Parasites in sympatric populations of native and invasive freshwater bivalves

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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, $df_{continuity\ corrected} = 0.863$, $p = 0.353$).

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 (± 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 largval/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 ≥ 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 ≥ 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 ≥ 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. anantina*, *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 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 reinvade 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).

References of supplementary materials

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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 *p*-value of 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 Bivalve (n)	*Rhipid. fenn.* Trem Lar Gonad	*Cerc. duplic.* Trem Lar Gonad	*Phyllod. macroc.* Trem Ad Gonad	Water-mite L,E Mantle	Water-mite L,E G	Water-mite Ad, G Ecto	N taxa	Prev. sum
Native								
A.ana (60)	27	7	0	95	8	2	5	139
Invasive								
D.pol (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 *p*-value of 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 Bivalve (n)	Water-mite L,E Mantle	Water-mite L,E,G	Water-mite Ad, G Ecto	*Rhod. amar.* Fish G	N taxa	Prev. sum
Native						
A.cyg (30)	50	97	100	17	4	264
Invasive						
S.woo (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 (± standard error s.e.) values and 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 Bivalve (n)	*Rhipid. camp.* Trem Lar Gonad	*Cerc.. duplic.* Trem Lar Gonad	*Aspido-gaster* Trem Ad Ecto	*Chaeto-gaster* Olig Ad Ecto	Unkn. Nemat Lar Gonad	Unkn. Nemat Lar G	*Glypto-tendipes* Chi Lar Ecto	*Con-choph.* Ciliate Ecto	Water-mite L,E Mantle	Water-mite L,E G	Water-mite Ad, G Ecto	*Rhod. amar.* Fish G	N taxa	Prev. sum
Native														
A.ana (30)	3	3	0	0	0	3	0	73	100	0	100	0	6	282
U.pic (30)	0	0	17	0	0	0	10	97	43	53	0	20	6	240
U.tum (30)	10	0	27	3	0	0	3	57	7	17	13	13	9	150
Mean±s.e.	4.3±3.0	1.0±1.0	15±8	1.0±1.0	0±0	1.0±1.0	4.3±3.0	76±12	50±27	23±16	38±31	11±5.9	7.0±1.0	224±39
Invasive														
S.woo (30)	3	0	0	27	0	0	7	0	43	0	90	0	5	170
D.pol (30)	0	0	0	0	3	0	0	0	0	0	0	0	1	3
C.flu (55)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean±s.e.	1.0±1.0	0±0	0±0	9.0±9.0	1.0±1.0	0±0	2.3±2.3	0±0	14±14	0±0	30±30	0±0	2.0±1.5	58±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*[a]	-	>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*[a]	-	-	>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*[a]	-	-	>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 (± 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 Bivalve (n)	*Rhipid. camp.* Trem Lar Gonad	*Glypto-tendipes* Chi Lar Ecto	*Con-choph.* Ciliate Ecto	Water-mite L,E Mantle	Water-mite L,E G	Water-mite Ad, G Ecto	*Rhod. amar.* Fish G	N taxa	Prev. sum
Native									
A.ana (30)	0	7	0	13	0	40	0	3	60
U.pic (30)	0	17	3	7	0	0	7	4	34
U.tum (30)	20	10	0	3	0	0	3	4	36
Mean±s.e.	6.7±6.7	11±3	1.0±1.0	7.7±2.9	0±0	13±13	3.3±2.0	3.7±0.3	43±8
Invasive									
S.woo (30)	3	20	0	7	3	3	0	5	36
D.pol (30)	0	0	0	0	0	0	0	0	0
C.flu (30)	0	0	0	0	0	0	0	0	0
Mean±s.e.	1.0±1.0	6.7±6.7	0±0	2.3±2.3	1.0±1.0	1.0±1.0	0±0	1.7±1.7	12±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 (± standard error s.e.) values for the four native bivalves, and 2-sided *p*-value of 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 Bivalve (n)	*Rhipid. fenn.* Trem Lar Gonad	*Cerc. duplic.* Trem Lar Gonad	Water-mite L,E Mantle	Water-mite L,E G	Water-mite Ad, G Ecto	N taxa	Prev. sum
Native							
A.ana (30)	13	3	97	7	80	5	200
A.cyg (30)	0	0	90	100	100	3	290
U.pic (30)	0	0	3	0	0	1	3
U.tum (30)	0	0	37	43	47	3	127
Mean±s.e.	3.3±3.3	0.8±0.8	57±22	38±23	57±22	3.0±0.8	155±61
Invasive							
S.woo (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 Bivalve (n)	*Chaeto-gaster* Olig Ad Ecto	*Glypto-tendipes* Chi Lar Ecto	*Con-choph.* Ciliate Ecto	Water-mite L,E Mantle	Water-mite L,E G	Water-mite Ad, G Ecto	N taxa	Prev. sum
Native								
U.tum (30)	17	23	3	0	0	0	3	43
P.com (30)	7	10	3	13	3	27	6	63
Invasive								
D.pol (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 (± 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 Bivalve (n)	*Rhipid. fenn.* Trem Lar Gonad	*Buceph. polym.* Trem Lar Gonad	*Phyllod. macroc.* Trem Lar Gonad	*Chaeto-gaster* Olig Ad Ecto	*Glypto-tendipes* Chi Lar Ecto	*Con-choph.* Ciliate Ecto	Water-mite L,E Mantle	Water-mite L,E G	Water-mite Ad, G Ecto	N taxa	Prev. sum
Native											
A.ana (37)	8	0	0	32	11	84	89	3	38	7	265
U.pic (34)	0	0	0	21	6	94	24	0	3	5	148
U.tum (34)	0	0	0	18	3	12	15	3	6	6	57
P.com (36)	0	0	0	3	3	0	64	3	44	5	117
Mean±s.e.	2.0±2.0	0±0	0±0	19±6	5.8±1.9	48±24	48±17	2.3±0.8	23±11	5.8±0.5	147±44
Invasive											
D.pol (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.,*Conchopthirus* 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 (± 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 Bivalve (n)	*Rhipid. fenn.* Trem Lar Gonad	*Aspido-gaster* Trem Ad Ecto	*Chaeto-gaster* Olig Ad Ecto	*Glypto-tendipes* Chi Lar Ecto	*Con-choph.* Ciliate Ecto	Water-mite L,E Mantle	Water-mite L,E G	Water-mite Ad G Ecto	N taxa	Prev. sum
Native										
A.ana (25)	20	36	28	0	100	8	0	0	5	192
U.tum (19)	0	16	21	0	74	26	11	5	6	153
P.com (23)	0	0	13	9	26	17	70	65	6	200
Mean±s.e.	6.7±6.7	17±10	21±4	3.0±3.0	67±22	17±5	27 ±22	23±21	5.7±0.3	182±15
Invasive										
D.pol (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 Võrtsjärv*	58.1565 N, 26. 0866 E	Summer 2017	Large, shallow, eutrophic lake	0-21	Aa, Ab, Blb, Gc, Gg, Li, Pf, Sc, Rr	*D.polymorpha* in 1950s
Czarny Młyn Pond	54º36'42"N 17º10'42"E	May 2016	Eutrophic, naturalized former fish pond	4-24	Ab, Cy, El, Ld, Pf, Rr, Ra, Tt	*S.woodiana* 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	*S.woodiana* and *D.polymorpha* in 1980s, *C.fluminea* 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	*S.woodiana* in 1980s, *D.polymorpha* 1960s, *C.fluminea* 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	S.woodiana 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	*D. polymorpha* 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	*D.polymorpha* 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	*D.polymorpha* 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 - *Leucaspius delineatus*; Lc-*Leuciscus cephalus;* Li – *Leuciscus idus*; Ll – *Leuciscus leucisus*; 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