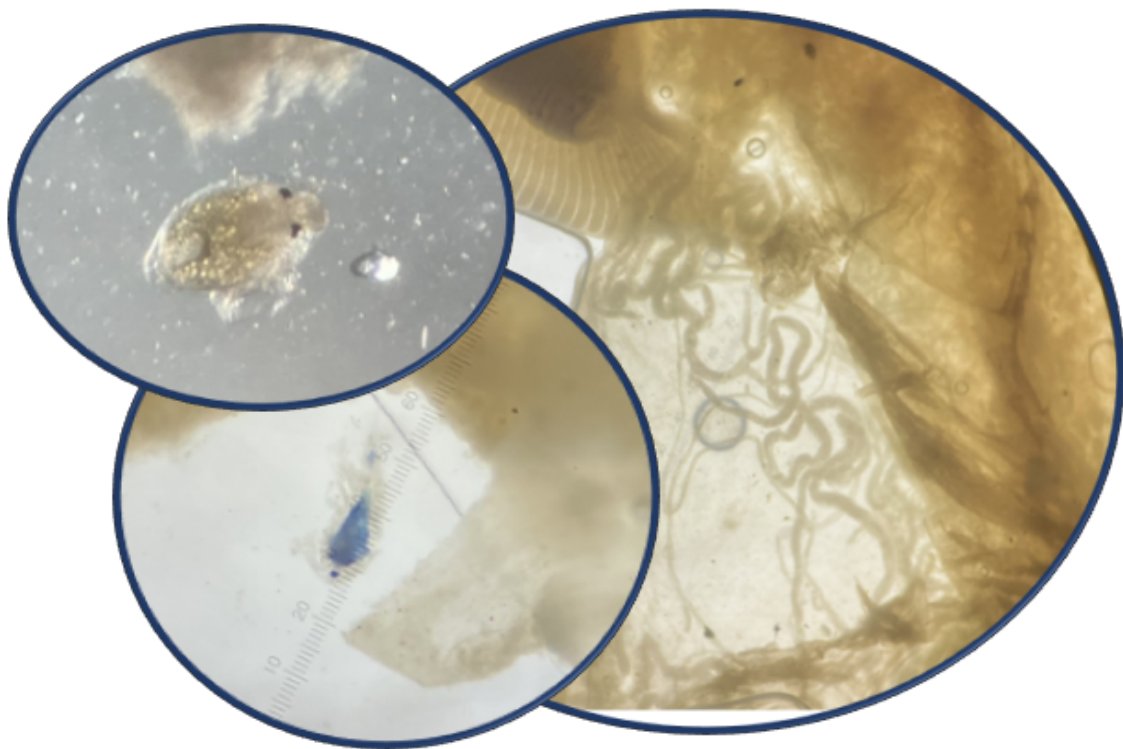


JYU DISSERTATIONS 799

Binglin Deng

Enemy Release Hypothesis: Parasitism in Invasive and Native Freshwater Bivalves



UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
AND SCIENCE

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ABSTRACT

Deng, Binglin

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Yhteenveto: Enemy Release -hypoteesi: loisinta vierassimpukoissa ja alkuperäis-simpukoissa

Diss.

The invasion of exotic bivalves can cause large changes in freshwater ecosystems and threaten native freshwater mussels (Unionida). The Enemy Release Hypothesis (ERH) proposes that (i) invasive species may lose enemies during the invasion process, and (ii) the invaders enjoy a competitive advantage caused by a lower enemy pressure than in the native species, contributing to the success of biological invasions. Freshwater mussels are infected by diverse parasite fauna, *i.e.* protozoans, trematodes, nematodes, watermites, associated with varying degrees of harm to the host. Thus, ERH was tested by using the invasive freshwater bivalves Chinese pond mussel *Sinanodonta woodiana*, Asian clam *Corbicula fluminea* and zebra mussel *Dreissena polymorpha*. First, parasite pressure, measured as parasite taxon richness and sum of prevalences of infection by different parasite taxa, was compared between native and invasive freshwater bivalves living in sympatry in two separate field studies covering a total of 11 European waterbodies inhabiting a total of 6 native Unionidae species. 16 parasite taxa were found, and in the native bivalves the mean site-specific parasite taxon richness was 2.3–3.4 times and the mean sum of prevalences of infection of different parasites was 2.4–2.6 times that of those in the invasive bivalves. Second, the parasite pressure in the invasive bivalves *S. woodiana* and *C. fluminea* was compared between populations in the original range (China, 5 waterbodies) and the invaded range (Europe, 11 waterbodies). For *S. woodiana*, the average site-specific parasite taxon richness in China was 2.1 times and the sum of prevalences of infection was 3.0 times of those in Europe. For *C. fluminea*, the average site-specific parasite taxon richness was 1.3 and the sum of prevalences of infection was 13.8 in China, while all the studied European *C. fluminea* populations were free of parasites. These results indicate a reduction in the parasite pressure among invasive species, and thus support ERH.

Keywords: Biological invasions; Enemy Release Hypothesis; freshwater bivalve; parasite; Unionida.

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TIIVISTELMÄ

Deng, Binglin

Enemy Release -hypoteesi: Loisinta vierassimpukoissa ja alkuperäissimpukoissa
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Yhteenvedo: Enemy Release -hypoteesi: loisinta vierassimpukoissa ja
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Diss.

Vieraslajit muuttavat ekosysteemejä ja uhkaavat mm. vesistöjen alkuperäisiä simpukkalajeja. *Enemy Release* -hypoteesin (ERH) mukaan (i) vieraslajeille haitallisten lajien määrä vähenee invaasioprosessin aikana ja (ii) vieraslajit saavat kilpailuetua, koska niihin kohdistuu vähäisempää haittaa muiden lajien taholta kuin alkuperäislajeihin. Järvi- ja jokisimpukoilla on laaja kirjo erilaisia loisia: alkueläimiä, imumatoja, sukkulamatoja, harvasukamatoja, vesipunkkeja, sulkasääskiä, vesiperhosia ja kaloja – jotka kaikki ovat ainakin jossakin määrin haitallisia simpukoille. Tästä syystä tässä väitöskirjassa testattiin ERH:n toimivuutta haitallisilla vierassimpukkalajeilla *Sinanodonta woodiana*, *Corbicula fluminea* ja *Dreissena polymorpha*. Ensimmäiseksi verrattiin vierassimpukoiden ja alkuperäissimpukoiden loispainetta 11:ssä sellaisessa eurooppalaisissa vesistössä, jossa molemmat simpukkatyypit esiintyvät rinnakkain. Kaksiosaisessa tutkimuksessa tavattiin edellä mainittujen vieraslajien lisäksi kaikkiaan kuusi paikallista alkuperäissimpukkalajia ja 16 loistaksonia. Keskimääräinen paikkakohtainen loistaksonien määrä oli 2,3–3,4-kertainen ja keskimääräinen loisten lajikohtaisten infektoitujen yksilöiden osuuksien (prevalenssi) summa 2,4–2,6-kertainen alkuperäissimpukoilla verrattuna vierassimpukoihin. Toiseksi verrattiin vierassimpukoiden loispainetta niiden alkuperäisellä elinalueella Kiinassa (5 vesistöä) niiden uusiin elinalueisiin Euroopassa (11 vesistöä). Kiinassa *S. woodiana* -lajin keskimääräinen loislajimäärä oli 2,1-kertainen ja keskimääräinen loislajien prevalenssien summa 3,0-kertainen Eurooppaan verrattuna. *C. fluminea* -vierassimpukan keskimääräinen loislajimäärä oli kiinalaisissa populaatioissa 1,3 ja prevalenssien summa 13,8, mutta eurooppalaisista populaatioista ei tavattu yhtään loista. Tulokset viittaavat loispaineen vähenemiseen invaasioprosessin seurauksena ja vierassimpukoiden alhaisempaan loispaineeseen samalla paikalla eläviin alkuperäissimpukoihin verrattuna. Väitöskirjan tulokset tukevat *Enemy Release* -hypoteesia.

Avainsanat: Biologinen invaasio; *Enemy Release* -hypoteesi; loinen; jokisimpukka; järvisimpukka; vieraslaji.

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CONTENTS

ABSTRACT

TIIVISTELMÄ

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

1	INTRODUCTION	9
1.1	Host-parasite association and parasites in ecosystems	9
1.2	Role of freshwater mussels in aquatic ecosystems	10
1.3	Conservation status and threats of freshwater mussels.....	11
1.3.1	Success of certain invasive freshwater bivalves	12
1.4	Bio-invasion, and the Enemy Release Hypothesis.....	13
1.5	Parasitism in freshwater bivalves.....	17
1.6	Aim of the study	19
2	MATERIALS AND METHODS	20
2.1	Terminology in parasitology.....	20
2.2	Field Work	20
2.2.1	Sampling in Europe	20
2.2.2	Sampling in China.....	21
2.3	Laboratory work	23
2.4	Statistical methods and data analysis	23
3	RESULTS AND DISCUSSION	25
3.1	Parasite pressure in sympatric native and invasive freshwater bivalves in European water bodies	25
3.2	“Loss” (and “gain”) of parasites in invasive <i>Sinanodonta woodiana</i> and <i>Corbicula fluminea</i>	27
4	CONCLUSIONS.....	32
	ACKNOWLEDGEMENTS.....	34
	YHTEENVETO (RÉSUMÉ IN FINNISH).....	35
	REFERENCES.....	37

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-III.

In study I, I participated in planning, laboratory work, data analysis, writing and manuscript revision. In II, I participated in planning, laboratory work and data analysis. I wrote the first version of the manuscript, was responsible for manuscript revision and correspondence; in III, I was responsible for planning, I organized sampling, performed laboratory work, data analysis and wrote the first version of the manuscript.

- I Taskinen J., Urbańska M., Ercoli F., Andrzejewski W., Özgo M., Deng B., Choo J.M. & Riccardi N. 2021. Parasites in sympatric populations of native and invasive freshwater bivalves. *Hydrobiologia* 848: 3167–3178. <https://doi.org/10.1007/s10750-020-04284-0>
- II Deng B., Riccardi N., Urbańska M., Marjomäki T.J., Andrzejewski W. & Taskinen J. 2024. Lower parasite pressure in invasive freshwater bivalves than in sympatric native Unionidae mussels in southern European lakes. Submitted manuscript.
- III Deng B., Riccardi N., Pin N., Urbańska M., Marjomäki T.J., Andrzejewski W., Nakamura K. & Taskinen J. 2024. Enemy release: Loss of parasites as a result of invasion in freshwater bivalves *Sinanodonta woodiana* and *Corbicula fluminea*. Manuscript.

1 INTRODUCTION

1.1 Host-parasite association and parasites in ecosystems

The parasite is an organism that lives in (or on) another organism, a host, receiving at least some resources from – and causing at least some harm to – the host (*e.g.* Schmid-Hempel 2021). Parasites are ubiquitous parts of practically all ecosystems, they are present basically everywhere on the planet. Parasitism is probably the most common lifestyle, with the majority of living organisms being parasites. Thus, there are more parasite species than host species living on our planet (Windsor 1998, Lafferty *et al.* 2006, Schmid-Hempel 2021). Despite being typically smaller than host, parasites can occupy up to 20 % of the biomass of an ecosystem, and even exceed the biomass of top predators (Kuris *et al.* 2008) and have an important role in, *e.g.* energy flow in food webs (Marcogliese and Cone 1997, Lafferty *et al.* 2008). In fact, parasites not only contribute to the species richness in food webs but even dominate the food web links, so that parasite–host links can outnumber the predator–prey links (Lafferty *et al.* 2006, McLaughlin *et al.* 2020, Morton *et al.* 2021). There are some other important, but frequently “hidden” roles provided by parasite in ecosystem functioning as well, *e.g.* in nutrient cycling (Mischler *et al.* 2016).

Parasites can also regulate their host populations and shape patterns of host species distribution and density at larger spatial scales (Grenfell *et al.* 1995), as well as manipulate the behavior of host (Thomas *et al.* 2005, Lefevre *et al.* 2009, Gopko *et al.* 2017). Parasites can generate strong selection pressure on their hosts (Schmid-Hempel 2021). Parasites have been suggested to maintain phenotypic polymorphism and to be behind the evolution of sexual selection and sexual reproduction (Wegner *et al.* 2003, Lively *et al.* 2004, Blanchet *et al.* 2009, Hamilton and Zuk 1982). The pervasiveness of parasites in all ecosystems (Kuris *et al.* 2008) underlines the ecological and evolutionary importance of parasites.

1.2 Role of freshwater mussels in aquatic ecosystems

Freshwater mussels (Mollusca: Bivalvia: Unionida) are sessile benthic animals having a parasitic larva, *glochidium*, which requires a fish host (Bauer 2001). Freshwater mussels are large filter feeders that use their muscular foot and shell to burrow into the sediment (Vaughn and Hakenkamp 2001, Allen and Vaughn 2009). One freshwater mussel can filter up to 55 l water per day (Tankersley and Dimock 1993), which clarifies water and removes particles from both the water column and interstitial sediments (Vaughn *et al.* 2008). However, they have different filtration rate depending on environmental variables such as temperature (Spooner and Vaughn 2008). Mussels are also known of excreting soluble nutrients to the water column (Vaughn and Hakenkamp 2001). Atkinson *et al.* (2014) found that mussel excretion could account for 40 % of the nitrogen in a nutrient limited river reach and that they supplied up to 19 % of the nitrogen in specific compartments in food web which are close to the mussel bed. Therefore, these animals contribute to nutrient recycling and support food web. Mussels are prey for other organisms (Tyrell and Hornbach 1998, Atkinson 2013), and also act as common daily human food supply in many Asian countries (Bolotov *et al.* 2014). In a word, freshwater mussels provide many supporting, regulating, provisioning and cultural ecosystem services, including nutrient cycling, nutrient storage, habitat modification and regulation of aquatic ecosystem processes through biofiltration (Vaughn 2018, Fig. 1).

In summary, freshwater mussels are remarkable animals, some species living up to 250 years, providing an opportunity to reveal past physical and chemical conditions, over both large spatial and temporal scales through their shells (Brown *et al.* 2005). However, as more and more anthropogenic impact is threatening mussel habitats, the populations of many mussel species are declining. Therefore, more and more spotlights and research attention should be shed on freshwater mussel.

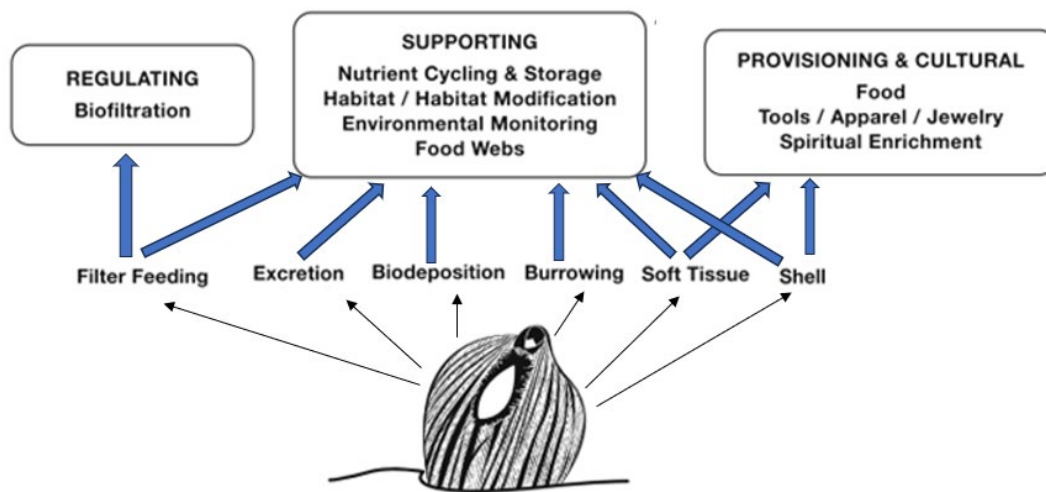


FIGURE 1 Mussel tissue and activities that mussels perform can be translated into ecosystem services. Modified from Vaughn (2018).

1.3 Conservation status and threats of freshwater mussels

Freshwater organisms are generally more endangered and periled than terrestrial organisms (Dudgeon *et al.* 2006, Dudgeon 2019), and 33 % of freshwater mollusk species are globally endangered (Böhm *et al.* 2021). Since unionids are highly endemic and sensitive to human impact and anthropogenic changes, many species and population have declined or disappeared in modern times (Strayer 2008). Thus, freshwater mussels is one of the most endangered groups of animals (Lydeard *et al.* 2004). Three major causes of freshwater mussel declines have been recognized: 1) Habitat loss because of anthropogenic construction, *e.g.* dams and channelization (Geist and Kuehn 2005), and more specifically, dams can be a major barrier to the dispersal of fish carrying glochidia (Watters 1996). 2) Pollution and eutrophication. There is a wide range of contaminants that can potentially affect freshwater mussels (Lopes-Lima *et al.* 2017). For example, the case of heavy metal leaking out in the River Tisza in 2000 lead to a complete destruction of local freshwater populations (Fleit and Lakatos 2003). 3) Invasion by non-native bivalve species. The most important invasive freshwater bivalves in Europe and the most studied invasive species as well are *Dreissena polymorpha* (Pallas 1771), *Corbicula fluminea* (Müller 1774), *Dreissena rostriformis bugensis* (Andrusov 1897), *Limnoperna fortune* (Dunker 1857) and *Sinanodonta woodiana* (Lea 1834) (Sousa *et al.* 2014). These invasive bivalves, especially *S. woodiana* and *C. fluminea*, are expanding their range in Europe rapidly (*e.g.* Bernal *et al.* 2018, Urbańska and Andrzejewski 2019, Urbańska *et al.* 2021). The introduction of non-indigenous species not only has direct negative impact such as competition over food and resources on native freshwater mussels, but indirect impact such as induction of cross-resistance in host fish of

native mussels from *S. woodiana* (Donrovich *et al.* 2017). The decline of freshwater mussels not only comes with the loss of species diversity (Bogan 1993), but the abundance of mussel individuals in certain populations (Karatayev *et al.* 2012).

1.3.1 Success of certain invasive freshwater bivalves

This chapter focuses mainly on three invasive freshwater bivalves introduced to Europe: Asia clam, *C. fluminea*, Chinese pond mussel, *S. woodiana*, and Zebra mussel, *D. polymorpha*. All these bivalves can be especially detrimental to native Unionida freshwater mussels (Lopes-Lima *et al.* 2017).

C. fluminea is the most successful non-native invasive bivalve in aquatic environments (Ferreira-Rodríguez *et al.* 2019a). It is native to Southeast Asia and was first found in Europe in the early 1980s (review in Modesto *et al.* 2023). The species is now present from Portugal in the west to Russia in the east in the European continent, and it has spread into United Kingdom and Ireland as well (Ilarri and Sousa 2012, Crespo *et al.* 2015). Human activities such as global trade, ballast water discharges, aquarium releases and sport fisheries, have been considered main factors and transport pathway of *C. fluminea* (Karatayev *et al.* 2007, Ferreira-Rodríguez *et al.* 2019b).

The introduction of *C. fluminea* has led to competition with native freshwater bivalves for space and/or food resources (Strayer 1999, Novais *et al.* 2016). Densities of *C. fluminea* are usually very high, which may lead to lower growth and lower physiological condition of local freshwater mussels (Ferreira-Rodríguez *et al.* 2018). Management of *C. fluminea* has been problematic. Physical removal has been attempted, but the population recovered within 15 months (Wittmann *et al.* 2012). Biological control methods have been tested as well, but they only worked on a small scale (Sousa *et al.* 2014, Ferreira-Rodríguez and Pardo 2018). Modesto *et al.* (2023) suggested that more innovative methods to reduce fecundity, such as genetic editing, should be applied.

S. woodiana, the Chinese pond mussel, an invasive species indigenous to Asia, first spread into Europe as glochidium in carp (*Cyprinus carpio*) (Watters 1997). The current distribution reaches from Spain in the southwest to Ukraine in the east and Sweden in the north (Lajtner and Crnčan 2011). As a habitat generalist, *S. woodiana* can inhabit ponds, reservoirs, lakes, irrigation channels and rivers (Urbańska and Andrzejewski 2019) and with the development of cold tolerance, it demographically outperforms native mussels in Europe and a dominance shift has been predicted in a Polish lake (Urbańska *et al.* 2019). A dense population of *S. woodiana* will not only compete with native Unionida mussels for food but also increases resistance to glochidia in potential host fish towards native mussels (Donrovich *et al.* 2017). A recent study by Reichard *et al.* (2012) shows that *S. woodiana* successfully used European bitterling (*Rhodeus amarus*) as a host in larval stages while glochidia of other native European unionids were rejected by the European bitterling. On the other hand, European bitterling cannot use *S. woodiana* as a host, but can use the native European mussels. Therefore, the invasion of *S. woodiana* is not only affecting negatively

the native unionid mussels but bitterling as well, and the biodiversity of aquatic system at the end.

D. polymorpha is native to the Ponto-Caspian region (e.g. Strayer 2009) and introduced to Europe and North America (US and Canada) (Sousa *et al.* 2014). It established widely throughout Europe already in the 18th and 19th centuries and is now present even in Russia and Scandinavia (reviewed in Aldridge *et al.* 2004). In addition to direct competition for food with native Unionida mussels (Strayer and Malcom 2007), dreissenid bivalves possess byssus threads which allow them to attach to the valves of native mussels (Lopes-Lima *et al.* 2017). Fouling from *D. polymorpha* can affect Unionida mussels' filtration rate, locomotion and burrowing behavior and eventually lead to suffocation and occlusion of unionids (Schloesser *et al.* 1996, Sousa *et al.* 2014). Fouling by *Dreissena* will result in depletion of biomass and energy stores of the host Unionida mussels and eventually lead to their localized extirpations (Sousa *et al.* 2011, Bódis *et al.* 2014).

1.4 Bio-invasion, and the Enemy Release Hypothesis

Invasive species refers to a species that manages to establish a colony and to reproduce in new environment outside the original area (Blackburn *et al.* 2009, Hulme *et al.* 2009, Jeschke *et al.* 2012, Lockwood *et al.* 2013) – typically as a result of human-mediated translocation (Jeschke and Heger 2018). According to Blackburn *et al.* (2011) and Jeschke and Heger (2018), invasive species experience stages of transport, introduction, establishment and spread, with specific barriers to overcome in each stage (Fig. 2). Thus, a biological invasion is a series of stages, a “pathway”, rather than just linear outcome of establishment (Jeschke and Heger 2018, Catford *et al.* 2009).

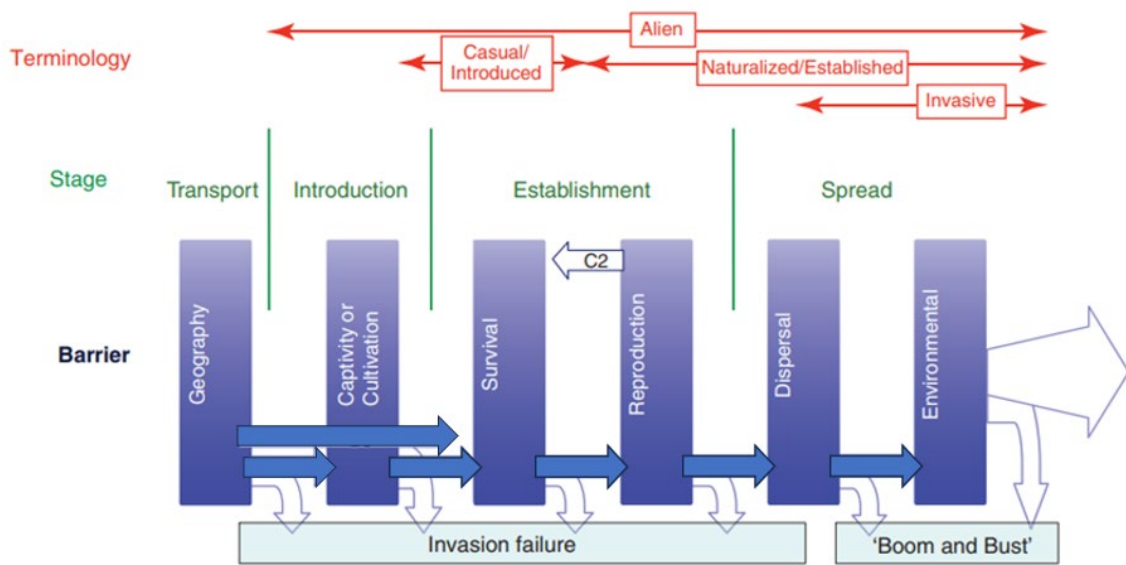


FIGURE 2 Pathway and series of stages in biological invasion which reflect the ecological barriers that a successful invasive species need to overcome in general. Modified from Blackburn *et al.* (2011)

It has been a major challenge in bio-invasion related research to understand what the decisive trait of the successful exotic species and what factors and characteristics contribute to its the success (*e.g.* Kolar and Lodge 2001, Mack *et al.* 2000). The emergent question is: is there a general rule in bio-invasion, is there a common trait on the invasive species that successfully colonise new environments? Numerous hypotheses address success and mechanisms of bio-invasions (Richardson and Pysek 2006). These theories can be divided into three categories: 1) theories that focus on the trait of invasive species (*e.g.* the Propagule Pressure Hypothesis and the Phenotypic Plasticity Hypothesis), 2) theories that focus on the environmental characteristics of invaded ranges (*e.g.* the Biotic Resistance Hypothesis) and 3) theories that focus on invasion-interactions process (*e.g.* the Enemy Release Hypothesis and the Evolution of Increased Competitive Ability Hypothesis). Other than individual studies on a single mechanism, there are also studies on holistic framework and synthesis of theories (*e.g.* Catford *et al.* 2009, Jeschke and Heger 2018).

There are eight leading extensively studied hypotheses addressing the success of biological invasions (Table 1). The most well-known hypothesis is the Enemy Release Hypothesis (ERH, Elton 1958, Heger *et al.* 2024). This thesis specifically focuses on ERH and studies its applicability in an empirical field study.

ERH suggests that the invaders are released from their natural enemies, *e.g.* predators, parasites or pathogens, during the invasion process, which will lead to a low enemy pressure on invaders in the invaded area and give the invaders an advantage when competing with local, native species (Elton 1958, Keane and

Crawley 2002, Mitchell and Power 2003, Heger and Jeschke 2014, Jeschke 2014). There are three rationales behind ERH related to parasites as enemies: First, parasites are harmful to host individuals and therefore can regulate the host populations. Indeed, such a regulation has been observed in natural populations (*e.g.* Grenfell *et al.* 1995). Second, parasites are generally highly host-specific, *i.e.* they can only live in certain species or even genotypes that exist in certain geographic area or environment (*e.g.* Price 1980). Thus, their ability to parasitize the invaders is limited. Third, the invaders normally cannot bring their own parasites with them to the new environment. This is either because they are introduced at the larval stage which is frequently parasite-free, or because the new environment lacks some suitable intermediate hosts for parasites having complex multi-host life cycle.

Reduction of the abundance of natural enemies will lead to competitive advantage for the invader in new area and environment (*e.g.* Jeschke and Heger 2018). Especially giving the fact that parasites can regulate host population as illustrated in previous chapter, there is a growing interest on role of parasites in the biological invasion (Dunn 2009).

TABLE 1 Leading hypotheses in explaining the success of bio-invasions. Adopted from Catford *et al.* (2009).

Hypothesis	Argument	Source and study
Biotic Resistance Hypothesis	Ecosystems with high biodiversity build up higher resistance to invaders than ecosystems with low biodiversity	Elton 1958, Levine and D'Antonio 1999, Lonsdale 1999
Tens Rule	Around 10 % of species successfully make it to next step in invasion process	Williamson and Brown 1986, Williamson 1996
Enemy Release Hypothesis	Invasive species benefit from loss of natural enemy in new environment	Elton 1958, Maron and Vila 2001, Keane and Crawley 2002
Evolution of Increased Competitive Ability	After getting rid of natural enemies, invaders can allocate more energy into growth	Blossey and Notzold 1995, Callaway and Ridenour 2004, Joshi and Vrieling 2005
Propagule Pressure Hypothesis	Species with higher propagule pressure will lead to higher chance of successful invasion	Lockwood <i>et al.</i> 2005, Colautti <i>et al.</i> 2006, Pysek and Richardson 2006, Richardson and Pysek 2006
Invasion Meltdown Hypothesis	Direct or indirect symbiotic or facilitative relationships among invaders cause an 'invasion domino effect'	Simberloff and Von Holle 1999, Mack 2003
Disturbance Hypothesis	Disturbance events increase resource availability and reset succession, giving invading species a better chance of success at colonization and establishment	Sher and Hyatt 1999, Hood and Naiman 2000, Colautti <i>et al.</i> 2006
Phenotypic Plasticity Hypothesis	Invasive species have higher phenotypically plasticity than native or non-invasive or native species.	Tollrian and Harvell 1999, DeWitt and Scheiner 2004, Engel <i>et al.</i> 2011

Two types of studies to test ERH have been conducted: First, studies that investigate the potentially reduced infestation of the invasive species with enemies in the invaded range (Jeschke 2014), comparing the enemy pressure in the invasive ranges with that in the native ranges (*e.g.* Torchin *et al.* 2001, Ebeling *et al.* 2008). This is based on the prediction of ERH that the invader has lower enemy load in invasive ranges compared with in native range due to the loss of enemies. Indeed, lower parasite and pathogen pressure in the invader in the invaded area when compared to its original area has been observed across a wide range of bioinvasions (Mitchell and Power 2003, Torchin *et al.* 2003, Liu *et al.* 2006, Blumenthal *et al.* 2009, Lowry *et al.* 2013). Second type of ERH studies is the comparison of enemy pressure between the populations of invasive and native

species living in sympatry in the introduced range (e.g. Ashton and Lerdaun 2008). This is based on the prediction of ERH that the invasive species suffer less from natural enemies than native species.

In addition, other than ERH, there are several hypotheses regarding the host–parasite relationships in the framework of invasion ecology, depending whether the invader is the host or the parasite, and whether the negative effect of parasite or pathogen is directed to the invader or to the native species – or towards the parasites of the native species – in the community of the introduced area (e.g. dilution effect, disease facilitation, parasite spillback and spill over, suppressive spillover; Chalkowski *et al.* 2018).

Hence, since the populations of native freshwater mussels Unionida are declining because of the invasive bivalves, it is worth to investigate the mechanism behind the success of invasive bivalves, such as the zebra mussel, Chinese pond mussel and Asian clam, including parasites. Invasive freshwater bivalves challenge both human and ecosystem health (Hulme *et al.* 2014, Mazza *et al.* 2014). Therefore, more research on management methods and on factors contributing to their success, should be carried out.

1.5 Parasitism in freshwater bivalves

Freshwater bivalves host diverse parasite fauna (Grizzle and Brunner 2009, Edwards and Vidrine 2013, Karatayev *et al.* 2024). However, parasites and endosymbionts of freshwater mussels (Unionida) are poorly known (Brian and Aldridge 2022). The most well-studied parasite taxa in freshwater mussels, and their possible impact to the host are listed below.

Ciliates: The most common protozoans in unionids are *Conchophthirus* spp. (family Conchophthiridae). The species in this genus are only found in freshwater bivalves and are among the most common symbionts in unionids. The body of these ciliates is laterally flattened, elliptical in profile, and they normally present with huge abundance (Fenchel 1965, Antipa and Small 1971).

Bitterling: Bitterling fishes (*Rhodeus* spp.) are freshwater fishes that have a larva parasitizing freshwater mussels; female fish place their eggs in the mussel gill demibranchs via the mussel's exhalant siphon and males release their sperm into the inhalant siphon of the mussel to fertilize the eggs (Smith *et al.* 2004). There are different bitterling species geographically, e.g. European bitterling *R. amarus*, and Chinese bitterling *R. sinensis*. It has been reported that bitterling have negative impact on feeding and respiration of the mussel (Stadnichenko and Stadnichenko 1980, Smith *et al.* 2001).

Chironomidae: Chironomids are dipteran insects having an aquatic larva (Armitage *et al.* 2012). Chironomidae is the most widely distributed and abundant group of freshwater insects (Pinder 1986). Some chironomid larvae such as *Glyptotendipes* sp. are parasitic in freshwater mussels and eat gill tissue of the host (Pinder 1986).

Leech: Leech (Hirudinae) belong to Annelida. Freshwater mussels are reported as host of a diverse leech assemblage (Bolotov *et al.* 2019). Mussel-associated leech may feed on mussel's tissue, and they seem to be host specific (Bolotov *et al.* 2019).

Mites: Mussel-parasitic watermites belong to genus *Unionicola* (family Unionicolidae) or *Najadicola* (family Pionidae) which have a global distribution and use a variety of freshwater mussel species as hosts (Edwards and Vidrine 2006, Edwards and Vidrine 2013). The life cycle of watermites also includes a larval stage that is parasitic on insects (*e.g.* chironomids) (Edwards and Vidrine 2013). Typically, parasitic mites experience four stages: egg, larva, nymph and adult (Böttger 1977, Walter and Proctor 2013). Watermites have been reported to feed upon on host's tissue (Fisher *et al.* 2000) and to be associated with reduced reproductive output and physiological condition of freshwater mussel host (Gangloff *et al.* 2008).

Nematoda: Nematodes have been reported as intestinal inhabitants of freshwater mussels (Clark and Wilson 1912, Coker *et al.* 1921) and in zebra mussel (*e.g.* Karatayev *et al.* 2000). One mussel can host up to 100 nematodes individuals. Tissue damage and pathological changes along with infections have been observed (McElwain *et al.* 2019).

Oligochaeta: *Chaetogaster limnaei* (family Naididae) is commonly found in freshwater bivalves, in the mantle cavity, on the gills, and in the kidney (Kelly 1899, Sickel and Lyles 1981). *C. limnaei* is reported to produce gill damage and increased respiration rate of the bivalve (Liquin *et al.* 2021).

Aspidogaster: Aspidogastreaan trematodes require only one host in their life cycle, and they infect bivalves, fishes and snails both in marine and freshwater environments worldwide (Alves *et al.* 2015). *A. conchicola* is the most common *Aspidogaster* sp. parasitizing freshwater bivalves. *A. conchicola* is reported to feed on hemocytes, hemolymph and epithelium of the host (Gentner 1971, Bakker and Davids 1973, Huehner *et al.* 1989).

Bucephalidae: Bucephalid trematodes have a complex life cycle including three different hosts with freshwater bivalves as the first intermediate host. A typical representative of bucephalids is *Rhipidocotyle campanula*, which parasitizes European freshwater bivalves from Finland to Italy (Taskinen *et al.* 1991, II). The intra-molluscan stage, sporocyst, invade the gonad of mussel host, causing sterility (Müller *et al.* 2015). Cercaria larvae produced by sporocysts emerge from the mussel host to infect the second intermediate host, a cyprinid fish (Taskinen *et al.* 1991). When the infected second intermediate host is eaten by the definitive host, perch (*Perca fluviatilis*) or pikeperch (*Stizostedion lucioperca*), metacercariae of *R. campanula* are released in the gut of fish, mate and release eggs which enter the water. A miracidium larva, which is hatched from the egg, seeks for freshwater mussel host (Taskinen *et al.* 1991). Infection of *R. campanula* and *R. fennica* lead to reduced survival and growth suppression of the mussel host with gonad destroyed (Taskinen 1998, Jokela *et al.* 2005, Taskinen and Valtonen 1995). *Bucephalus polymorphus* is a bucephalid parasitizing the dreissenid bivalve *D. polymorpha* (Taskinen *et al.* 1991).

Gorgoderidae: The common gorgoderid trematode species in freshwater bivalves are *Phyllodistomum* spp and *Cercaria duplicata*. *C. duplicata* is a parasite of *Anodonta* and *Phyllodistomum macrocotyle* is a parasite of *D. polymorpha*. Bivalves act as the first intermediate host in the life cycle of the gorgoderids parasite (Grizzle and Brunner 2009, Petkevičiūtė *et al.* 2015). Infection of *Phyllodistomum* spp cause similar gonad damage as bucephalid trematodes (Grizzle and Brunner 2009).

Echinostomatidae: Echinostomatidae trematodes normally have three-host life cycle as well. The first intermediate host is commonly a snail, freshwater bivalves (such as zebra mussel) are second intermediate hosts, and the final hosts normally are birds (duck) (Toledo and Esteban 2016). Specifically, human being can be final host of *Echinostoma revolutum* through consuming insufficiently cooked molluscs or fish, which will lead to echinostomiasis disease (Toledo and Esteban 2016).

1.6 Aim of the study

Regardless the wide range employment of ERH in many cases to explain the success of invasive species, the role of releasing parasites on the success of invasions by freshwater mussels and clams has received only limited attention. In this thesis, I employed ERH as fundamental concept and tested its applicability in the context of the successful freshwater bivalve invasions, especially zebra mussel *D. polymorpha*, Asian clam *C. fluminea* and Chinese pond mussel *S. woodiana*. Therefore, based on the definition of ERH, there are two sub-hypotheses tested in this study: 1. Invasive freshwater bivalves have lower parasite pressure than the native mussel in the invaded area (I, II). 2. Invasive freshwater bivalves have higher parasite pressure in their original range than in the invaded range (III).

The aim of the study I was to compare the parasite load in sympatric populations of invasive freshwater bivalves and local mussels in eight waterbodies in northern Europe (Estonia, Poland).

The aim of II was to compare the parasite load in sympatric populations of invasive freshwater bivalves and local mussels in three waterbodies in southern Europe (Italy).

The aim of III was to compare the parasite load in *C. fluminea* and *S. woodiana* between the native range (China) and introduced range (Europe).

2 MATERIALS AND METHODS

2.1 Terminology in parasitology

The definitions of the variables to quantify the parasite pressure in population level that are used in I, II and III and widely in parasitological studies are:

Parasite taxon richness: Number of parasite taxa found in each host population from one sampling site.

Prevalence of infection: Proportion (%) of host individuals infected in a sample.

Sum of prevalences: Sum of the prevalences of all parasite taxa for each host species, can be more than 100 %.

2.2 Field Work

2.2.1 Sampling in Europe

Sampling of freshwater mussels in Europe (invaded range of *S. woodiana*, *C. fluminea* and *D. polymorpha*) was carried out in three different countries, Estonia, Poland and Italy, in total of 11 waterbodies (Table 2), where both native and invasive bivalves are present sympatrically. All collected bivalves were randomly selected in site regardless of their age and size.

The sampling in the sites in Estonia and Poland (Northern Europe) was conducted in May and July during 2017–2018 in eight waterbodies. These data were utilized in I, and partially in III with results of *S. woodiana* and *C. fluminea*.

Collection was performed by hand picking (snorkeling or wading with or without aquascope) and in Lake Gosławskie and Lake Siecino, Poland, also by snorkeling and scuba diving. The target sample size was 30 mussel individuals per species per site and the realized sample sizes ranged from 16 to 60 individuals.

The material for II was collected from three Italian lakes (Southern Europe), Lake Maggiore, Lake Varese and Lake Lugano. The data for *S. woodiana* and *C. fluminea* were utilized in III as well (Table 2). In Lake Maggiore, sampling was conducted in 2016, 2017 and 2018, in Lake Varese and Lake Lugano in 2017 and 2017–2018, respectively. The bivalves were collected by hand or by sieving the sediments through a 2 mm² mesh size net within 0.5 m² squares. Sampling was mainly performed by handpicking and in Lake Maggiore scuba diving was performed as well. The composition of the bivalve community was variable in different sites, so that the number of native and invasive species varied from 1 to 4 and from 2 to 3, respectively, per lake and the sample size of bivalve individuals per species per lake from 12 to 187 (Table 2).

2.2.2 Sampling in China

Sampling of bivalves in China was carried out in five waterbodies of two river Basin: The River Beijiang in Pearl River basin; Lake Dongting, Lake Poyang, the Rivers Tuojiang and Nanhe in Yangtze River Basin, from April 2020 to March 2022. These data were utilized in III. Lakes Dongting and Poyang are hot spots of aquatic species richness worldwide, and the middle portion of the River Yangtze basin is one of the most species-rich regions for freshwater mussels on Earth (Zieritz *et al.* 2018), including many endemic species (He and Zhuang 2013, Shu *et al.* 2014). The River Tuojiang is one of the major tributaries of the upper River Yangtze located in Sichuan Province. The sampling of *S. woodiana* in the Yangtze River Basin was conducted in three sites, Lake Dongting, Lake Poyang, and the River Tuojiang (Table 2). A total of 81 *S. woodiana* individuals were collected in their original range by hand picking. The sample size did not meet the goal of 30 individuals per site in Lake Dongting, but it is assumed that it represents well the intracommunity of parasites in *S. woodiana*, and this population was included in the following analysis. China is one of the original distribution areas of *C. fluminea* (Modesto *et al.* 2023). A total of 778 individuals of *C. fluminea* were collected by handpicking in their original range (China) from four sites, the River Beijiang, Lake Dongting, the River Tuojiang and the River Nanhe (Table 2). The River Beijiang is in the southern sub-tropical part of China, which is the northern tributary of the Pearl River.

TABLE 2 Sampling sites in Europe and China combined, with bivalve species, sample size (n) and study assigned. Abbreviation: BS = Baltic Sea drainage, MS = Mediterranean Sea drainage, YR = Yangtze River drainage, PR = Pearl River drainage, I = Invasive species, N =Native species.

Country	Waterbody	Bivalve Species	Sample size	Study	
Estonia	Lake Võrtsjärv, BS	<i>A. anatina</i> (N)	60	I	
		<i>D. polymorpha</i> (I)	39	I	
Poland	Czarny Młyn Pond, BS	<i>A. cygnea</i> (N)	30	I	
		<i>S. woodiana</i> (I)	31	I, III	
	Warta- Gopło Canal, BS	<i>A. anatina</i> (N)	30	I	
		<i>U. pictorum</i> (N)	30	I	
		<i>U. tumidus</i> (N)	30	I	
		<i>S. woodiana</i> (I)	30	I, III	
		<i>D. polymorpha</i> (I)	30	I	
		<i>C. fluminea</i> (I)	55	I, III	
		Lake Gosławskie, BS	<i>A. anatina</i> (N)	30	I
			<i>U. pictorum</i> (N)	30	I
	<i>U. tumidus</i> (N)		30	I	
	<i>S. woodiana</i> (I)		30	I, III	
	Nowy Lipsk Pond, BS	<i>D. polymorpha</i> (I)	30	I	
		<i>C. fluminea</i> (I)	30	I, III	
		<i>A. anatina</i> (N)	30	I	
		<i>A. cygnea</i> (N)	30	I	
		<i>U. pictorum</i> (N)	30	I	
		<i>U. tumidus</i> (N)	30	I	
	Siecino Lake, BS	<i>S. woodiana</i> (I)	31	I, III	
		<i>U. tumidus</i> (N)	30	I	
<i>P. complanata</i> (N)		30	I		
<i>D. polymorpha</i>		30	I		
The River Szezszipa, BS	<i>A. anatina</i> (N)	37	I		
	<i>U. pictorum</i> (N)	34	I		
	<i>U. tumidus</i> (N)	34	I		
	<i>P. complanata</i> (N)	36	I		
	<i>D. polymorpha</i> (I)	25	I		
	<i>A. anatina</i> (N)	25	I		
The River Rospuda, BS	<i>U. tumidus</i> (N)	19	I		
	<i>P. complanata</i> (N)	23	I		
	<i>D. polymorpha</i>	16	I		
	<i>A. anatina</i> (N)	37	II		
	<i>A. cygnea</i> (N)	35	II		
	<i>A. exulcerata</i> (N)	21	II		
Italy	Lake Maggiore, MS	<i>U. elongatulus</i> (N)	187	II	
		<i>S. woodiana</i> (I)	62	II, III	
		<i>D. polymorpha</i> (I)	53	II	
		<i>C. fluminea</i> (I)	30	II	
		Lake Varese, MS	<i>U. elongatulus</i> (N)	48	II
			<i>S. woodiana</i> (I)	26	II, III
	Lake Lugano, MS	<i>D. polymorpha</i> (I)	18	II	
		<i>C. fluminea</i> (I)	99	II, III	
		<i>A. cygnea</i> (N)	12	II	
		<i>D. polymorpha</i> (I)	32	II	
China	Lake Dongting, YR	<i>C. fluminea</i> (I)	19	II, III	
		<i>S. woodiana</i> (N)	17	III	
	Poyang Lake, YR	<i>C. fluminea</i> (N)	34	III	
		<i>S. woodiana</i> (N)	34	III	
	The River Tuojiang, YR	<i>S. woodiana</i> (N)	30	III	
		<i>C. fluminea</i> (N)	30	III	
	The River Beijiang, PR	<i>C. fluminea</i> (N)	682	III	
	The River Nanhe, YR	<i>C. fluminea</i> (N)	32	III	

2.3 Laboratory work

All bivalves were stored in plastic bags on ice and transported to the laboratory alive, except for the *C. fluminea* samples from Italian sites, Lake Maggiore, Lake Varese and Lake Lugano, and from the Spanish sites Canal Imperial de Aragón and the River Ebro, which were stored in ethanol after sampling. The length, width, height (to 1 mm) of the bivalves were measured by caliper before the parasite examination process. Parasite examination was performed by first counting the ectoparasites (e.g. adult watermites) and then dissecting the tissue into pieces and pressing the pieces between two large glass plates and examining under binocular microscope with transmitted light (Taskinen *et al.* 1991). Small individuals, such as most of *C. fluminea* and *D. polymorpha* specimens, were examined as whole. Thus, the mantle, gills, digestive gland and gonad, which are the typical sites of infection in freshwater mussels (e.g. Taskinen *et al.* 1997, Edwards and Vidrine 2013) were examined. Numbers of parasites were counted (e.g. adult watermites and watermite eggs and larval stages in the gill and mantle). If the mantle and gills were examined only from one side of the mussel (some results from the European sites), the numbers were doubled. If it was not possible to count the individuals (e.g. sporocysts of bucephalid trematodes), the intensity of infection was categorized as 1 (low), 2 (moderate) or 3 (heavy infection).

2.4 Statistical methods and data analysis

As mentioned above, parasite taxon richness (number of parasite taxa) and sum of prevalences (sum of the prevalence of all parasite taxa for each host species) were used as the response variables when comparing the parasite pressure between populations of native and invasive species. They have generally been used as the measures of parasite pressure in studies exploring ERH (Lafferty *et al.* 2010, Torchin *et al.* 2003).

The parasite load in freshwater bivalves can depend negatively on depth (Taskinen and Valtonen 1995). Therefore, only the samples collected from the depth zone 0–1 m by wading and from the depth zone 1–15 m by scuba diving were included in analysis.

In I, the average parasite taxon richness and the average sum of prevalences of native and invasive bivalve populations in each lake were calculated firstly. If only one native or invasive mussel species was present, its values represented the averages for native/invasive group in that lake. Secondly, a paired *t*-test was applied to analyze differences in the mean average parasite taxon richness and sum of prevalences for the native and invasive bivalve populations over the eight study sites. In addition to this “whole picture” analysis, a site-specific analysis was performed to get an idea of the interaction between mussel and parasite community in a given waterbody. This was done by comparing prevalences of infection of every possible parasite taxon for every pair of native vs. invasive

mussel species using χ^2 -test. If the number of cells with expected count less than five was greater than one, a Yates continuity correction was applied. In addition, if there were at least three native and invasive mussel species in a given site, the difference in the mean parasite taxa-specific prevalence of native and invasive mussels was analyzed using t -test for that particular site.

In II, the original sample sizes of different bivalve species were very different in different sites. Therefore, to make the results to be comparable to each other before further statistical analyses, the parasite taxon richness was standardized by scaling them to equal sample size by bootstrapping. In this study, the smallest acceptable sample size was set to $n = 12$, which was the number of *A. cygnea* collected from Lake Lugano (Table 2). In the bootstrapping process, 2000 resamples (with replacement) of 12 individuals were drawn from the samples of all other populations and the averages of the 2000 results were used instead of the original values when comparing the parasite taxon richness between invasive and native bivalve species. The species- and lake-specific parasite taxon richness and sum of prevalence, after rank-transformation (Conover and Iman 1981), were used as the response variables in Two-way ANOVA, where 'invasion status' (invasive/native) and 'lake' (study site) were used as fixed factors. In addition, to have a detailed view of each waterbody and each parasite taxon, site-specific χ^2 -tests were performed on the parasite presence-absence data of all parasite taxa in each lake between each pair of invasive and native species.

In III, standardization of parasite taxon richness by bootstrapping was applied before further analysis as well. In this study, the comparison was between native and invasive populations of *S. woodiana* and *C. fluminea* respectively, and the standard sample sizes for bootstrapping were $n = 19$ and $n = 17$, respectively. Both Student's t -test with rank-transformation and Mann-Whitney test were applied to compare the population-specific parasite taxon richness (after standardization) and the sum of parasite prevalences between native and invasive ranges, China and Europe. Similar analysis was applied to compare the prevalence of each parasite taxon of *S. woodiana* (without standardization), as the expected value of parasite-specific prevalence is independent of n) between native and invasive sampling sites. In addition, generalized Linear Regression analysis with Poisson distribution and log-link function was performed on individual level to explore whether individually, invaders have lower parasite taxon richness than conspecifics in their native range. Generalized linear regression with parasite taxon (each host individual carried) as response variable, range of sites (native or invasive) as fixed effect and sampling site (waterbody) as nested random effect was performed in R statistical computing software (version 4.3.1; R Core Team 2023) among results of *S. woodiana* and *C. fluminea* respectively.

3 RESULTS AND DISCUSSION

3.1 Parasite pressure in sympatric native and invasive freshwater bivalves in European water bodies

In I, 5 native (*A. anatina*, *A. cygnea*, *P. complanata*, *U. pictorum* and *U. tumidus*) and 3 invasive (*S. woodiana*, *D. polymorpha* and *C. fluminea*) bivalve species were found, and a total of 15 parasite taxa were discovered from the 8 waterbodies (detailed list of parasite taxa in Table 1 in I).

Mean (\pm s.e.) average parasite taxon richness over the 8 study sites was for the native bivalves 2.3-fold that for the invasive bivalves (4.8 ± 0.5 vs. 2.1 ± 0.6 taxa; range 3–7 and 0–5 taxa, respectively). Furthermore, the mean (\pm s.e.) average sum of prevalences of infections of different parasites over the study sites was for the native species 2.4-fold that for the invasive bivalves (151 ± 27 vs. 64 ± 24 %, range 43–264 and 0–170 %, respectively). Both of these two indicators show the tendency of the invasive freshwater bivalves in general having lower parasite pressure than the sympatric population of native species in the Northern European waterbodies in Estonia and Poland.

In II, 4 native bivalve host species (*A. anatina*, *A. cygnea*, *A. exulcerated* and *U. elongatulus*) and 3 invasive species (*S. woodiana*, *D. polymorpha*, *C. fluminea*) were found from the three study lakes, and 11 parasite taxa were observed (detailed parasite taxa in supplementary table in II).

Within a lake, the average parasite taxon richness per bivalve species was lower in the invasive bivalves than among the native ones (II). Among the native species, the standardized parasite taxon richness ranged from 3 to 5.39, while among invasive species it was from 0 to 4.17. ANOVA results indicated that invasion status (invasive or native) had a statistically significant effect on the parasite taxon richness. The native species harbored significantly more parasite species – having on average, 2.6-fold parasite taxon richness in comparison to invasive bivalves in the same site. In addition, the parasite taxon richness did not

differ significantly between lakes, and the interaction of invasion status and lakes was not significant.

Similarly, the second indicator of parasite pressure – sum of prevalences – differed statistically significantly between native and invasive species, being in native mussels on average 3.4-fold that in invasive bivalves (II), while the effect of lake and interaction of lake and mussel status were not significant.

Lake specific between-bivalve species comparisons (I, II) revealed that most parasite taxa had statistically significantly higher infection prevalence in the native than in invasive host species. The invader *D. polymorpha* had in some cases significantly higher prevalence of the trematodes *B. polymorphus* and *P. macrocotyle*, but these trematodes are *Dreissena*-specific and do not infect the native unionids. However, in the case of *S. woodiana*, there were some notable exceptions that parasite prevalence in *S. woodiana* population was significantly higher than that in the native mussel population. For example, in Warta-Gopło Canal, Poland, parasite prevalence in *S. woodiana* was significantly higher than in *U. pictorum* for the taxon of adult watermites; *U. tumidus* for the taxa of both adult watermites and larval/egg stages of watermites in the mantle, and in the comparison of *Chaetogaster oligochaete* taxon, the prevalence of *S. woodiana* was significantly higher than all native species (I). In summary of lake specific between-bivalve species comparisons in all 11 studied sites and excluding the results from *Dreissena*-specific parasite, there were 122 between-host species comparisons results found significantly different, and specifically, 107 cases were that the prevalence was higher in native species over invasive species and only 15 cases were higher in invaders over native ones.

I and II combined, a conclusion can be drawn that there is a lower parasite pressure in invasive freshwater bivalves in European waterbodies than in the native ones. Invasive *C. fluminea* did not have any parasites at all. One prediction of the Enemy Release Hypothesis is that introduced species should benefit from enemy-mediated competitive release because they are less likely to be affected by natural enemies than their native competitors (Elton 1958, Keane and Crawley 2002). In I and II, the prediction of lower parasite pressure in invasive bivalves was met and therefore, ERH was supported on the perspective of missing parasites (enemies). A prerequisite of one central assumption of ERH – that the invaders can benefit from the “missing enemies” – is that the enemies (in this case parasites) must be harmful. Indeed, *e.g.* adult watermites have a strong negative impact on their bivalve-host by ingesting their mucus and tissues (Fisher *et al.* 2000) or have an impact on the host’s glycogen condition and thus reproductive output (Gangloff *et al.* 2008), and in most of the waterbodies, the prevalence of adult watermites was higher in native species than in the invasive species (I, II). Among all the 43 cases of between host species comparison of adult watermite prevalence in all 11 studied sites, 23 results were significantly higher in the invasive species, and 3 were significantly higher in the native ones: in Warta-Gopło Canal, Poland, there was higher prevalence of adult watermite in *S. woodiana* than in native mussel *U. pictorum* and *U. tumidus*; and in Nowy Lipsk Pond, Poland, higher prevalence of adult watermite in *S. woodiana* than *U.*

pictorum also. Larval trematodes, such as bucephalids and gorgoderids, can cause partial or complete sterility, parasite-induced mortality and lowered growth to the bivalve host (Davids and Kraak 1993, Taskinen and Valtonen 1995, Taskinen 1998, Jokela *et al.* 2005, Müller *et al.* 2014). In this study, the trematode *R. fennica* was only found in native species (*A. anatina* in Lake Vörtsjärvi, Nowy Lipsk Pond, The River Szeszupa and Rospuda). Also *R. campanula* mainly infected the native mussels, but in some cases *S. woodiana* as well but with lower infection prevalence (*e.g.* result in Gosławskie Lake). Therefore, in the case of adult watermite and larval trematodes, native mussels of I and II should receive more negative impact from these parasite taxa.

3.2 “Loss” (and “gain”) of parasites in invasive *Sinanodonta woodiana* and *Corbicula fluminea*

In the native range, China, a total of 9 parasite taxa were discovered from *S. woodiana* (n = 81, 3 water bodies): a Gorgoderidae type trematode (sporocysts in the gonad), *Aspidogaster* type trematode (adult trematode in the pericardial cavity), *Glyptotendipes* type chironomid (mantle cavity), *Conchophthirus* type ciliate, larval/egg stages of watermites in the mantle, larval/egg stages of watermites in the gills, adult watermites on the gills and larvae of bitterling *R. sinensis* in the gills, as well as hirudinae from the mantle cavity (III). In the invaded range, a total of 6 parasite taxa were discovered from *S. woodiana* (n = 210, 6 water bodies): the gonad-dwelling bucephalid trematode *Rhipidocotyle campanula*, *Chaetogaster* type oligochaete, *Glyptotendipes* type chironomid, larval/egg stages of watermites in the mantle, larval/egg stages of watermites in the gills, and adult watermites (III).

Parasite taxa of *S. woodiana* missing from the invaded area – “lost parasites” – were Gorgoderidae type trematode, *Aspidogaster* type trematode, bitterling, Hirudinae leech and the *Conchophthirus* type ciliate. To my knowledge, this was the first time that Gorgoderidae type trematode was reported in *S. woodiana*, but not very surprising as parasites of bivalves are not well-studied in east Asia, and most studies have focused on *Unionicola* watermites (*e.g.* Wen *et al.* 2006). In any case, lack of Gorgoderidae trematodes in Europe should give an important advantage to *S. woodiana* as gorgoderids infect the gonads of bivalve causing sterility (Grizzle and Brunner 2009). *Aspidogaster* type adult trematode was “missing” from invasive *S. woodiana* as well, while it was found in all native populations, the prevalence ranging from 3 to 38%. Preliminary identification of Hirudinae parasites of *S. woodiana* indicates that there were at least two different Hirudinae species parasitizing *S. woodiana* in China (Hämäläinen, Deng and Taskinen, unpublished), but none in European populations of *S. woodiana*. This should benefit *S. woodiana* since leeches are known to feed on tissues of the mussel host (Wu *et al.* 2018, Bolotov *et al.* 2019). An important group of parasites missing from European *S. woodiana* populations was ciliates. The densities of

Ciliata parasites, e.g. *Conchophthirus* within a freshwater bivalve, can be very high (Molloy *et al.* 1997). Karatayev *et al.* (2024) stated that *Conchophthirus* spp. tend to be fairly host-specific, which could explain the lack of these ciliates in *S. woodiana* in Europe. European bitterling (*R. amarus*) deposits its eggs to the gill chamber of unionid mussel, which is harmful to the host (Smith *et al.* 2004, Reichard *et al.* 2006). *Rhodeus* species are adapted to their local host fish population (Rouchet *et al.* 2017), and the invasive mussel *S. woodiana* is able to reject eggs of *R. amarus* (Reichard *et al.* 2007). This should benefit *S. woodiana* since bitterlings have negative effect on feeding and respiration of the mussel (Stadnichenko and Stadnichenko 1980, Smith *et al.* 2001).

However, there was not only “loss” of enemies, but also “new enemies” appeared after introduction of *S. woodiana* to Europe (Table 3): both *R. campanula* and *Chaetogaster* type oligochaete have been found in multiple sites of range of invasion but not from Chinese *S. woodiana* populations. They both are important, harming the host, but especially *R. campanula*, as a sterilizing parasite (Müller *et al.* 2015) should be a burden to *S. woodiana*. On the other hand, the other sterilizing trematodes, *R. fennica* and gorgoderid, did not infect *S. woodiana* in Europe, compensating for the parasite pressure by *R. campanula*.

Population-specific standardized parasite taxon richness (\pm s.e.) among invasive *S. woodiana* populations in Europe was 3.0 ± 0.3 , while in the native populations in China it was 6.4 ± 0.3 . Population-specific sum of infection prevalences (\pm s.e.) among invasive *S. woodiana* populations in Europe and in the native populations in China were $120 \pm 28\%$ and $358 \pm 43\%$, respectively (III). The Student’s *t*-test for ranked standardized data indicated a significant difference in both parasite taxon richness and sum of prevalence between native and invasive *S. woodiana*. In individual-level analysis, mean (\pm s.e.) parasite taxon richness per *S. woodiana* individual from native and invasive range were 3.23 ± 0.14 and 1.32 ± 0.06 , respectively (III).

TABLE 3 Prevalence (%) of “gained” and “loss” parasite taxa in *S. woodiana* during invasion process from China to Europe, as well as *t*-test *p*-values and Mann-Whitney (M-W) test *p*-values of comparisons each parasite taxon between native and invasive population. Trem = trematode, Olig = oligochaete.

Sites (n)	“Gained” parasite taxa		“Loss” parasite taxa				
	Bucephalidae Trem	<i>Chaetogaster</i> type Olig	Gorgoderidae type Trem	Aspido- gaster type Trem	Hirudinae	<i>Rhodeus</i> spp.	<i>Conchoph.</i> type ciliate
Native							
Dongting (17)	0	0	0	35	82	0	47
Poyang (34)	0	0	0	38	12	0	21
Tuojiang (30)	0	0	39	3	0	3	42
Mean±s.e.	0	0	13±13	25±11	32±26	1±1	37±8
Invasive							
Varese (48)	0	4	0	0	0	0	0
Maggiore (62)	16	1	0	0	0	0	0
Cazrny meyn (31)	0	0	0	0	0	0	0
Warta- Gopło (30)	3	27	0	0	0	0	0
Novy lipsk (31)	0	0	0	0	0	0	0
Goslawskie (30)	3	0	0	0	0	0	0
Mean±s.e.	4 ±2	5±4	0±0	0±0	0±0	0±0	0±0
<i>p</i> (<i>t</i> -test)	0.18	0.18	0.18	<0.001	<0.01	0.17	<0.01
<i>p</i> (M-W)	0.17	0.29	0.16	0.006	0.034	0.16	0.006

For *C. fluminea*, the difference was very clear (III). In the native range, as many as 7 parasite taxa were discovered from *C. fluminea* (n = 778, 4 water bodies): Bucephalidae type trematode, *Aspidogaster* type trematode (adult trematode in the pericardial cavity), *Glyptotendipes* sp., larval/egg stages of watermites in the mantle, larval/egg stages of watermites in the gills, adult watermites) (III). Specifically, to my knowledge, this was the first report of bucephalid trematode parasitizing *C. fluminea*. Meanwhile, in the introduced range (Europe), no parasites were discovered from any *C. fluminea* individual (n = 345, 7 water bodies). In addition to this study, *C. fluminea* has been reported to host *Phyllodistomum mingensis* (Tang 1985), *Echinostoma* trematodes (Keeler and Huffman 2009) and *Aspidogaster* spp. (Tang 1992) in China. Therefore, the previous finding and this study have shown that *C. fluminea* harbors a diverse parasite fauna in its original range, but outside the original range, the pattern of less parasite pressure in introduced *C. fluminea* has been observed also in North and South America; only the parasite *Chaetogaster limnaei* was reported in *C. fluminea* in South America (Liquin *et al.* 2021); Karatayev *et al.* (2012) found echinostomatid metacercariae from *C. fluminea* only in one out of 27 sites from Great Lakes area and Danford and Joy (1984) found only the generalist parasite aspidogastrid trematodes *A. conchicola* and *Cotylapsis insignis* in North America. In summary, the introduced *C. fluminea* populations harbor lower parasite pressure globally than the Chinese (native) populations.

In the present study, both parasite taxa number and sum of prevalences were zero for the invasive, European *C. fluminea* populations, but among the native Chinese *C. fluminea*, the mean population-specific standardized parasite taxon richness was 1.28 ± 0.34 , and mean sum of prevalences $13.75 \pm 6\%$ (III). In an individual level, the mean (\pm s.e.) number of parasite taxa in the native range was 0.09 ± 0.011 per *C. fluminea* individual, while zero per *C. fluminea* individuals in the invasive range (III).

In this study, parasites of *D. polymorpha* were studied only in the invaded range, Europe. Thus, it was not possible to evaluate the loss of parasites. However, the review by Karatayev *et al.* (2024) reported 20 taxa of endosymbionts (commensals and parasites) from Europe (including the original Ponto-Caspian range of *D. polymorpha*), and the corresponding figure in North America was 5, this indicates a marked reduction in the number of parasite taxa during the invasion process also in the invasive bivalve *D. polymorpha*.

The actual parasite pressure after the invasion is mainly determined by 1) the number of invader's own parasites carried from the original range, capable of surviving and reproducing in the new environment and 2) the number of new parasite species acquired from native mussels within the invaded environment, capable of using the invasive species as a host. In the case of *C. fluminea*, both variables seemingly get value zero – within the precision of the current methodology no parasites were detected from *C. fluminea* in Europe while a total of 7 parasite taxa were present in China. *S. woodiana* was introduced to Europe as a parasitic glochidium larva with carp (Watters 1997), which makes bringing any parasites from China to Europe highly improbable because glochidia are

surrounded by the tissue of host fish and thus in a ‘sterile’ environment, not in contact with water. It is possible that some of the parasites of *S. woodiana* were introduced to Europe as glochidium within the carp, if carp was an intermediate host, but such parasites are not known or the other required hosts in the life cycle of such parasites were lacking in Europe. Therefore, the number of *S. woodiana*’s own parasites brought from the original range, capable of surviving and reproducing in the new environment is probably zero.

Thus, it can be concluded that *S. woodiana* has acquired at least 6 parasite taxa from the native European unionid mussels after introduction to Europe if they are assumed to have arrived parasite free. *C. fluminea* and *S. woodiana* were introduced to Europe at approximately same time (in around 1980s). However, given the same duration of time, *S. woodiana* has accumulated a diverse parasite community as a host (present study and Cichy *et al.* 2015), but *C. fluminea* has not been “recognized” as a host by European parasites of freshwater mussels. As *S. woodiana* belongs to the same family as the native European freshwater mussels, Unionidae, probably the genetic relatedness is an important factor enabling European parasites of unionids to infect the invasive *S. woodiana*. In line with this, the dreissenid bivalve zebra mussel, *D. polymorpha*, another notorious invasive species in Europe and North America, which invaded Europe much earlier than *S. woodiana* and *C. fluminea* (1800s to Europe, late 1900s to North America), share 3 parasite taxa with the native freshwater mussels in this study (*Chaetogaster* type oligochaete, *Glyptotendipes* type chironomid and *Conchophthirus* type ciliate). In reality, it can be that there are only two shared parasites since the *Conchophthirus* sp. parasitizing *D. polymorpha* is *C. acuminatus*, a strictly host-specific parasite of dreissenids (Karatayev *et al.* 2024). The unionid mussel *S. woodiana* shared, or acquired, a total of 6 parasite taxa with native mussels in Europe (*R. campanula* trematode, *Chaetogaster limnaei*, *Glyptotendipes* type chironomid, and all the 3 watermite types) even though it was introduced quite late – emphasizing the potential role of genetic relatedness for acquisition of parasites in new environment; host switch from native host species to invasive species is maybe more probable between hosts belonging to same family than between host families.

In summary, ERH is supported by III since a great reduction in the parasite pressure measures has been observed in both invasive *C. fluminea* and *S. woodiana*, especially for *C. fluminea* which has “lost” all parasites during the invasion process. Combined with the studies showing that many parasites have a negative impact on their bivalve host (*e.g.* Grizzle and Brunner 2009), this study suggests that the lower parasite pressure (as compared to native ones) may contribute to the success of invasive freshwater bivalves.

4 CONCLUSIONS

The central idea of EHR is that invaders lose enemies as a result of the invasion process (Elton 1958). This reduction in the enemies can take place so that the 'old' enemies from the original range are not at all or rarely introduced together with the host to the invaded area, accompanied by low or no acquisition of 'new' enemies of the introduced area during the invasion process. Aquatic species, in general, have a high potential for enemy release if introduced without their enemies or enemies do not cope in new environment (Prior *et al.* 2015).

Combining the results from I, II and III, I can conclude that the invasive freshwater bivalves seem to have lower parasite pressure than the native mussels in the invaded area, and that the alien bivalves seem to have higher parasite pressure in their original range than the same bivalve species in the introduced range. This supports ERH, *i.e.* the loss of enemies as a result of their invasion process from Asia to Europe. Because many of these parasites have been shown to be harmful, it cannot be ruled out that the observed "enemy release" is contributing to the successful invasion of *S. woodiana* and *C. fluminea*. A clear reduction in parasites of *D. polymorpha* observed in other studies supports enemy release also in this invasive species.

A premier fundament of the ERH is that parasitic infection is harmful to the host and reduce host's fitness. Even though the impact that parasites post on the bivalve was not investigated in this study, it has been shown by a great amount of previous research that endosymbionts or parasites can have a negative impact on physiological condition, growth, reproduction and survival of bivalves host. Furthermore, along with the hypothesis of Evolution of Increased Competitive Ability, when lacking natural enemies, introduced species are able to reallocate resources from defense into growth and development (Blossey and Notzold 1995). This can possibly explain, for example, the large size of *C. fluminea* in European waterbodies compared with their conspecific in native range (Modesto *et al.* 2023).

Research on invasion mechanisms is important since it may lead to a reduction of the occurrence of new invasions and thus is a proactive way to prevent further invasion (Sakai *et al.* 2001) and benefits invasive species

management. Under the fact that there is not enough evidence for the ERH for the invasive species among most the taxonomic group (Prior *et al.* 2015), I believe this study provides a new dimension toward invasion of freshwater bivalves and offers a piece to the puzzle of bioinvasion ecology. It may help expand our understanding of the complex processes that likely underlie biological invasions and improve our ability to manage natural ecosystems. Since invasive species can lead to reduced reproductive success and overall population decline of native mussels (Lopes-Lima *et al.* 2018), conservation effort must be addressed, and the potential spread of invasive bivalves must be prevented.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Enemy Release -hypoteesi: loisinta vierassimpukoissa ja alkuperäissimpukoissa

Vieraslajit muuttavat ekosysteemejä ja uhkaavat esimerkiksi vesistöjen alkuperäisiä simpukkalajeja. *Enemy Release* -hypoteesin mukaan vieraslajeilla on alhaisempi vihollispaine, koska niiden alkuperäiset viholliset eivät yleensä siirry samanaikaisesti uudelle alueelle vieraslajin kanssa, eli vieraslajit menettävät ainakin osan vihollisistaan invaasioprosessin aikana. Alhaiseen vihollispaineeseen "valloitetulla" alueella myötävaikuttaa myös se, että yhteisen evolutiivisen historian puuttumisen takia uuden alueen viholliset eivät osaa tunnistaa ja hyödyntää vieraslajia. Vieraslajit hyötyvät alhaisesta 'vihollispaineesta' suhteessa paikallisiin alkuperäisiin lajeihin, mikä *Enemy Release* -hypoteesin mukaan voi edesauttaa vieraslajien leviämistä ja menestymistä. Järvi- ja jokisumpukoilla on laaja kirjo erilaisia loisia: alkueläimiä, imumatoja, sukkulamatoja, harvasukamatoja, vesipunkkeja, sulkasääskiä, vesiperhosia ja kaloja – kaikki ovat enemmän taikka vähemmän haitallisia simpukoille. Tästä syystä testasin väitöskirjatyössäni *Enemy Release* -hypoteesin toimivuutta haitallisten invaasiosimpukoiden *Sinanodonta woodiana*, *Corbicula fluminea* ja *Dreissena polymorpha* menestymisen selittäjänä tutkimalla käytännössä seuraavia hypoteesin väittämiä: vieraslajilla on "valloitetuilla" alueilla vähemmän loisia kuin niiden alkuperäisillä elinalueilla (vihollispaineen pieneneminen) ja vieraslajeilla on "valloitetuilla" alueilla vähemmän loisia kuin alkuperäislajeilla.

Ensimmäiseksi vertasin invaasiosimpukoiden ja paikallisten simpukoiden loispainetta 11:ssä eurooppalaisessa vesistössä, jossa molemmat simpukkatyypit esiintyvät rinnakkain. Paikallisia alkuperäislajeja edustivat simpukat *Anodonta cygnea*, *A. anatina*, *A. exculcerata*, *Pseudanodonta complanata*, *Unio tumidus* ja *U. pictorum*, mutta sekä alkuperäisten että vierassimpukoiden lajisto vaihteli vesistöstä toiseen. Kaksiosaisessa tutkimuksessa tavattiin kaikkiaan 16 loislajia/taksonia vesistökohtaisen lukumäärän vaihdellessa kolmesta kahdeksaan. Pohjoiseurooppalaisissa vesistöissä keskimääräinen loislajien määrä oli 2.3-kertainen – ja eri loistaksonien prevalenssien (=infektoituneiden yksilöiden osuus) summa 2.4-kertainen paikallisissa alkuperäissimpukkapopulaatioissa verrattuna vierassimpukkapopulaatioihin. Eteläeurooppalaisissa vesistöissä vallitsi sama trendi vastaavien loispainemittareiden osoittaessa 3.4- ja 2.6-kertaista tasoa alkuperäissimpukoilla vieraslajeihin verrattuna. *C. fluminea* -vierassimpukalta ei Euroopasta tavattu yhtään loista. Nämä tulokset tukevat *Enemy Release* -hypoteesin ennustetta, että loispaine on vieraslajeilla alhaisempi kuin alkuperäisillä simpukoilla, mikä voi antaa kilpailuedun vierassimpukoille.

Toiseksi tutkin *Enemy Release* -hypoteesin ennustetta, että vieraslajit menettävät vihollisiaan invaasioprosessin aikana, vertaamalla kahden vierassimpukan (*S. woodiana* ja *C. fluminea*) loispainetta niiden alkuperäisellä elinalueella Kiinassa (5 vesistöä) ja niiden "valloittamalla" alueella Euroopassa (11 vesistöä). Loistak-

sonien kokonaismäärä *S. woodiana* -lajilla Kiinassa oli 8 ja keskimääräinen populaation loislajimäärä 2,1-kertainen ja keskimääräinen loislajien prevalenssien summa 3,0-kertainen Eurooppaan verrattuna. *C. fluminea* -vierassimpukalta löydettiin Kiinassa 7 loislajia. Keskimääräinen loislajimäärä kiinalaisissa populaatioissa oli 1,3 ja prevalenssien summa 13,8. Sen sijaan eurooppalaisista populaatioista ei tavattu yhtään loista.

Väitöskirjani tulokset viittaavat loispaineen vähenemiseen invaasioprosessin seurauksena tutkituilla vierassimpukkalajeilla. Tulokset viittaavat lisäksi siihen, että ainakin eurooppalaisissa vesistöissä vierassimpukoihin kohdistuva loispaine on alhaisempi kuin alkuperäisiin simpukkalajeihin kohdistuva loispaine. Nämä tulokset tukevat *Enemy Release* -hypoteesia ja viittaavat siihen, että alhainen loispaine voi osaltaan auttaa vierassimpukoiden menestymistä.

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ORIGINAL PAPERS

I

PARASITES IN SYMPATRIC POPULATIONS OF NATIVE AND INVASIVE FRESHWATER BIVALVES

by

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Parasites in sympatric populations of native and invasive freshwater bivalves

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Abstract An increasing threat to local, native freshwater mussels (Unionida)—an ecologically important but globally alarmingly declining group—is the invasion by exotic bivalves. The Enemy Release Hypothesis predicts that introduced species should benefit from enemy-mediated competition because they are less likely to be harmed by natural enemies, such as parasites, than their native competitors. We investigated within-site differences in parasitism between sympatric native (tot. five spp.) and invasive

(tot. three spp.) bivalves in eight northern European waterbodies, which harboured totally 15 parasite taxa. In paired comparisons using within-site averages, the mean number of parasite species in the native bivalves was 2.3 times higher, and the sum of parasite prevalences 2.4 times higher, than in the invasive bivalves. This may lead to enemy-mediated competitive release of invaders and contribute to the success of invasive freshwater bivalves, in general. However, while the invasive clam *Corbicula fluminea* was completely free from parasites, parasite parameters of the other invader, *Sinanodonta woodiana*, were relatively high, indicating that the role of parasites can be invader-specific and urges further research. Understanding the factors affecting success of freshwater bivalve invasions, such as parasitism, can aid invasion

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control and conservation of local, native (endangered) bivalves.

Keywords Enemy release · Exotic species · Freshwater mussels · Introduced alien species · Non-indigenous species · Parasite benefit

Introduction

Biological invasions threaten biodiversity and cause drastic changes in the invaded ecosystems, leading to local extirpation of native species, biogenic homogenization and extensive economic costs (e.g. Olden et al., 2004; Pimentel et al., 2005; Keller et al., 2011; Lockwood et al., 2013). The most supported explanation (see e.g. Blakeslee et al., 2013) for the success of invasive species (when they have colonized the new, invaded area) is provided by the Enemy Release Hypothesis that invaders benefit from lower pressure of natural enemies when compared to native species (Elton, 1958). Rationale behind the Enemy Release Hypothesis is that (1) natural enemies are harmful and can control populations of their host/target species, and that (2) natural enemies, such as parasites, frequently are specialized to utilize only a small number of host/target species, or even genotypes that are available in their local environment. Therefore, as (3) invaders usually do not bring their own enemies with them to the new area during the colonization process, it (4) results in a situation where the pressure by the natural enemies in the invaded area can be remarkably lower for the invaders than for the local, native species (as well as when compared to the parasite pressure of the invader in its original range). Since parasites and disease are able to control and regulate host populations (Anderson and May, 1979; May and Anderson, 1979; Hudson et al., 1998) and can be highly specialized (i.e., host specific) (Price, 1980), they provide a promising ‘enemy candidate’. Two extensive meta-analyses clearly show that the invaders (a) lose their own, natural parasites and pathogens when introduced to a new geographic area, and (b) that their colonization by new parasites in the introduced area does not make up that loss (Mitchell and Power, 2003; Torchin et al., 2003). The introduced animal populations were less heavily parasitized and had only half of the parasite species as compared to their

original, natural range (Torchin et al., 2003). In line with this, the invasive plant species which were more completely released from pathogens were more widely reported as harmful invaders (Mitchell and Power, 2003).

Many freshwater mussels (Unionoida) have declined dramatically, so that they are the most imperiled animal group in the world (Lydeard et al., 2004; Lopes-Lima et al., 2017). Invasive bivalves can remarkably harm the native mussels (Haag et al., 1993; Ferreira-Rodriguez et al., 2018), either directly—biofouling by e.g. zebra mussel *Dreissena polymorpha* (Pallas, 1771)—or indirectly via competition over food, space or host fish or by changing the bottom habitat (Sousa et al., 2014; Ożgo et al., 2020). Therefore, the introduction of invasive bivalves is a serious and increasing threat to local, native bivalves, as non-indigenous bivalve species expand their range at increasing pace (Sousa et al., 2014). In addition, when considering ecosystem functions, some freshwater bivalves can be ranked among the world’s most problematic biological invaders. For example, recent invasions of the zebra mussel *D. polymorpha* and Asian clam *Corbicula fluminea* (Müller, 1774) have altered entire aquatic ecosystems by their filtration and burrowing activities, and they have severely affected native bivalve communities by altering bottom habitats and competing for resources (Karatayev et al., 1997; Strayer et al., 1998; Ward and Ricciardi, 2007; Sousa et al., 2009; Higgins and Vander Zanden, 2010; Ferreira-Rodriguez et al., 2018; Modesto et al., 2019).

Successful establishment and further population growth of introduced freshwater bivalves has been linked, for example to fouling (*D. polymorpha* prefers shells of unionids over conspecifics), induction of cross-resistance (infection with glochidia of the Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) early in the season creates acquired immunity against native unionids in host fishes), potentially longer breeding season, high fecundity and a wide spectrum of suitable fish hosts (*S. woodiana*), as well as free-living instead of parasitic larva (*D. polymorpha*, *C. fluminea*) (Douda et al., 2012; Sousa et al., 2014; Yanovych 2015; Donrovich et al., 2016; Dzierżyńska-Białończyk et al., 2018; Labecka and Domagała, 2018; Urbańska et al., 2018, 2019). However, the role of parasites in success of bivalve invasions has not been thoroughly explored. This is surprising, considering that freshwater bivalves harbor a rich community of

parasites (Molloy et al., 1997; Grizzle and Brunner, 2009; Carella et al., 2016; Brian and Aldridge, 2019). Indeed, parasitism was recently named as one of the priority research topics on freshwater mussel conservation assessment (Ferreira-Rodríguez et al., 2019). Some of these parasites are capable of markedly reducing the reproductive output, growth and survival of the bivalve host (Taskinen and Valtonen, 1995; Taskinen, 1998b; Jokela et al., 2005; Müller et al., 2014). In addition, parasites of freshwater bivalves can be host specific and adapted to their local host population (Taskinen et al., 1991; Saarinen and Taskinen, 2005), a prerequisite of the Enemy Release Hypothesis. Thus, if occurring at a higher rate in the native bivalves than in the invasive competitors, parasites could benefit the invaders. Parasitism of invasive bivalves has been studied in the invaded areas (e.g. Molloy et al., 1997; Burlakova et al., 2006; Mastitsky et al., 2010; Karatayev et al., 2012; Cichy et al., 2016), and there is evidence for parasite escape, i.e., fewer parasites in the invaded area as compared to the original range in the case of *D. polymorpha* (Molloy et al., 1997), but to our knowledge their parasite pressure has not been compared to sympatric native competitors at the same site.

One prediction of the Enemy Release Hypothesis is that introduced species should benefit from enemy-mediated competition because they are less likely to be affected by natural enemies than their native competitors (Elton, 1958; Keane and Crawley, 2002). The studies examining enemy-mediated competitive release, i.e., that invasive species benefit from competitive release as the native competitors are preferred by (native) enemies, compared enemy prevalence or enemy effects on invasive and native species in the introduced range (see Prior et al., 2015). In a recent review, studying parasites of sympatric native and invasive bivalve populations was pointed as one of the most important research needs with respect to invasion threats in conservation of endangered freshwater mussels conservation (Brian and Aldridge, 2019). Therefore, the aim of this study was to compare parasite pressure in sympatric populations of native and invasive bivalves, i.e. within-site differences in parasite species richness and summed prevalence of infection (see e.g., Torchin and Lafferty, 2009). This study can potentially contribute to the conservation of imperiled native mussels, as it should increase our knowledge and understanding of bivalve-parasite

relationships, explore the explanations for success of invasions and possibly increase our ability to manage invasions.

Our study included eight European waterbodies which were inhabited by at least one of the three invasive bivalves—*C. fluminea* (Corbiculidae), *D. polymorpha* (Dreissenidae) and *S. woodiana* (Unionidae)—and at least one of the five native Unionidae mussels [*Anodonta anatina* (Linnaeus, 1758), *A. cygnea* (Linnaeus, 1758), *Pseudanodonta complanata* (Rossmässler, 1835), *Unio pictorum* (Linnaeus, 1758) and/or *U. tumidus* Philipsson, 1788]. *D. polymorpha* is originally from Black Sea and Caspian Sea drainages and has invaded Europe gradually since 1700s (Karatayev et al., 2007). *C. fluminea* and *S. woodiana* are both native to Asia and have colonized Europe since 1970–1980s (Watters, 1997; Lucy et al., 2012). Our hypothesis was that the invasive bivalves would have, on average, fewer parasite species and lower prevalence of infection than the native ones.

Materials and methods

Collection and examination of bivalves

The study was performed by collecting bivalves from sites where at least one native and one invasive bivalve species are living in sympatry, in the same particular site, to ensure that they are exposed to same parasites when they live in the same environment. Bivalve samples were collected from eight waterbodies, of which one was in Estonia and seven in Poland, between May and July during 2017–2018 (Table 1, Supplementary Tables 1–8). Collection was performed by hand picking (visual detection; with or without an aquascope), and—in Lake Gosławskie and Lake Siecino, Poland—also by snorkelling and scuba diving. The target sample size was 30 bivalve individuals per species per site. Characteristics of the study sites, with e.g., fish community and introduction of the invasive bivalves, are given in Supplementary Table 9.

Bivalves were stored on ice and examined fresh, except for samples from Lake Vörtsjärv, which were stored frozen. Parasite examination was performed by dissecting the bivalve, counting adult watermites (crawling on gills and mantle cavity), separating different tissues and examining the tissues, piece by piece, by pressing between two large glass plates and

Table 1 Occurrence (X) of the 15 observed parasite taxa in each native (*Anodonta anatina*, *Anodonta cygnea*, *Pseudanodonta complanata*, *Unio pictorum* and *Unio tumidus*) andinvasive (*Sinanodonta woodiana*, *Dreissena polymorpha* and *Corbicula fluminea*), all eight waterbodies combined

Bivalve	N_{sites}	N_{bivalves}	Trematoda sporocysts					Ac	Cl	N1	N2	Gly	Con	M1	M2	M3	Ra	Tot.
			Rf	Rc	Bp	Cd	Pm											
Native																		
<i>A.ana</i>	6	218	X	X	–	X	–	X	X	–	X	X	X	X	X	X	–	11
<i>A.cyg</i>	2	67	–	–	–	–	–	X	X	–	–	–	X	X	X	X	X	7
<i>P.com</i>	3	89	–	–	–	–	–	X	–	–	X	X	X	X	X	–	–	6
<i>U.pic</i>	4	136	–	–	–	–	–	X	X	–	–	X	X	X	X	X	X	8
<i>U.tum</i>	6	173	–	X	–	–	–	X	X	–	–	X	X	X	X	X	X	9
<i>N of host species</i>			1	2	0	1	0	4	5	0	1	4	5	5	5	5	3	
Mean±s.e.	5.0±0.8	136±27																8.2±0.9
Invasive																		
<i>S.woo</i>	4	121	–	X	–	–	–	–	X	–	–	X	–	X	X	X	–	6
<i>D.pol</i>	6	170	–	–	X	–	X	–	–	X	–	X	X	–	–	–	–	5
<i>C.flu</i>	2	85	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
Mean±s.e.	4.0±1.2	125±25																3.7±1.9
<i>N of host species</i>			0	1	1	0	1	0	1	1	0	2	1	1	1	1	0	
Tot. <i>N of hosts</i>			1	3	1	1	1	4	6	1	1	6	6	6	6	6	6	
<i>t</i> -test <i>p</i> -value	0.501	0.792																0.043

Numbers of waterbodies per bivalve species, the total number of individuals studied and the total number of observed parasite taxa per bivalve species are given in columns N_{sites} , N_{bivalves} and Tot., respectively, and for these parameters the average values (Mean ± s.e.) are given, as well as the *t* test result (2-sided *p*-value) comparing the means of native and invasive bivalve species. Number of host species observed per parasite taxa is given separately for the native and the invasive bivalves. In addition, the total number of host species for each parasite taxon (combining the invasive and native bivalves)—a measure of host specificity of the parasite—is given on the row Tot. *N of hosts*

Trematoda sporocysts = Tissue-dwelling, larval, (castrating) trematodes, Rf, *Rhipidocotyle fennica*; Rc, *Rhipidocotyle campanula*; Bp, *Bucephalus polymorphus*; Cd, *Cercaria duplicata* and Pm, *Phyllodistomum macrocotyle*; Ac, *Aspidogaster conchicola* (adult trematode), Cl, *Chaetogaster limmaei* (Oligochaeta), N1, unknown larval nematode in gonad, N2, unknown larval nematode in gills; Gly, *Glyptotendipes* sp. (Chironomidae); Con, *Conchophthirus* sp. (Ciliata)

M1 = larval/egg stages of watermites in the mantle, M2 = larval/egg stages of watermites in the gills, M3 = adult watermites, Ra = *Rhodeus amarus* (bitterling)

inspecting with a preparation microscope using transmitted light (Taskinen et al., 1991). The tissues examined included mantle, gills, digestive gland and gonad, which are the typical sites of parasites in freshwater bivalves (e.g., Edwards and Vidrine, 2013; Taskinen et al., 1997). Bivalve sex was determined microscopically (presence and abundance of oocytes in gonad), and larval production as percentage of gill marsupia filled with glochidia was evaluated. In addition, shell measurements (length, width, height) and age determination (from annual growth rings on

shell) were performed, but these aspects are not included in the present study. Numbers of parasites were counted or their abundance was estimated. If the mantle and gills were examined only from one side of the bivalves, the numbers were doubled. However, in the present analyses, only presence-absence data were used (prevalence of infection, i.e. the proportion of infected individuals), because summed prevalences have been previously used as a measure of parasite load in studies of Enemy Release Hypothesis (Lafferty et al., 2010; Torchin et al., 2003). Ciliates cannot be

reliably observed from frozen samples. Therefore, ciliate parasites were not recorded for Lake Vörtsjärv since the bivalves from this lake were stored frozen.

Statistical analyses

We used parasite taxa richness (number of parasite taxa) and sum of prevalences (sum of the prevalence of all parasite taxa for each host species) as the response variables as they have generally been used as the measures of parasite pressure in studies exploring ‘enemy release’ (Lafferty et al., 2010; Torchin et al., 2003). First, a site-specific analysis was performed to get an idea of the bivalve and parasite community in a given waterbody. This was done by comparing prevalences of infection of every possible parasite taxa for each pair of native vs. invasive bivalve species using χ^2 -test. If the number of cells with an expected count value lower than five was greater than one, a Yates continuity correction (Sokal and Rohlf, 1981) was applied.

In addition, if there was at least three native and invasive bivalve species in a given site, difference in the mean prevalence of a given parasite taxon between native and invasive bivalves was analysed using t-test (for example, see Gosławskie Lake, Supplementary Table 3). This approach did not differentiate the bivalve species. For instance, mean value for the invasive species *S. woodiana*, *D. polymorpha* and *C. fluminea* against the mean value of the native species *A. anatina*, *U. pictorum* and *U. tumidus* was used in the case of Gosławskie Lake, without taking into consideration possible differences between the three invasive or between the three native bivalve species. However, this approach was in line with our hypothesis, that “invasive bivalves would have, on average, fewer parasite species and lower prevalence of infection than the native ones”, which does not separate bivalve species within the native or invasive group. Detailed reports of these site-specific analyses, where all possible native vs. invasive bivalve combinations are analysed for each parasite taxon, are given in “Site-specific results” of the Supplementary materials and in Supplementary Tables 1–8.

Second, to get a comprehensive picture over the all studied waterbodies, the average number of parasite taxa and the average sum of prevalences pooled for all invasive vs. native bivalves per site were used as the response variables in comparisons performed over all

sites between invasive and native bivalves (for example, see Fig. 1). If only one native or invasive bivalve species was present, its values represented the average for native/invasive group in that lake. Then these averages were used in a *t*-test to analyse within-site differences in the mean average parasite taxa richness and summed prevalences between the native and invasive bivalves. In this approach, for each site, only those bivalve species were included in the statistical analyses for which the number of individuals was at least 15. This was done to avoid biases due to different sample size, because even though the aim was to collect and study 30 individuals per bivalve species per site, this was not always possible (see Table 1 and Supplementary Tables 1–8).

The second procedure (above) was also applied to the number of bivalve individuals collected to estimate the possible difference in sample sizes for the native and invasive bivalves. Mean (\pm s.e.) average number of bivalves studied was 32 ± 4 and 30 ± 3 for the native and invasive bivalves, respectively. The difference was statistically not significant indicating that sample sizes for the native and invasive bivalves over the eight study sites did not differ from each other (paired *t*-test, $n = 8$, $t = 0.506$, $df = 7$, $p = 0.629$) (see Supplementary Tables 1–8 for details).

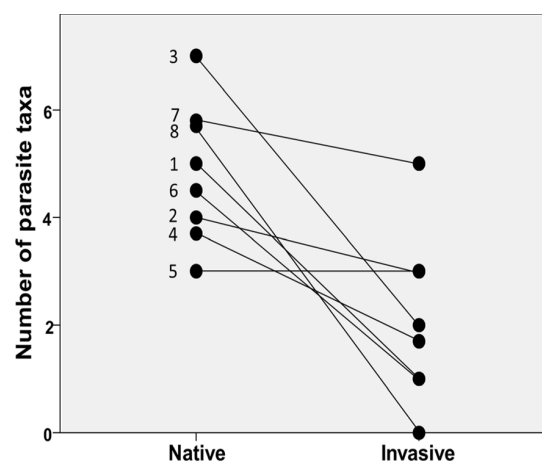


Fig. 1 Mean parasite taxa richness for native and invasive freshwater bivalves in eight northern European waterbodies. 1—Lake Vörtsjärv, 2—Czarny Młyn Pond, 3—Warta-Gopło Canal, 4—Gosławskie Lake, 5—Nowy Lipsk Pond, 6—Siecino Lake, 7—Szeszupa River, 8—Rospuda River. Values connected by a line belong to the same study site. Mean number of parasite taxa was significantly higher in native bivalves as compared to invasive bivalves (paired *t*-test, $p = 0.008$)

Differences were regarded as statistically significant, when $p < 0.050$. When $0.050 \leq p \leq 0.099$, the difference was interpreted as marginally significant, indicating that there is a trend, but the risk of wrong conclusion is increased.

Results

Five native (*A. anatina*, *A. cygnea*, *P. complanata*, *U. pictorum* and *U. tumidus*) and three invasive (*S. woodiana*, *D. polymorpha* and *C. fluminea*) freshwater bivalve species and a total of 15 parasite taxa (*Rhipidocotyle fennica* Gibson et al., 1992, *R. campanula* (Dujardin, 1845), *Bucephalus polymorphus* von Baer, 1827, *Cercaria duplicata* von Baer, 1827, *Phyllodistomum macrocotyle* (Lühe, 1909), *Aspidogaster conchicola* von Baer, 1827, *Chaetogaster limnaei* von Baer, 1827, unknown larval nematode from gonad, unknown larval nematode from gills, *Glyptotendipes* sp. (Lenz), *Conchophthirus* sp. (Claparède and Lachmann), larval/egg stages of watermites in the mantle, larval/egg stages of watermites in the gills, adult watermites and larvae of bitterling *Rhodeus amarus* (Bloch, 1872) were discovered from the eight waterbodies (Table 1).

Mean (\pm s.e.) average parasite taxa richness over the eight study sites was 2.3 times higher for the native than for the invasive bivalves (4.8 ± 0.5 vs. 2.1 ± 0.6 taxa; range 3–7 and 0–5 taxa, respectively), the difference being statistically significant (paired t test, $n = 8$, $t = 3.703$, $df = 7$, $p = 0.008$) (Fig. 1). Furthermore, the mean (\pm s.e.) average summed prevalence of infections of different parasites over the eight study sites was 2.4 times higher for the native than for the invasive bivalves (151 ± 27 vs. 64 ± 24 %, range 43–264 and 0–170 %, respectively), the difference being statistically significant (paired t test, $n = 8$, $t = 2.378$, $df = 7$, $p = 0.049$) (Fig. 2).

Anodonta anatina ($n_{\text{tot}} = 218$ individuals) was found from six out of eight sites and harboured in total 11 different parasite taxa, while *A. cygnea* ($n_{\text{tot}} = 67$) was found from two waterbodies and harboured altogether seven parasite taxa (Table 1). Corresponding figures for *P. complanata* ($n_{\text{tot}} = 89$) were three lakes/ rivers and six parasite taxa, for *U. pictorum* ($n_{\text{tot}} = 136$) four sites and eight parasite taxa and for *U. tumidus* ($n_{\text{tot}} = 173$) six waterbodies and nine parasite taxa (Table 1). Of the invasive mussels, *S. woodiana*

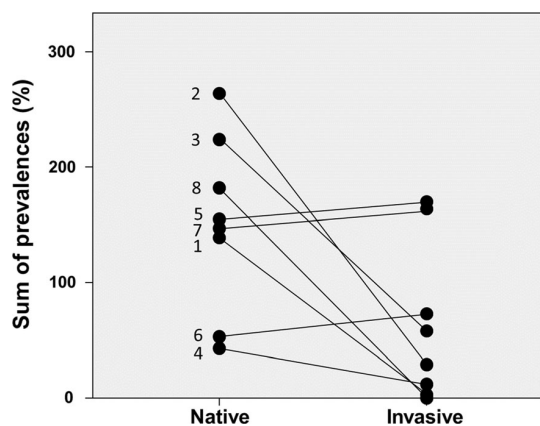


Fig. 2 Mean sum of prevalences of infection of different parasites for native and invasive freshwater bivalves in eight northern European waterbodies. 1—Lake Vörtsjärvi, 2—Czarny Młyn Pond, 3—Warta-Gopło Canal, 4—Gosławskie Lake, 5—Nowy Lipsk Pond, 6—Siecino Lake, 7—Szeszupa River, 8—Rospuda River. Values connected by a line belong to the same study site. Mean sum of infection prevalences of parasites was significantly higher in native bivalves than in the invasive ones (paired t -test, $p = 0.049$)

($n_{\text{tot}} = 121$ individuals) was found from four lakes/ rivers and harboured six different parasite taxa, and *D. polymorpha* ($n_{\text{tot}} = 170$) was found from six waterbodies and harboured five parasite taxa, while *C. fluminea* ($n_{\text{tot}} = 85$) was found from two sites and did not harbour any parasites (Table 1). The total parasite pool infecting native mussels consisted of 12 taxa while that of invasive bivalves included 10 parasite taxa (Table 1). When combined over the eight waterbodies, the total number of parasite taxa infecting each native and invasive mussel species varied from six to 11 and from zero to six per bivalve species, respectively (Table 1). The mean (\pm s.e.) total number of observed parasite taxa for native and invasive bivalves (per bivalve species, combined over all sites) was 8.2 ± 0.9 and 3.7 ± 1.9 , respectively, the difference being statistically significant (t -test, $t = 2.553$, $df = 6$, $p = 0.043$) (Table 1).

Site-specific results with parasite taxon-specific comparisons between all possible combinations of native and invasive mussels revealed statistically significant differences with respect to all parasite taxa other than *B. polymorphus*, *C. duplicata* and the unknown nematodes from gonad and gills (Supplementary Tables 1–8).

Discussion

Detailed discussion on results of each of the eight sites is provided in Supplementary Materials. In addition, parasite-specific discussion with respect to life cycles, hosts, host specificity and earlier findings of geographic distributions (Dimock and LaRochelle, 1980; Davids et al., 1988; Taskinen et al., 1991; Gibson et al., 1992; Conn et al., 1996; Vidrine, 1996; Fried and Graczyk, 1997; Molloy et al., 1997; Taskinen, 1998a; Jokela et al., 2005; Edwards and Vidrine, 2006; Ibrahim, 2007; Reichard et al., 2007; Mastitsky et al., 2010; Mastitsky and Veres, 2010; Edwards and Vidrine, 2013; Cichy et al., 2016; Rouchet et al., 2017) is also given in Supplementary Materials.

Enemy Release Hypothesis (Elton, 1958) suggests that invasive species should lose parasites during the invasion process, which should lead to a lower parasite load in the new, introduced range, benefitting the invaders in competition against the local, native competitors. Large body of empirical evidence indicates that the invaders, indeed, are frequently released from parasites when introduced to new area (Mitchell and Power, 2003; Torchin et al., 2003). One prediction of the Enemy Release Hypothesis is that introduced species should benefit from enemy-mediated competitive release because they are less likely to be affected by natural enemies than their native competitors (Elton, 1958; Keane and Crawley, 2002). Therefore, we compared parasite pressure of invasive and native freshwater bivalves living in sympatry. The overall mean site-specific average parasite taxon richness and sum of prevalences were, both, more than two times higher among the native bivalves than in the invasive bivalves. Site-specific comparisons between pairs of native and invasive bivalves at individual parasite taxon level frequently also indicated higher parasite prevalence in the native bivalve than in the invasive counterpart. Thus, the results support the Enemy Release Hypothesis and the view that the invasive bivalves benefit from a lower parasite pressure than the sympatric populations of native species.

A prerequisite of enemy-mediated competitive release is that parasitism is a cost, i.e., parasites must be harmful. Larval trematodes using bivalve as the 1st intermediate host (bucephalids, gorgoderids) are inevitably very harmful, causing partial or complete sterility, parasite-induced mortality and lowered growth (Davids and Kraak, 1993; Taskinen and

Valtonen, 1995; Taskinen, 1998b; Jokela et al., 2005; Müller et al., 2014). Adult watermites ingest mucus and tissues of their bivalve host (Fisher et al., 2000). Results by Gangloff et al. (2008) suggest that ectoparasitic adult watermites (*Unionicola* spp.) and ectoparasitic adult trematodes (*Aspidogaster* spp.) are strongly negatively associated with both physiological condition (glycogen) and reproductive output of bivalve. Numbers of eggs/larval stages of watermites in the present study were very high—up to thousands of eggs/larval mites embedded in mantle tissue of a single mussel individual, for example, suggesting potential for high virulence. The effect of echinostomatid trematode metacercariae on mussel host is usually benign (Laruelle et al., 2002), but bitterling (*R. amarus*) embryos can be costly to a mussel (Reichard et al., 2006). Therefore, many of the parasites of the present study are potentially costly to host bivalve, and the higher parasite prevalences in native bivalves should benefit the invasive competitors.

Another prerequisite of enemy-mediated competitive release is that there must be competition between native and invasive bivalves. Freshwater bivalves can be assumed to compete with each other as they all are benthic filter feeders, thereby sharing the same ecological niche in terms of food and space. As mentioned earlier, invasive bivalves can remarkably harm native mussels either directly (biofouling by e.g. zebra mussel *Dreissena polymorpha*) or indirectly via competition over food, space or host fish or by changing the bottom habitat (reviewed by Sousa et al., 2014). Competition between native and invasive mussels is indicated by the negative correlation between their densities on local scale (Vaugh and Spooner, 2006) and illustrated, for example, by the rapid decline of native mussels after introduction of *Dreissena* to North American Great Lakes (Strayer and Malcom, 2007).

Invasive species can acquire parasites in the invaded area either by bringing them along themselves or by infection by parasites of the native species. If bivalves are introduced as larvae, probability that they transport their own parasites to the invaded area is close to zero. Introduction of *D. polymorpha* to North America and *S. woodiana* to Europe have happened presumably by veliger and glochidium larvae, respectively (Watters, 1997; Karatayev et al., 2007), though the introduction and spread of *D. polymorpha* in Europe might have also occurred at an adult stage

(Burlakova et al., 2006). Consequently, North American *D. polymorpha* populations have no records of original parasites of *D. polymorpha* while those in Europe have (Burlakova et al., 2006) and they were also observed in the present study (trematodes *B. polymorphus* and *P. macrocotyle*). Based on the few studies on invasive hosts and parasites, adaptation of novel hosts/parasites to those in the introduced region is, in general, a long process (Taraschewski, 2006), but Karatayev et al. (2012) observed that *D. polymorpha* has acquired echinostomatids from native hosts in North America 26 year after it was first time recorded. Thus, it is possible that some of the parasites of native mussels have jumped to *D. polymorpha* also in Europe during the up to 200 years of invasion. Results of Petkeviciute et al. (2014) support the view that the gorgoderid trematode *P. macrocotyle* is a specific parasite of *D. polymorpha*, while *C. duplicata* is specific to unionid mussels. Similarly, Taskinen et al. (1991) concluded that the bucephalid trematode *B. polymorphus* is a specific parasite of *D. polymorpha*, while *R. fennica* and *R. campanula* are specific to unionids. So, at least *B. polymorphus* and *P. macrocotyle* were brought to Europe by *D. polymorpha*—they did not jump to *D. polymorpha* from native mussels—meaning that *D. polymorpha* was not released from these two enemies during the invasion process. In spite of this, the prevalence of infection was always (when possible to analyse) higher in the native counterpart(s) than in *D. polymorpha*—except for *P. macrocotyle*, a *Dreissena*-specific trematode, and *Conchophthirus* ciliate—in Szeszupa River (Suppl. Table 7). There are at least five *Dreissena*-specific ciliate parasites including *Conchophthirus acuminatus* (Claparède and Lachmann, 1858) and *C. klimentinus* (Molloy et al., 1997). Thus, it is possible that the high *Conchophthirus* infection of *D. polymorpha* in Szeszupa River was caused by a ciliate species specific to *D. polymorpha*.

In addition to almost always lower parasite load in *D. polymorpha* (except for the *D. polymorpha*-specific trematodes), when compared to native bivalves the complete absence of parasites in *C. fluminea* was striking (Suppl. Table 3, 4) and strongly supports the view of parasite benefit for *C. fluminea* when competing with native bivalves. In its original range, *C. fluminea* has been reported to host *Phyllodistomum mingensis* (Tang, 1985), five species of *Echinostoma* (Keeler and Huffman 2009) and seven species of

Aspidogastrea (Tang, 1992). Parasite escape by introduced *C. fluminea* has been observed also in North America. Despite a long history of *C. fluminea* research since their introduction in 1930s, only the host-generalist aspidogastrid trematodes *A. conchicola* and *Cotylapsis insignis* Leidy, 1857, have been reported from North America (Danford and Joy, 1984). In addition, Karatayev et al. (2012) also did not find any parasites from *C. fluminea* from Great Lakes area except echinostomatid metacercariae in one out of 27 sites. Thus, besides the European waterbodies studied here, *C. fluminea* seem to enjoy a remarkable reduction of parasite pressure also in other regions it has invaded globally.

A meta-analysis revealed that a significantly higher extent of parasite escape is evident in aquatic than in terrestrial environments, possibly due to less frequent introductions per invader and introduction as a larval form free of parasites (Lafferty et al., 2010). Results by Mastitsky et al. (2010) indicate that over one third of invasive freshwater invertebrates did not successfully introduce any of their coevolved parasites to the invaded areas. Our results suggest that in the introduced range, the invasive freshwater bivalves may enjoy lower parasite pressure, i.e., have lower number of parasite species and lower combined prevalence of infection, than their native counterparts, giving a ‘parasite benefit’ for the invasive bivalves in competition against the native ones. However, the within-site variation between the native species was also relatively high in our study—not all native species were always strongly infected. In addition, variation between the invasive species was also large, from not-a-single-parasite in the Asia clam *C. fluminea* to considerably high values of parasite parameters in the Chinese pond mussel *S. woodiana* in Warta-Gopło Canal and Nowy Lipsk Pond (Suppl. Table 3, 5). This will rise many questions, for example: Are the parasites of European unionids better able to infect another unionid, *S. woodiana*, than phylogenetically distinct dreissenids or corbiculids—even if *S. woodiana* is a recent invader compared to e.g. *Dreissena*? Therefore, to better understand the role of parasites in invasions of freshwater bivalves, the importance of parasitism should be studied in relation to the genetic (and geographic) distance between the introduced and native bivalves, and in relation to time since introduction and routes of invasions—from both the host bivalves’ and parasites’ perspectives.

Freshwater mussels Unionida are important in maintaining aquatic biodiversity and ecosystem functions (Vaughn, 2018). Invasive bivalves can have devastating effects on the native mussels and on the whole freshwater ecosystems (Higgins and Vander Zanden, 2010; Sousa et al., 2014). So, research on the interplay between parasites, bivalves and invasions will potentially increase our understanding how host-parasite relationships are shaping the outcomes of invasions. This may thereby possibly contribute to our ability to manage invasions and protect imperiled native bivalves and aquatic ecosystems.

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Parasites in sympatric populations of native and invasive freshwater bivalves

Taskinen et al.

Supplementary materials

Site-specific results

Lake Võrtsjärv, Estonia: One native (*A. anatina*) and one invasive bivalve species (*D. polymorpha*) was discovered from this lake with numbers of ≥ 15 individuals (Supplementary Table 1). Five parasite taxa were found from the native mussel *A. anatina* and one from the invasive *D. polymorpha* (Supplementary Table 1). Summed prevalences of infection for *A. anatina*, and *D. polymorpha* were 139 and 3 %, respectively (Supplementary Table 1). Statistically significant differences in parasite-specific prevalences of infection between *A. anatina* and *D. polymorpha* were detected in the case of the castrating trematode, *R. fennica*, in gonads, and in larval/egg stages of watermites in mantle— with higher prevalences in the native *A. anatina* than in the invasive *D. polymorpha* (Supplementary Table 1). In addition to those given in Table 1, the catch from Lake Võrtsjärv included four *U. pictorum* individuals, which were infected (prevalence in parentheses) with three parasite taxa; larval/egg stages of watermites in the mantle (25 %) and in the gills (25 %) and adult watermites (50 %). The only statistically significant parasite specific difference between *U. pictorum* and *D. polymorpha* was that of adult watermites, with a higher prevalence in the native *U. pictorum* (χ^2 -test, $df_{\text{continuity corrected}} = 10.731, p = 0.001$). Ciliates could not be reliably studied from these mussels due to the frozen samples, and were excluded from statistical analyses concerning this site.

Czarny Młyn Pond, Poland: One native (*A. cygnea*) and one invasive mussel species (*S. woodiana*) was discovered from this lake in numbers of ≥ 15 individuals (Supplementary Table 2). Total number of parasite taxa of *A. cygnea* and *S. woodiana* was

four and one, respectively, and summed prevalences of infection as 264 and 29 %, respectively (Supplementary Table 2). When *A. cygnea* and *S. woodiana* were compared by each parasite taxon, statistically significant, or marginally significant differences in parasite-specific prevalences of infection were detected in all four parasite taxa that occurred in these mussels—larval/ egg stages of watermites in the mantle and in the gonad, adult watermites and bitterling— with always a higher prevalence in *A. cygnea* than in the *invasive* *S. woodiana* (Supplementary Table 2). In addition to those given in Table 1, the catch from Czarny Młeyn Pond included six *U. pictorum* individuals, which were infected (prevalence in parentheses) with one parasite taxon; the castrating trematode *R. fennica*, with a prevalence of (17 %) which did not differ from *R. fennica* prevalence in *S. woodiana* (0 %) (χ^2 -test, $df_{\text{continuity corrected}} = 0.863$, $p = 0.353$).

Warta-Gopło Canal, Poland: Three native (*A. anatina*, *U. pictorum* and *U. tumidus*) and three invasive bivalve species (*S. woodiana*, *D. polymorpha* and *C. fluminea*) were discovered from this lake in numbers of ≥ 15 individuals (Supplementary Table 3). Number of parasite taxa varied among the native bivalves from six to nine and within the invasive bivalves from zero in *C. fluminea* and one in *D. polymorpha* to five in *S. woodiana*. Mean number of parasite taxa was 3.5 times higher in the native bivalves than in the invasive ones, the difference being statistically almost significant (Supplementary Table 3). Sum of prevalences varied from 150 to 282 % in the native bivalves and from zero to 170% in the invasive ones, so that the mean summed prevalence was almost four times higher in the native bivalves than in the invasive ones, the difference being statistically marginally significant (Supplementary Table 3). The mean (\pm s.e) prevalence of *Conchophthirus* ciliates was statistically significantly higher (76 ± 12 % vs. 0 %) among the three native bivalves than in the three invasive bivalves (Supplementary Table 3). Parasite-specific differences using χ^2 -test between each native and invasive bivalve were always (when applicable) statistically

significant or almost significant in the case of *Conchophthirus*, *Aspidogaster* and larval/egg stages of watermites in gills, with a higher prevalence in the native bivalve—as well as in most cases of bitterling (Supplementary Table 3). When the castrating trematode *R. campanula* and the parasitic chironomid *Glyptotendipes* were analysed, a higher prevalence in the native bivalve was evident in *U. tumidus/C. fluminea* and *U. pictorum/C. fluminea* combinations (Supplementary Table 3) This was also the case with respect to majority of native vs. invasive comparisons of adult watermites and larva/egg stages of watermites in the mantle, with a significantly higher prevalence of infection in the native bivalves than in the invasive bivalves (Supplementary Table 3). However, a notable exception to this was *S. woodiana*, in which the prevalence of infection was significantly higher than in the native counterpart when compared to *U. pictorum* (adult watermites) and *U. tumidus* (adult watermites and larval/egg stages of watermites in the mantle) (Supplementary Table 3). Finally, all comparisons with respect to *Chaetogaster* oligochaete which were statistically significant, signaled a higher infection prevalence in the invasive *S. woodiana* than in the native bivalves (Supplementary Table 3). Significantly higher prevalence in *S. woodiana* was also observed in *U. tumidus-S. woodiana* (larval/egg stages of watermites in mantle) and *U. pictorum-S. woodiana* (adult watermites) and *U. tumidus-S. woodiana* (adult watermites) comparisons.

In addition to those given in Supplementary Table 3, the catch from Warta-Gopło Canal included seven *A. cygnea* individuals, which were infected (prevalence in parentheses) with six parasite taxa; *Aspidogaster* (29 %), *Chaetogaster* (29 %), *Conchophthirus* (29 %), larval/egg stages of watermites in mantle (43 %) and in gills (100 %) and adult watermites (100 %), none of which differing statistically significantly from respective prevalences in *S. woodiana*., but all or almost all comparisons with respect to these

parasites showed significantly higher values in *A. cygnea* than in the other invasive bivalves, *D. polymorpha* and *C. fluminea* (χ^2 -test, $p \leq 0.037$).

Gosławskie Lake, Poland: Three native (*A. anatina*, *U. pictorum* and *U. tumidus*) and three invasive bivalve species (*S. woodiana*, *D. polymorpha* and *C. fluminea*) were discovered from this lake in sufficient numbers ($n \geq 15$) (Supplementary Table 4). Number of parasite taxa varied among the native bivalves from three to four and within the invasive bivalves from zero in *C. fluminea* and *D. polymorpha* to five in *S. woodiana*. Mean number of parasite taxa was 2.2 times higher in the native bivalves than in the invasive ones, but the difference was statistically not significant (Supplementary Table 4). Sum of prevalences varied from 34 to 60 % in the native bivalves and from zero to 36 % in the invasive ones, so that the mean summed prevalence was 3.6 times higher in the native bivalves than in the invasive ones, the difference being statistically marginally significant (Supplementary Table 4). None of the parasite-specific t-tests revealed statistically significant differences in the mean prevalences of infection between the native and invasive bivalve species (Supplementary Table 4). Parasite-specific differences analysed using χ^2 -test between each pair of native and invasive bivalves were statistically significant in the case of adult watermites when *A. anatina* was compared to any of the invasive bivalves, and mostly in the case of *Glyptotendipes* chironomid when *U. pictorum* was compared to invasive bivalves *D. polymorpha* and *C. fluminea*, as well as frequently in the case of the castrating trematode *R. campanula* when *U. tumidus* was compared to *D. polymorpha* and *C. fluminea*—always with a higher prevalence in the native bivalve (Supplementary Table 4). In all of the statistically significant χ^2 -test comparisons the native bivalve had a higher prevalence of infection than the invasive counterpart.

Nowy Lipsk Pond, Poland: Four native (*A. anatina*, *A. cygnea*, *U. pictorum* and *U. tumidus*) and one invasive mussel species (*S. woodiana*) was discovered from this lake in

sufficient numbers ($n \geq 15$) (Supplementary Table 5). Number of parasite taxa in *S. woodiana* was one, but varied from one to five among the native mussels. Mean number of parasite taxa in the native mussel species was the same as in the invasive *S. woodiana* (Supplementary Table 5). Parasite-specific analyses (using χ^2 -test) between each native and invasive mussel indicated that the prevalence of adult watermites was higher in the native *A. anatina* and *A. cygnea* than in *S. woodiana*, and that also the prevalence of larval/egg stages of watermites in gills was higher both in *A. cygnea* and in *U. tumidus* than in *S. woodiana* (Supplementary Table 5). However, prevalence of infection was statistically significantly or almost significantly higher in *S. woodiana* than in *U. pictorum* with respect to watermite larval/egg stages in the mantle and in the gills, and with respect to adult water mites. Significantly higher prevalence in *S. woodiana* was evident also in larval/egg stages of watermites in the mantle when compared to *U. tumidus* (Supplementary Table 5).

Siecino Lake, Poland: Two native (*U. tumidus* and *P. complanata*) and one invasive mussel species (*D. polymorpha*) was discovered from this lake in sufficient numbers ($n \geq 15$) (Supplementary Table 6). Number of parasite taxa in *D. polymorpha* was only one, but varied from three to six in the native mussels. Parasite-specific analyses (using χ^2 -test) on paired combinations of each native and invasive mussel indicated that the prevalence of *Chaetogaster* and *Glyptotendipes* was significantly or almost significantly higher in the native *U. tumidus* than in *D. polymorpha*. In addition, the prevalence adult watermites was significantly higher in the native *P. complanata* than in *D. polymorpha* (Supplementary Table 6). However, prevalence of *Conchophthirus* ciliate was significantly higher in the invasive *D. polymorpha* than in either of the native mussels (Supplementary Table 6).

Szeszupa River, Poland: Four native (*A. anatina*, *U. pictorum*, *U. tumidus* and *P. complanata*) and one invasive bivalve species (*D. polymorpha*) was discovered from this lake in sufficient numbers ($n \geq 15$); *D. polymorpha* with a slightly lower abundance than the

native bivalve species (Supplementary Table 7). Number of parasite taxa in *D. polymorpha* was five, and varied from five to seven in the native bivalves. Parasite-specific analyses (using χ^2 -test) on paired combinations of each native and invasive bivalve indicated that the prevalence of adult watermites was significantly higher in the native bivalves *A. anatina* and *P. complanata* than in *D. polymorpha*, and that the prevalence of larval/egg stages of watermites in the mantle was higher in *A. anatina*, *U. pictorum* and *P. complanata* than in the invasive *D. polymorpha*. However, prevalence of *P. macrocotyle*, a parasite specific to *D. polymorpha*, was significantly higher in *D. polymorpha* than in any of the native bivalves. In addition, prevalence of the ciliate parasite *Conchophthirus* was significantly or marginally significantly higher in *D. polymorpha* than in three of the four native bivalves (Supplementary Table 7).

Rospuda River, Poland: Three native (*A. anatina*, *U. tumidus* and *P. complanata*) and one invasive mussel species (*D. polymorpha*) was discovered from this lake in sufficient numbers ($n \geq 15$), but with varying numbers so that the abundances of *U. tumidus* and *D. polymorpha* were quite low (Supplementary Table 8). Number of parasite taxa was between five and six in the native mussel species but zero in the invasive *D. polymorpha*. Parasite-specific analyses (using χ^2 -test) on paired combinations of each native and invasive mussel indicated that the zero prevalence in *D. polymorpha* was statistically significantly lower than in any of the native mussels in the case of *Conchophthirus* ciliate, and significantly or marginally significantly lower than in *A. anatina* also in the case of *Aspidogaster* and *Chaetogaster* parasites (Supplementary Table 8). Furthermore, statistically significantly or marginally significantly lower prevalence of infection in the invasive *D. polymorpha* was also revealed with respect to larval/egg stages of watermites in the mantle and in the gills (*U. pictorum*, *U. tumidus*), and in larval/egg stages and adult watermites in gills (*P. complanata*) (Supplementary Table 8). As *D. polymorpha* had no parasites, in all of

the statistically significant χ^2 -test comparisons the native mussel had, naturally, a higher prevalence of infection than the invasive counterpart.

In addition to those given in Supplementary Table 8, the catch from Rospuda River included eight *A. cygnea* individuals, which were infected (prevalence in parentheses) with six parasite taxa; *Aspidogaster* (13 %), *Chaetogaster* (25 %), *Conchophthirus* (63 %), larval/egg stages of watermites in mantle (75 %) and in gills (38 %) and adult watermites (13 %). The prevalence of *Conchophthirus* and larval/egg stages of watermites in the mantle and in the gills, was statistically significantly higher in *A. cygnea* than in the invasive *D. polymorpha* (χ^2 -test, $p \leq 0.050$).

Discussion about site-specific results from parasitological point of view

Rich parasite community, a total of 15 taxa—representing several phyla, varying virulence and diverse types of life cycles—was inhabiting the freshwater bivalves of the present study. Bucephalid trematodes (*Bucephalus*, *Rhipidocotyle*) and gorgoderid trematodes (*Phyllodistomum*, *Cercaria duplicata*) use bivalve as their 1st intermediate host where they occur as the sporocyst stage, producing large numbers of free-living larvae, cercariae, and have a total of three different hosts in their life cycle, usually a fish or amphibian as the 2nd intermediate and definitive host (Fried and Graczyk 1997; Gibson et al. 1992; Molloy et al. 1997; Taskinen 1998a; Taskinen et al. 1991). Trematode metacercariae found from gonad and gills are probably echinostomatids. For example, *Echinoparyphium recurvatum* (Linstow, 1873) infects both native unionids and *D. polymorpha* in Europe (Mastitsky and Veres 2010). *Aspidogaster* trematode requires only single host to complete its life cycle, and is not host specific, infecting widely both unionids and dreissenids (e.g., Molloy et al. 1997). *Chaetogaster limnaei* oligochaete is also a host generalist, infecting not only bivalves but also aquatic snails (Conn et al. 1996; Ibrahim 2007). *Glyptotendipes* is a chironomid larva which

was earlier found from Polish lakes, including Lake Gosławskie, by Cichy et al. (2016). Ciliate taxon, called here as *Conchophthirus* after the study of Cichy et al. (2016) is a microscopic protozoan. There are at least five species of ciliates, including two *Conchophthirus* species, specific to dreissenid mussels (Molloy et al. 1997). Watermites were the most prevalent group of parasites in this study. All of the European watermites parasitizing mussels belong to the genus *Unionicola* (Edwards and Vidrine 2013). We have separated two different larval/egg watermite ‘taxa’, one in the mantle and second in the gills, which is justified by the fact that they are different species of *Unionicola* that exploits mantle and gills as oviposition site (Vidrine 1996). Thus, together with the adult watermites, we had three different functional groups which we regarded as ‘taxa’. Diversity of *Unionicola* watermites is overwhelming (244 species), there can be as many as five species infecting a single mussel individual and strong host specificity is a common feature of *Unionicola* (Dimock and LaRochelle 1980; Edwards and Vidrine 2006; Edwards and Vidrine 2013). For example, adult *U. ypsilophora* (Bonz, 1783) colonizes only *A. cygnea* while *U. intermedia*, Koenike, can colonize both *A. cygnea* and *A. anatina*, but *U. ypsilophora* will exclude any co-occurring *U. intermedia* from *A. cygnea* (Davids et al. 1988). Life cycle of *Unionicola* watermites is very complex. Females deposit eggs in specific tissues of the host, larvae emerge from eggs in spring and summer and are briefly parasitic on chironomids, to invade the mussel, embed in host tissues and enter the transitional nymphochrysalis stage from which the sexually immature nymphs emerge. Nymphs enter a transitional teleochrysalis stage from which the sexually mature adult (imago) emerges (Edwards and Vidrine 2013). Finally, there was also a parasitic fish, *Rhodeus amarus*, a European bitterling species, infecting the present bivalves. *R. amarus* deposits eggs to the gill chamber of unionid mussels. It is known that *Rhodeus* species are adapted to local host fish population (Rouchet et al. 2017), and that the invasive mussel *S. woodiana* is able to eject eggs of *R. amarus* better

than the European native mussels (Reichard et al. 2007). The diverse parasite fauna of bivalves (as many as nine parasite taxa in *U. tumidus* in Warta-Gopło Canal) and high frequency of occurrence (summed prevalence as high as 330 in *A. cygnea* of Warta-Gopło Canal) observed in the present study emphasizes the importance and the potential impact that parasites have on the bivalves. The higher the number of parasite taxa per bivalve, the stronger the challenge for the bivalve's immune system, physiological state and condition and presumably is (see e.g., Jokela et al. 2005).

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Supplementary Tables

Supplementary Table 1. Prevalences (%) of infection of parasites—*Rhipidocotyle fennica*, *Cercaria duplicata*, *Phyllodistomum macrocotyle* and watermites—in native (*Anodonta anatina* and *Unio pictorum*) and invasive (*Dreissena polymorpha*) freshwater bivalves of Lake Võrtsjärv, Estonia, with the number of studied bivalves (n), total number of parasite taxa (N taxa) and sum of prevalences of different parasites per bivalve species (Prev. sum), and 2-sided χ^2 -test comparing the parasite-specific prevalences between *A. anatina* and *D. polymorpha* (Aa-D). In the two comparisons in which a significant difference in the prevalence of infection was observed, the prevalence of infection was higher in the local bivalve than in the invasive counterpart. Unlike the other waterbodies, *Conchophthirus* ciliates could not be studied from Lake Võrtsjärv since the samples were stored frozen.

Lake Võrtsjärv	<i>Rhipid.</i> <i>fenn.</i> Trem Lar Gonad	<i>Cerc.</i> <i>duplic.</i> Trem Lar Gonad	<i>Phyllod.</i> <i>macroc.</i> Trem Ad Gonad	Water- mite L,E Mantle	Water- mite L,E G	Water- mite Ad, G Ecto	N taxa	Prev. sum
Native								
<i>A.ana</i> (60)	27	7	0	95	8	2	5	139
Invasive								
<i>D.pol</i> (39)	0	0	3	0	0	0	1	3
χ^2 -test Aa-D	<0.001	0.261*	0.827*	<0.001	0.167*	>0.999*		

Trem = Trematoda, Lar = larval stage, Ad = adult, L,E* = larval/nymphal stages and eggs,

G = gills, Ecto = ectoparasitic on soft body surfaces

* p -value after continuity correction

Supplementary Table 2. Prevalences (%) of infection of parasites—watermites and bitterling—in native (*Anodonta cygnea* and *A. anatina*) and invasive (*Sinanodonta woodiana*) freshwater bivalves of Czarny Młyn Pond, Poland, with the number of studied bivalves (n), total number of parasite taxa (N taxa) and sum of prevalences of different parasites per bivalve species (Prev. sum), and 2-sided χ^2 -test comparing the parasite-specific prevalences between *A. cygnea* and *S. woodiana* (Ac-S). In all four comparisons, the prevalence of infection was significantly or marginally significantly higher in the local bivalve than in the invasive counterpart.

Czarny Młyn Pond	Water-mite L,E	Water-mite L,E,G	Water-mite Ad, G	<i>Rhod. amar.</i> Fish G	N taxa	Prev. sum
Bivalve (n)	Mantle		Ecto			
Native						
<i>A.cyg</i> (30)	50	97	100	17	4	264
Invasive						
<i>S.woo</i> (31)	19	3	7	0	3	29
χ^2 -test Ac-S	0.012	<0.001	<0.001	0.057*		

Trem = Trematoda, Lar = larval stage, Ad = adult, L,E* = larval/nymphal stages and eggs

G = gills, Ecto = ectoparasitic on soft body surfaces

* p -value after continuity correction

Supplementary Table 3. Prevalences (%) of infection of parasites—*Rhipidocotyle campanula*, *Cercaria duplicata*, *Aspidogaster conchicola*, unknown larval nematode from gonad and gills, *Glyptotendipes* sp., watermites and bitterling—in native (*Anodonta anatina* (Aa), *A. cygnea* (Ac), *Unio pictorum* (Up) and *U. tumidus*(Ut) and invasive (*Sinanodonta woodiana* (S), *Dreissena polymorpha* (D) and *Corbicula fluminea* (C)) freshwater bivalves of Warta- Gopło Canal, Poland, with the number of studied bivalves (n), total number of parasite taxa (N taxa), sum of prevalences of different parasites per bivalve species (Prev. sum), mean (\pm standard error s.e.) values and t-test *p*-values comparing the mean values of native and invasive bivalves, as well as χ^2 -test *p*-values comparing parasite-specific prevalence differences between each native-invasive bivalve species combination. All *p*-values are 2-sided. In 36 out of the 42 comparisons in which a significant or marginally significant difference in the prevalence of infection was observed, the local bivalve had a higher prevalence than in the invasive counterpart. In six cases, marked with ^a, the prevalence of infection was significantly higher in the invasive bivalve (*S. woodiana*).

Warta-Gopło Canal	<i>Rhipid. camp.</i>	<i>Cerc. duplic.</i>	<i>Aspido-gaster</i>	<i>Chaeto-gaster</i>	Unkn. Nemat Lar	Unkn. Nemat Lar	<i>Glypto-tendipes</i> Chi Lar	<i>Con-choph.</i> Ciliate	Water-mite L,E	Water-mite L,E	Water-mite Ad, G	<i>Rhod. amar.</i> Fish	N taxa	Prev. sum
Bivalve (n)	Trem Lar Gonad	Trem Lar Gonad	Trem Ad Ecto	Olig Ad Ecto	Lar Gonad	Lar G	Chi Lar Ecto	Ciliate Ecto	Mantle	G	Ecto	G		
Native														
<i>A.ana</i> (30)	3	3	0	0	0	3	0	73	100	0	100	0	6	282
<i>U.pic</i> (30)	0	0	17	0	0	0	10	97	43	53	0	20	6	240
<i>U.tum</i> (30)	10	0	27	3	0	0	3	57	7	17	13	13	9	150
Mean \pm s.e.	4.3 \pm 3.0	1.0 \pm 1.0	15 \pm 8	1.0 \pm 1.0	0 \pm 0	1.0 \pm 1.0	4.3 \pm 3.0	76 \pm 12	50 \pm 27	23 \pm 16	38 \pm 31	11 \pm 5.9	7.0 \pm 1.0	224 \pm 39
Invasive														
<i>S.woo</i> (30)	3	0	0	27	0	0	7	0	43	0	90	0	5	170
<i>D.pol</i> (30)	0	0	0	0	3	0	0	0	0	0	0	0	1	3
<i>C.flu</i> (55)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean \pm s.e.	1.0 \pm 1.0	0 \pm 0	0 \pm 0	9.0 \pm 9.0	1.0 \pm 1.0	0 \pm 0	2.3 \pm 2.3	0 \pm 0	14 \pm 14	0 \pm 0	30 \pm 30	0 \pm 0	2.0 \pm 1.5	58 \pm 56
t-test Nat-In	0.346	0.423	0.136	0.427	0.423	0.423	0.624	0.003	0.309	0.274	0.868	0.134	0.052	0.072
χ^2 -test Aa-S	>0.999*	0.313*	-	0.008 ^{aa}	-	>0.999*	0.472*	<0.001	<0.001	-	0.236*	-		
χ^2 -test Aa-D	>0.999*	>0.999*	-	-	>0.999*	>0.999*	-	<0.001	<0.001	-	<0.001	-		
χ^2 -test Aa-C	0.757*	0.757*	-	-	-	0.757*	-	<0.001	<0.001	-	<0.001	-		
χ^2 -test Up-S	>0.999*	-	0.062*	0.008 ^{aa}	-	-	>0.999*	<0.001	1.000	<0.001	<0.001 ^a	0.031*		
χ^2 -test Up-D	-	-	0.062*	-	>0.999*	-	0.236*	<0.001	<0.001	<0.001	-	0.031*		
χ^2 -test Up-C	-	-	0.008*	-	-	-	0.076*	<0.001	<0.001	<0.001	-	0.003*		
χ^2 -test Ut-S	0.605*	-	0.008*	0.030 ^{aa}	-	-	>0.999*	<0.001	0.001 ^a	0.062*	<0.001 ^a	0.121*		
χ^2 -test Ut-D	0.236*	-	0.008*	>0.999*	>0.999*	-	>0.999*	<0.001	0.472*	0.062*	0.121*	0.121*		
χ^2 -test Ut-C	0.076*	-	<0.001	0.757*	-	-	0.757*	<0.001	0.234*	0.008*	0.025*	0.025*		

Trem = Trematoda, Olig = Oligochaeta, Nemat = Nematoda, Chi = Chironomidae

Lar = larval stage, L,E = larval/nymphal stages and eggs, Ad = adult

G = gills, Ecto = ectoparasitic on soft body surfaces

**p*-value after continuity correction

^a = significantly higher infection prevalence in the invasive bivalve

Supplementary Table 4. Prevalences (%) of infection of parasites—*Rhipidocotyle campanula*, *Glyptotendipes* sp., *Conchophthirus*, watermites and bitterling—in native (*Anodonta anatina*, *Unio pictorum* and *U. tumidus*) and invasive (*Sinanodonta woodiana*, *Dreissena polymorpha* and *Corbicula fluminea*) freshwater bivalves of Gosławskie Lake, Poland, with the number of studied bivalves (n), total number of parasite taxa (N taxa), sum of prevalences of different parasites per bivalve species (Prev. sum), mean (\pm standard error s.e.) values and t-test results comparing the mean values of native and invasive bivalves, as well as χ^2 -test *p*-values comparing parasite-specific prevalence differences between each native-invasive bivalve species combination. All *p*-value are 2-sided. In all eight comparisons in which a significant difference in the prevalence of infection was observed, the prevalence of infection was higher in the local bivalve than in the invasive counterpart.

Gosławskie Lake	<i>Rhipid. camp.</i> Trem Lar	<i>Glypto-tendipes</i> Chi Lar	<i>Con-choph.</i> Ciliate	Water-mite L,E	Water-mite L,E	Water-mite Ad, G	<i>Rhod. amar.</i> Fish	N taxa	Prev. sum
Bivalve (n)	Gonad	Ecto	Ecto	Mantle	G	Ecto	G		
Native									
<i>A.ana</i> (30)	0	7	0	13	0	40	0	3	60
<i>U.pic</i> (30)	0	17	3	7	0	0	7	4	34
<i>U.tum</i> (30)	20	10	0	3	0	0	3	4	36
Mean \pm s.e.	6.7 \pm 6.7	11 \pm 3	1.0 \pm 1.0	7.7 \pm 2.9	0 \pm 0	13 \pm 13	3.3 \pm 2.0	3.7 \pm 0.3	43 \pm 8
Invasive									
<i>S.woo</i> (30)	3	20	0	7	3	3	0	5	36
<i>D.pol</i> (30)	0	0	0	0	0	0	0	0	0
<i>C.flu</i> (30)	0	0	0	0	0	0	0	0	0
Mean \pm s.e.	1.0 \pm 1.0	6.7 \pm 6.7	0 \pm 0	2.3 \pm 2.3	1.0 \pm 1.0	1.0 \pm 1.0	0 \pm 0	1.7 \pm 1.7	12 \pm 12
t-test Nat-In	0.486	0.557	0.423	0.226	0.423	0.453	0.176	0.305	0.099
χ^2 -test Aa-S	>0.999*	0.255*	-	0.667*	>0.999*	0.001	-		
χ^2 -test Aa-D	-	0.472*	-	0.121*	-	<0.001	-		
χ^2 -test Aa-C	-	0.472*	-	0.121*	-	<0.001	-		
χ^2 -test Up-S	>0.999*	0.739	>0.999*	>0.999*	>0.999*	>0.999*	0.472*		
χ^2 -test Up-D	-	0.062*	>0.999*	0.472*	-	-	0.472*		
χ^2 -test Up-C	-	0.062*	>0.999*	0.472*	-	-	0.472*		
χ^2 -test Ut-S	0.108*	0.470*	-	>0.999*	>0.999*	>0.999*	>0.999*		
χ^2 -test Ut-D	0.031*	0.236*	-	>0.999*	-	-	>0.999*		
χ^2 -test Ut-C	0.031*	0.236*	-	>0.999*	-	-	>0.999*		

Trem = Trematoda, Chi = Chironomidae

Lar = larval stage, L,E = larval/nymphal stages and eggs, Ad = adult

G = gills, Ecto = ectoparasitic on soft body surfaces

**p*-value after continuity correction

Supplementary Table 5. Prevalences (%) of infection of parasites—*Rhipidocotyle fennica*, *Cercaria duplicata* and watermites—in native (*Anodonta anatina*, *A. cygnea*, *Unio pictorum* and *U. tumidus*) and invasive (*Sinanodonta woodiana*, *Corbicula fluminea*) freshwater bivalves of Nowy Lipsk Pond, Poland, with the number of studied bivalves (n), total number of parasite taxa (N taxa), sum of prevalences of different parasites per bivalve species (Prev. sum) and mean (\pm standard error s.e.) values for the four native bivalves, and 2-sided χ^2 -test comparing the parasite-specific prevalences between *S. woodiana* and each of the native bivalves. In four out of the eight comparisons in which a significant difference in the prevalence of infection was observed, the local bivalve had a higher prevalence than in the invasive counterpart. In four cases, marked with ^a, the prevalence of infection was significantly higher in the invasive bivalve (*S. woodiana*).

Nowy Lipsk Pond	<i>Rhipid. fenn.</i>	<i>Cerc. duplic.</i>	Water-mite L,E	Water-mite L,E	Water-mite Ad, G	N taxa	Prev. sum
Bivalve (n)	Trem Lar Gonad	Trem Lar Gonad	Mantle	G	Ecto		
Native							
<i>A.ana</i> (30)	13	3	97	7	80	5	200
<i>A.cyg</i> (30)	0	0	90	100	100	3	290
<i>U.pic</i> (30)	0	0	3	0	0	1	3
<i>U.tum</i> (30)	0	0	37	43	47	3	127
Mean \pm s.e.	3.3 \pm 3.3	0.8 \pm 0.8	57 \pm 22	38 \pm 23	57 \pm 22	3.0 \pm 0.8	155 \pm 61
Invasive							
<i>S.woo</i> (31)	0	0	97	17	53	3	170
χ^2 -test Aa-S	0.121*	>0.999*	>0.999*	0.421*	0.028		
χ^2 -test Ac-S	-	-	0.605*	<0.001	<0.001		
χ^2 -test Up-S	-	-	<0.001 ^a	0.062* ^a	<0.001 ^a		
χ^2 -test Ut-S	-	-	<0.001 ^a	0.024	0.606		

Trem = Trematoda, Lar = larval stage, L,E = larval/nymphal stages and eggs, Ad = adult

G = gills, Ecto = ectoparasitic on soft body surfaces

* p -value after continuity correction

^a = significantly higher infection prevalence in the invasive bivalve

Supplementary Table 6. Prevalences (%) of infection of parasites—*Rhipidocotyle fennica*, watermites and bitterling—in native (*Unio tumidus* and *Pseudanodonta complanata*) and invasive (*Dreissena polymorpha*) freshwater bivalves of Siecino Lake, Poland, with the number of studied bivalves (n), total number of parasite taxa (N taxa) and sum of prevalences of different parasites per bivalve species (Prev. sum), as well as χ^2 -test results (2-sided *p*-value) comparing the parasite-specific prevalences between *D. polymorpha* and the native bivalves. In three out of the five comparisons in which a significant or marginally significant difference in the prevalence of infection was observed, the local bivalve had a higher prevalence than in the invasive counterpart. In two cases, marked with ^a, the prevalence of infection was significantly higher in the invasive bivalve (*D. polymorpha*).

Siecino Lake	<i>Chaetogaster</i> Olig Ad	<i>Glyptotendipes</i> Chi Lar	<i>Conchoph.</i> Ciliate	Water-mite L,E	Water-mite L,E	Water-mite Ad, G	N taxa	Prev. sum
Bivalve (n)	Ecto	Ecto	Ecto	Mantle	G	Ecto		
Native								
<i>U.tum</i> (30)	17	23	3	0	0	0	3	43
<i>P.com</i> (30)	7	10	3	13	3	27	6	63
Invasive								
<i>D.pol</i> (30)	0	0	73	0	0	0	1	73
χ^2 -test Ut-D	0.062*	0.016*	<0.001 ^a	-	-	-		
χ^2 -test Pc-D	0.472*	0.236*	<0.001 ^a	0.121*	>0.999*	0.008*		

Trem = Trematoda, Olig = Oligochaeta, Chi = Chironomidae

Lar = larval stage, L,E = larval/nymphal stages and eggs, Ad = adult

G = gills, Ecto = ectoparasitic on soft body surfaces

**p*-value after continuity correction

^a = significantly higher infection prevalence in the invasive bivalve

Supplementary Table 7. Prevalences (%) of infection of parasites—*Rhipidocotyle campanula*, *Cercaria duplicata*, *Aspidogaster conchicola*, unknown larval nematode from gonad and gills, *Glyptotendipes* sp., watermites and bitterling—in native (*Anodonta anatina*, *A. cygnea*, *Unio pictorum* and *U. tumidus*) and invasive (*Sinanodonta woodiana*, *Dreissena polymorpha* and *Corbicula fluminea*) freshwater bivalves of Szeszupa River, Poland, with the number of studied bivalves (n), total number of parasite taxa (N taxa), sum of prevalences of different parasites per bivalve species (Prev. sum), mean (\pm standard error s.e.) values for the three native bivalves, as well as χ^2 -test results (2-sided *p*-value) comparing the parasite-specific prevalences between *D. polymorpha* and each of the native bivalves. In five out of the 13 comparisons in which a significant or marginally significant difference in the prevalence of infection was observed, the local bivalve had a higher prevalence than in the invasive counterpart. In seven cases, marked with ^a, the prevalence of infection was significantly higher in the invasive bivalve (*D. polymorpha*).

Szeszupa River	<i>Rhipid. fenn.</i> Trem Lar	<i>Buceph. polym.</i> Trem Lar	<i>Phyllod. macroc.</i> Trem Lar	<i>Chaeto-gaster</i> Olig Ad	<i>Glypto-tendipes</i> Chi Lar	<i>Con-choph.</i> Ciliate	Water-mite L,E Mantle	Water-mite L,E G	Water-mite Ad, G Ecto	N taxa	Prev. sum
Bivalve (n)	Gonad	Gonad	Gonad	Ecto	Ecto	Ecto					
Native											
<i>A.ana</i> (37)	8	0	0	32	11	84	89	3	38	7	265
<i>U.pic</i> (34)	0	0	0	21	6	94	24	0	3	5	148
<i>U.tum</i> (34)	0	0	0	18	3	12	15	3	6	6	57
<i>P.com</i> (36)	0	0	0	3	3	0	64	3	44	5	117
Mean \pm s.e.	2.0 \pm 2.0	0 \pm 0	0 \pm 0	19 \pm 6	5.8 \pm 1.9	48 \pm 24	48 \pm 17	2.3 \pm 0.8	23 \pm 11	5.8 \pm 0.5	147 \pm 44
Invasive											
<i>D.pol</i> (25)	0	12	28	16	8	100	0	0	0	5	164
χ^2 -test Aa-D	0.392*	0.120*	0.003* ^a	0.147	>0.999*	0.093* ^a	<0.001	>0.999*	<0.001		
χ^2 -test Up-D	-	0.141*	0.004* ^a	0.655	>0.999*	0.613*	0.026*	-	>0.999*		
χ^2 -test Ut-D	-	0.141*	0.004* ^a	0.868	0.784*	<0.001 ^a	0.126*	>0.999*	0.613*		
χ^2 -test Pc-D		0.126*	0.003* ^a	0.169*	0.745*	<0.001 ^a	<0.001	>0.999*	<0.001		

Trem = Trematoda, Olig = Oligochaeta, Chi = Chironomidae

Lar = larval stage, L,E = larval/nymphal stages and eggs, Ad = adult

G = gills, Ecto = ectoparasitic on soft body surfaces

**p*-value after continuity correction

^a = significantly higher infection prevalence in the invasive bivalve

Supplementary Table 8. Prevalences (%) of infection of parasites—*Rhipidocotyle fennica*, *Aspidogaster conchicola*, *Chaetogaster*, *Glyptotendipes* sp., *Conchophthirus* sp. and watermites—in native (*Anodonta anatina*, *Unio pictorum*, *U. tumidus* and *Pseudanodonta complanata*) and invasive (*Dreissena polymorpha*) freshwater bivalves of Rospuda River, Poland, with the number of studied bivalves (n), total number of parasite taxa (N taxa), sum of prevalences of different parasites per bivalve species (Prev. sum) and mean (\pm standard error s.e.) values for the native bivalves, as well as χ^2 -test results (2-sided *p*-value) comparing the parasite-specific prevalences between *D. polymorpha* and each of the native bivalves. In all 11 comparisons in which a significant or marginally significant difference in the prevalence of infection was observed, the prevalence of infection was higher in the local bivalve than in the invasive counterpart.

Rospuda River	<i>Rhipid. fenn.</i>	<i>Aspido-gaster</i>	<i>Chaeto-gaster</i>	<i>Glypto-tendipes</i>	<i>Con-choph.</i>	Water-mite	Water-mite	Water-mite	N taxa	Prev. sum
Bivalve (n)	Trem Lar	Trem Ad	Olig Ad	Chi Lar	Ciliate	L,E	L,E	Ad G		
	Gonad	Ecto	Ecto	Ecto	Ecto	Mantle	G	Ecto		
Native										
<i>A.ana</i> (25)	20	36	28	0	100	8	0	0	5	192
<i>U.tum</i> (19)	0	16	21	0	74	26	11	5	6	153
<i>P.com</i> (23)	0	0	13	9	26	17	70	65	6	200
Mean \pm s.e.	6.7 \pm 6.7	17 \pm 10	21 \pm 4	3.0 \pm 3.0	67 \pm 22	17 \pm 5	27 \pm 22	23 \pm 21	5.7 \pm 0.3	182 \pm 15
Invasive										
<i>D.pol</i> (16)	0	0	0	0	0	0	0	0	0	0
χ^2 -test Aa-D	0.156*	0.007	0.058*	-	<0.001	0.677*	-	-		
χ^2 -test Up-D	-	0.718*	0.192*	-	0.003*	<0.001*	0.050*	0.718*		
χ^2 -test Ut-D	-	0.291*	0.157*	-	<0.001	0.083*	0.545*	>0.999*		
χ^2 -test Pc-D	-	-	0.372*	0.636*	0.077*	0.221*	<0.001	<0.001		

Trem = Trematoda, Olig = Oligochaeta, Chi = Chironomidae

Lar = larval stage, L,E = larval/nymphal stages and eggs, Ad = adult

G = gills, Ecto = ectoparasitic on soft body surfaces

**p*-value after continuity correction

Supplementary Table 9. Bivalve collection sites, their coordinates, sampling time, characteristics of waterbodies, annual temperature range (°C), fish community and approximate introduction of invasive bivalve species. Lake Võrstjärv is in Estonia, other sites are from Poland.

Site	Coordinates	Sampling	Characteristic	°C	Fish community	Appearance of invasive species
Lake Võrstjärv*	58.1565 N, 26. 0866 E	Summer 2017	Large, shallow, eutrophic lake	0-21	Aa, Ab, Blb, Gc, Gg, Li, Pf, Sc, Rr	<i>D.polymorpha</i> in 1950s
Czarny Młyn Pond	54°36'42"N 17°10'42"E	May 2016	Eutrophic, naturalized former fish pond	4-24	Ab, Cy, El, Ld, Pf, Rr, Ra, Tt	<i>S.woodiana</i> in 1980s
Warta-Gopło Canal	52°17'48"N 18°17'45"E	May 2016	Artificial cooling channel of a power plant, thermally polluted	12-34	Aa, Ab, Bbj, Ca, Cc, Ci, Gc, Pf, Rr, Tt	<i>S.woodiana</i> and <i>D.polymorpha</i> in 1980s, <i>C.fluminea</i> in 2010s
Gosławskie Lake	52°17'17"N, 18°14'36"E	May 2016	Eutrophic lake, thermally polluted by power plant	7-29	Aa, Ab, Ca, Cg, Ci, Cy, El, Hm, Hn, Pf, Rr, Sg, Sl, Tt	<i>S.woodiana</i> in 1980s, <i>D.polymorpha</i> 1960s, <i>C.fluminea</i> 2010s
Nowy Lipsk Pond	53°44'15.9"N 23°19'15.2"E	June 2017	Eutrophic fish pond established in 2000	4-26	Aa, Ci, Cy, El, Rr, Hn, Pf, Tt	<i>S.woodiana</i> in 2000-2010
Siecino Lake	53°35'16.5"N 16°01'11.0"E	May 2018	Deep, mesotrophic 'ribbon lake'	4-24	Ab, Aan, Cal, Cl, El, Llo, Oe, Pf, Rr, Se, Tt	<i>D. polymorpha</i> in 1970s or before
Szeszupa River	54°15'48.7"N 22°53'58.8"E	June 2018	Lowland river with batrachion vegetation communities	2-19	Aa, Aan, Bb, Bbj, Gc, Lc, Gg, Ala, Pf, Gc, Ll, Pp, Rr, Se	<i>D.polymorpha</i> in 1800-1900
Rospuda River	54°03'19.7"N 22°41'42.0"E	June 2018	Small lowland river in a natural state	2-19	Bbj, El, Li, Ll, Llo, Mf, Pf, Ra, Rr, Se, St, Tt	<i>D.polymorpha</i> in 1800-1900

Aa - *Alburnus alburnus*; Ab - *Abramis brama*; Aan - *Anuilla anguilla*; As - *Aspius aspius*; Bb - *Barbatula barbatula*; Bbj - *Blicca bjoerkna*; Ca - *Carassius carassius*; Cal - *Coregonus albula*; Cg - *Carassius gibelio*; Ci - *Ctenopharyngodon idella*; Cl - *Coregonus lavaretus*; Cy - *Cyprinus carpio*; El - *Esox lucius*; Gc - *Gymnocephalus cernua*; Gg - *Gobio gobio*; Hm - *Hypophthalmichthys molitrix*; Hn - *Hypophthalmichthys nobilis*; Ld - *Leucaspis delineatus*; Lc - *Leuciscus cephalus*; Li - *Leuciscus idus*; Ll - *Leuciscus leuciscus*; Llo - *Lota lota*; Mf - *Misgurnus fossilis*; Oe - *Osmerus eperlanus*; Pf - *Perca fluviatilis*; Pp - *Phoxinus phoxinus*; Ra - *Rhodeus amarus*; Rr - *Rutilus rutilus*; Se - *Scardinius erythrophthalmus*; Sg - *Silurus glanis*; Sl - *Stizostedion lucioperca*; St - *Salmo trutta*; Tt - *Tinca tinca*

*bivalve samples stored frozen



II

LOWER PARASITE PRESSURE IN INVASIVE FRESHWATER BIVALVES THAN IN SYMPATRIC NATIVE UNIONIDAE MUSSELS IN SOUTHERN EUROPEAN LAKES

by

Binglin Deng, Riccardi Nicoletta, Maria Urbańska, Timo J. Marjomäki,
Wojciech Andrzejewski & Jouni Taskinen 2024

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III

**ENEMY RELEASE: LOSS OF PARASITES AS A RESULT OF
INVASION IN FRESHWATER BIVALVES *SINANODONTA*
WOODIANA AND *CORBICULAR FLUMINEA***

by

Binglin Deng, Riccardi Nicoletta, Pin Nie, Maria Urbańska, Timo J.
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