

## The invasive Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) as a host for native symbionts in European waters

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### ABSTRACT

Biological invasions are commonly observed in both the natural habitats and those which are altered by human activities. An understanding of the mechanisms involved in the successful introduction, establishment and invasion of exotic taxa is essential in predicting of changes in biodiversity and community structure. Symbiont-mediated interactions between exotic and native hosts are of special interest due to the indirect effects on population dynamics. The aim of this study was to estimate the presence of symbionts in Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834), an exotic species of mussel in European fresh waters. The number of 340 individuals of *S. woodiana* was collected from Polish water bodies, including thermally heated lakes and fish ponds with natural thermal regime. The examination of mussels revealed the presence of Rhipidocotyle campanula sporocysts and cercariae (Digenea: Bucephalidae), water mites Unionicola ypsilophora (Acari: Hydracarina), oligochaetes Chaetogaster limnaei limnaei (Oligochaeta: Naididae) and chironomids Glyptotendipes sp. (Diptera: Chironomidae). The global prevalence of mussels inhabited by *Ch. limnaei limnaei* was 7.6%, by water mites and chironomids 3.5%, and by *R. campanula cercariae* 2.0%. The significant difference in the number of mussels with symbionts was identified between heated lakes and fish ponds ( $\chi^2=4.15$ ;  $df=1$ ,  $P=0.04$ ), with a higher global prevalence of mussels in fish ponds (22.3%) compared to heated lakes (13.7%). *R. campanula* or *U. ypsilophora* were only found in mussels collected from thermally polluted lakes or fish ponds, respectively. Chironomid larvae and oligochaetes occurred in both types of water bodies. However, *Glyptotendipes sp.* inhabited mussels with a higher global prevalence in fish ponds than in thermally polluted lakes, while *Ch. limnaei limnaei* was observed mainly in hosts from heated lakes, and only from one fish pond that were not drained. Our findings indicate that the alien Chinese pond mussel *S. woodiana* can be inhabited by different groups of symbionts native to Europe, including digenetic trematodes. The results show that *S. woodiana* can affect directly and indirectly water habitats and the vulnerability of infection with symbionts depends on ecosystem conditions. It occurs that even considerable climate differences do not pose a barrier for exotic mussels to spread.

**Key words:** *Sinanodonta woodiana*; Rhipidocotyle; Chaetogaster; Unionicola; Glyptotendipes; Poland.

Received: September 2015. Accepted: December 2015.

### INTRODUCTION

The increasing problem of the spread and colonization of new territories by exotic taxa is of great interest to researchers due to the direct and indirect impact of exotic species on native biota. The direct effect, which is usually caused by competition between exotic and native species for the same resources, is well documented (Huxel, 1999; Byers, 2000). The indirect impact is, in turn, linked to modification of complex interactions between exotic and native organisms and their environment, as exotic species may bring their own symbionts from their native range of distribution and/or may acquire native symbionts in new ranges (Conn *et al.*, 1996; Kelly *et al.*, 2009; Hatcher and Dunn, 2011; Paterson *et al.*, 2012; Pulkkinen *et al.*, 2013; Glodosky and Sandland, 2014; Mori *et al.*, 2015). An alien species in new habitats does not usually have natural enemies and thus the lack of top-down control of their

populations is an important factor facilitating their rapid spread and the increase in their local abundance (Torchin *et al.*, 2001; Torchin *et al.*, 2003; Blossey, 2011).

The Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) is an alien freshwater bivalve species, which was introduced into European waters in the early 1960s (Kraszewski, 2007). The distribution of *S. woodiana* in Poland in the initial stages of its invasion was restricted to artificially heated lakes (Zdanowski, 1996; Spyra *et al.*, 2012), while the presence of this exotic mussel in waters with natural thermal regime was first reported by Böhme (1998) and subsequently by Urbańska *et al.* (2012). The continuously growing number of new localities of *S. woodiana*, as well as the biology and ecology of the species (Dudgeon and Morton, 1983; Kiss, 1995; Sirbu *et al.*, 2005; Corsi *et al.*, 2007; Du *et al.*, 2011; Douda *et al.*, 2012), indicate that this unionid species easily spreads in freshwater habitats (Andrzejewski *et al.*, 2013). Data on the occurrence

of non-parasitic and parasitic symbionts of *S. woodiana* in its native ecosystems and European water bodies are scanty (Zhao and Tang, 2007; Yuryshynets and Krasutska, 2009; Wu *et al.*, 2012; Yanovich and Shevcuk, 2012) and usually do not include the diversity of inland waters inhabited by Chinese pond mussel. According to Paterson *et al.* (2012), several biological and geographical factors are thought to explain the successful invasion of exotic species and the acquisition of symbionts, mainly parasites, in invaded areas. However, the determinants affecting the acquisition of native symbionts by exotic hosts remain poorly understood, especially in the context of population dynamics of native hosts (Paterson *et al.*, 2012). Thus an important task now is to look for the possible interactions between *S. woodiana*, an exotic mussel native to China, and its native symbionts in Europe.

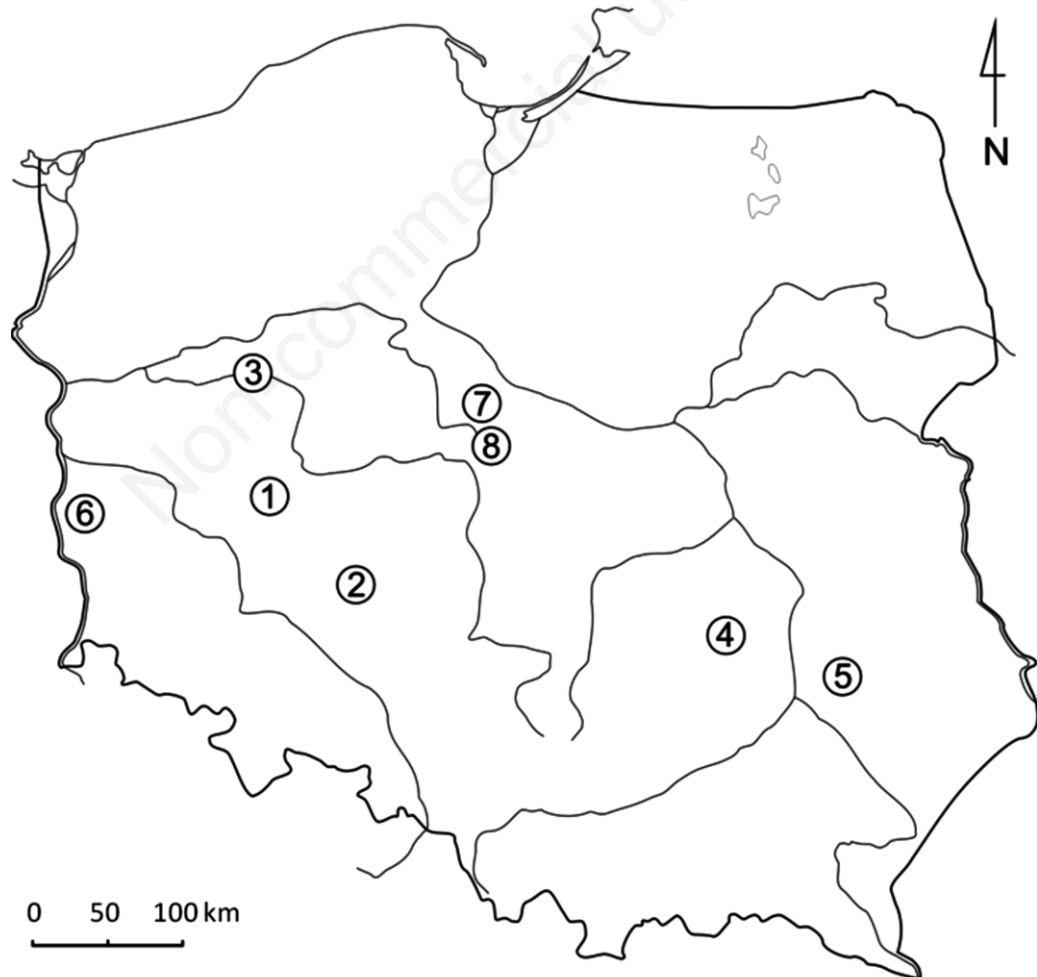
The aim of this study was to determine whether and to what extent *S. woodiana* can be colonized by symbionts

inhabiting water bodies of Poland, including thermally polluted lakes and fish ponds with natural thermal regime.

## METHODS

### Study area

*S. woodiana* individuals were collected in May and at the beginning of June of 2011 from 8 localities in Poland, including 2 thermally polluted lakes (warm water discharges from 2 electric power plants) and 6 fish ponds with natural thermal regime (Fig. 1). The water from all fish ponds, except for Samita, was regularly drained (Tab. 1). Long-term drainage took place in autumn and fish ponds were refilled with water in early spring next year, whereas short-term drainage was associated with the exchange of water in autumn within 2 weeks after drainage. The mean temperature of water in heated lakes during the sampling period was nearly 21°C, and was 6°C higher than in the



**Fig. 1.** Location of study sites in Poland. Fish ponds: 1, Wojnowicki; 2, Czesławicki; 3, Samita; 4, Polna Rzeka; 5, Zaklików; 6, Żary; heated lakes: 7, Gosławskie; 8, Licheńskie.

fish ponds under study. The spectrum of ichthyofauna in heated lakes is diverse and includes at least 14 native and exotic species (Kapusta *et al.*, 2008; Kapusta and Bogacka-Kapusta, 2015), while the number of fish species in the ponds under study ranged from 1 to 3, and only in Wojnowicki Pond it reached 8 species (Tab. 1).

## Material

Chinese pond mussels were gathered by hand in the littoral zone of the water bodies from a depth of 1.0-1.5 m and then they were transported to the laboratory in containers under a thin and wet textile. The mean shell dimensions of the collected mussels from both habitat types were comparable: 133.85 (SE 1.54) mm long and 76.94 (SE 1.32) mm height in heated lakes and 128.04 (SE 2.55) mm long and 71.23 (SE 1.58) mm height in fish ponds. The number of *S. woodiana* individuals in the thermally polluted lakes exceeds 40 individuals m<sup>-2</sup> and the biomass reaches 10 kg m<sup>-2</sup>, depending on the study sites (Kraszewski and Zdanowski, 2001), compared to 19 individuals m<sup>-2</sup> and 4.4 kg m<sup>-2</sup> in fish ponds (Spyra *et al.*, 2012). The symbionts inhabiting *S. woodiana* were initially searched for on the mantle surface and between the mantle and the inner side of the shell, and next inside the internal organs such as the gonad, hepatopancreas, gills and heart. Tissue sections were observed under a light microscope (Primostar Carl Zeiss). The species of symbionts were identified on the basis of the morphology of live digenean cercariae (Batur, 1977; Richardson, 1990; Taskinen *et al.*, 1991), oligochaetes (Kasprzak, 1981; Timm, 1999), chironomids (Wiederholm, 1983) and water mites (Hevers, 1979). The photos of symbionts were taken using the Primostar Carl Zeiss microscope and Motic Images Plus 2.0 software.

## Statistical analysis

A Chi-square test of contingency table was used to compare the numbers of mussels inhabited by symbionts

and of mussels without symbionts between heated lakes and fish ponds. A Principal Component Analysis (PCA), performed using MVSP 3.22 KCS software, was used to check for associations of symbiont species with particular types of habitat, *i.e.* heated lakes or fish ponds. Data were log-transformed in order to reduce the impact of particularly numerous taxa on results. The term *prevalence* was used for description of one population of hosts inhabited by a symbiont species, while *global prevalence* was used as a proportion of hosts inhabited by a symbiont species in relation to all the populations of hosts sampled.

## RESULTS

Nearly 17.0% of the 340 collected individuals of *S. woodiana* were inhabited by symbionts belonging to one of the 4 systematic groups of invertebrates, including oligochaetes, chironomids, water mites and digenetic trematodes (Fig. 2). Mixed invasions were not observed. The symbionts associated with *S. woodiana* were found in 6 of the 8 localities studied, with prevalence ranging from 12.0% to 45.4% (Tab. 2). Oligochaetes were the most prevalent symbionts of the collected mussels (global prevalence of 7.6%; Tab. 2). Hosts inhabited by water mites and chironomids occurred less frequently and had a similar global prevalence of 3.5%, while the cercariae diagnosed as larvae of *R. campanula* were noted in 2.0% of the collected mussels. The number of mussels with symbionts significantly differed between heated lakes and fish ponds ( $\chi^2=4,15$ ;  $df=1$ ,  $P=0.04$ ). *S. woodiana* individuals collected from fish ponds had a higher global prevalence (22.3%) than mussels gathered from heated lakes (13.7%). PCA analysis showed that samples collected from heated lakes and Samita Pond formed one group and differed from other samples along the first axis due to the presence of *R. campanula* and/or *Ch. limnaei limnaei* (Fig. 3). Along the second axis, samples from one fish pond (Polna Rzeka) and a heated lake (Gośląskie)

**Tab. 1.** Characteristics of the study sites.

Study sites	Coordinates		Habitat type	Bottom	Fish spectrum
Wojnowicki	51°56'39"N	16°43'06"E	FP (std)	Sandy-silty	Aa, Ab, Ca, Cy, El, Sg, Sl, Rr
Czesławicki	51°29'00"N	17°33'15"E	FP (ltd)	Silty	Cy
Samita	52°41'56"N	16°07'11"E	FP (nd)	Sandy-silty	Ca, Cy
Polna Rzeka	51°17'16"N	20°25'20"E	FP (std)	Sandy-loam	As, Cy, Li
Zaklików	50°45'53"N	22°08'16"E	FP (std)	Sandy-silty	Ci, Cy, Hm
Żary	51°19'17"N	15°16'18"E	FP (ltd)	Sandy-silty	Ca, Cy
Gośląskie	52°17'17"N	18°14'36"E	HL	Sandy-silty	Aa, Ab, Ca, Cg, Ci, Cy, El, Hm, Hn, Pf, Rr, Sg, Sl, Tt
Licheńskie	52°19'24"N	18°20'55"E	HL	Sandy-silty with a layer of leaves	Aa, Ab, As, Ca, Cg, Ci, Cy, El, Hm, Hn, Li, Pf, Rr, Sg, Sl, Tt

FP, fish ponds (std, short-term drainage), (ltd, long-term drainage), (nd, not drained); HL, heated lakes. Aa, *Anguilla anguilla*; Ab, *Abramis brama*; As, *Aspius aspius*; Ca, *Carassius carassius*; Cg, *Carassius gibelio*; Ci, *Ctenopharyngodon idella*; Cy, *Cyprinus carpio*; El, *Esox lucius*; Hm, *Hypophthalmichthys molitrix*; Hn, *Hypophthalmichthys nobilis*; Li, *Leuciscus idus*; Pf, *Perca fluviatilis*; Rr, *Rutilus rutilus*; Sg, *Silurus glanis*; Sl, *Stizostedion lucioperca*; Tt, *Tinca tinca*.

formed one group, while samples from other water bodies formed another group (Fig. 3). The presence of *U. ypsilophora* and/or *Glyptotendipes* sp. was the factor that differentiated the samples along the second PCA axis.

## DISCUSSION

Interactions between unionid mussels and symbionts are commonly observed in the environment (Piechocki and Dyduch-Falniowska, 1993). We found 4 groups of invertebrates occurring on the surface of the mantle and/or inside the internal organs of *S. woodiana*. The presence of *R. campanula* in the gonads is the first recorded case of occurrence of bucephalid larval stages in *S. woodiana* in Poland and probably in Europe. The prevalence of Chinese pond mussels inhabited by *R. campanula* was comparable with the results noted by Baturó (1977) and Müller *et al.* (2015) in native bivalve species (*Unio pictorum* and *Anodonta anatina*, respectively). In our re-

search, *R. campanula* was found in *S. woodiana* only in thermally polluted lakes. The ability to create new relationships in heated lakes may result from both i) the higher temperature of water, which facilitates the recruitment of parasites and promotes their transmission to hosts (Poulin, 2006); and ii) the more diversified spectrum of the second and final fish hosts of *R. campanula*, including *Rutilus rutilus*, *Perca fluviatilis* and probably *Stizostedion lucioperca* (Gibson *et al.*, 1992), in comparison to fish ponds. In native bivalve species, *e.g.* *A. anatina*, the presence of bucephalid trematodes decreases the production of glochidia (Taskinen and Valtonen 1995), causes partial or complete castration (Jokela *et al.*, 2005; Gangloff *et al.*, 2008; Müller *et al.*, 2015), increases mortality (Taskinen *et al.*, 1997) and causes changes in shell shape (Zieritz and Aldridge, 2011). The ecological consequences of the infestation of Chinese pond mussels by *R. campanula* remain unknown and further studies should address the question of whether this parasite significantly burdens



**Fig. 2.** Symbionts identified in *Sinanodonta woodiana*. A) *Chaetogaster limnaei limnaei*; scale bar: 100  $\mu$ m. B) *Glyptotendipes* sp.; scale bar: 200  $\mu$ m. C) *Rhipidocotyle campanula*; scale bar: 150  $\mu$ m. D) *Unionicola ypsilophora*; scale bar: 300  $\mu$ m.



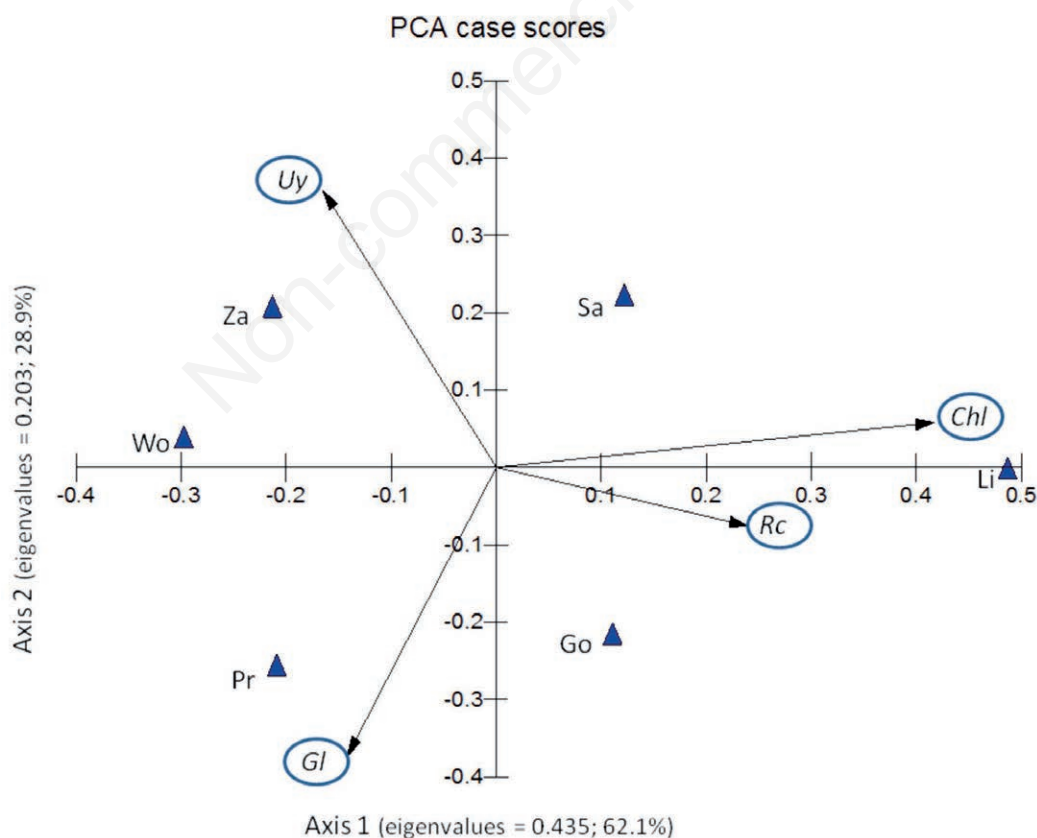
its exotic hosts and thus may limit their spread. The complex interactions between native hosts, exotic hosts and native parasites are another interesting issue. The impact of exotic hosts is not associated exclusively with direct effects (Douda *et al.*, 2012), as exotic hosts can also indirectly affect native hosts by altering the population dy-

namics of native symbionts (Krakau *et al.*, 2006; Thielges *et al.*, 2009; Telfer and Bown, 2012). It is highly probable that the presence of *R. campanula* in *S. woodiana* decreases the prevalence in native unionids, as the more diversified spectrum of hosts, the lower prevalence of each host species separately. It is suggested that the com-

**Tab. 2.** Prevalence of *S. woodiana* inhabited by symbionts in Poland.

Study site	Type of site	No. of collected mussels	Prevalence (%)			
			<i>Rc</i>	<i>Uy</i>	<i>Gl</i>	<i>Chl</i>
Wojnowicki	FP	31	0	16.1	9.7	0
Czesławicki	FP	30	0	0	0	0
Samita	FP	22	0	13.6	0	31.8
Polna Rzeka	FP	12	0	0	41.7	0
Zaklików	FP	14	0	28.6	0	0
Żary	FP	12	0	0	0	0
Gosławskie	HL	44	2.3	0	9.1	9.1
Licheńskie	HL	175	3.4	0	0	8.6
Total		340	2.0	3.5	3.5	7.6

FP, fish pond; HL, heated lake; *Rc*, *Rhipidocotyle campanula*; *Uy*, *Unionicola ypsilophora*; *Gl*, *Glyptotendipes* sp.; *Chl*, *Chaetogaster limnaei* *limnaei*.



**Fig. 3.** Principal Component Analysis (PCA) of samples (symbionts inhabiting *Sinanodonta woodiana*) collected from fish ponds (Wo, Wojnowicki; Za, Zaklików; Pr, Polna Rzeka; Sa, Samita) and heated lakes (Go, Gosławskie; Li, Licheńskie). *Chl*, *Chaetogaster limnaei* *limnaei*; *Gl*, *Glyptotendipes* sp.; *Uy*, *Unionicola ypsilophora*; *Rc*, *Rhipidocotyle campanula*.

munity diversity of hosts alters parasite transmission and mediates infection parameters, as the presence of other less competent host species may reduce the prevalence in the principal host (Telfer *et al.*, 2005; Johnson and Thieltges, 2010; Bouchard *et al.*, 2013).

During our research the naidid oligochaete *Chaetogaster limnaei limnaei* was also noted. This animal is commonly observed in molluscs, including bivalves (Fuller, 1974; Sickel, 1986; Conn *et al.*, 1996) and freshwater snails (Michelson, 1964; Rodgers *et al.*, 2005; Ibrahim, 2007). Previous data reported a different nature of the symbiotic relationship between this oligochaete and its bivalve hosts (Eng, 1979; Sickel and Lyles, 1981; Conn *et al.*, 1996). *Ch. limnaei limnaei*, associated with the mantle surfaces of freshwater molluscs, is considered to be a commensal, and feeds on digenean larvae (Khalil, 1961; Buse, 1972; Rajasekariah, 1978; Cichy, 2013). Although we found no mixed invasions, it is probable that the relatively high prevalence of *S. woodiana* inhabited by oligochaetes in heated lakes could be associated with the presence of *R. campanula* larvae, one of the sources of food for oligochaetes. The relationship between *Ch. limnaei limnaei* abundance and cercariae density was experimentally confirmed by Hopkins *et al.* (2013). Some authors (Conn *et al.*, 1996) indicate the seasonality of this annelid species in bivalve hosts and show a positive correlation of this phenomenon with water temperature changes. According to Sankurathri and Holmes (1976), the prevalence of *Ch. limnaei limnaei* decreased in a lake at temperatures above 24°C, whereas Poddubnaya (1968) indicates the optimum temperature for other *Chaetogaster* species of about 20°C. In our study the mean temperature of water in heated lakes was below 24°C, so the temperature of water probably was not a factor that limited the development of *Ch. limnaei limnaei* populations. Moreover, the diverse aquatic flora and the longer growing season in the thermally polluted lakes promotes higher numbers of *Chaetogaster* generations per year (Learner *et al.*, 1978), which probably contributed to the formation of the association with exotic *S. woodiana* in these habitats. In addition to both thermally polluted lakes, *Ch. limnaei limnaei* in *S. woodiana* was observed in only one fish pond (Samita). These 3 water bodies formed one group and differed from other samples along the first axis (PCA). Samita was the only fish pond not drained among the studied fish ponds. Thus the population of *Ch. limnaei limnaei* in mussels was, similarly to lakes, maintained in the habitat and did not have to rebuild after refilling the pond with water. In the two long-term drained fish ponds (Czesławicki, Żary) we found no symbionts, which may confirm that habitat disturbance (drainage) influences the acquisition of symbionts by hosts.

The association between insects and mussels is not often observed (Tokeshi, 1993; Riccardi, 1994). The chi-

ronomids *Glyptotendipes* sp. found during our study were recorded between the mantle and the inner side of *S. woodiana* shell. Beedham (1965, 1970, 1971) suggested that *Glyptotendipes* sp. is a facultative parasite of *Anodonta cygnea*, as the chironomid larvae fed on the hemocytes and caused the disintegration of the outer mantle epithelium. In turn, Riccardi (1994) indicated that the presence of chironomid larvae in *Dreissena polymorpha* has no effect on its condition. The prevalence of *Glyptotendipes* sp. in the collected samples of *S. woodiana* was higher in fish ponds with natural thermal regime than in heated lakes. The higher temperature of water in the thermally polluted lakes can affect the accelerated metamorphosis of the chironomid larva to the imago, which leaves the aquatic environment (Macky, 1977; Stevens, 1998). Moreover, Gudasz *et al.* (2010) found a positive correlation between the temperature of water and the mineralization of organic matter in lake sediments. A smaller amount of organic matter in the sediments of the thermally polluted lakes under study (Brzozowska *et al.*, 2007; Bojakowska and Krasuska, 2014), in comparison to reservoirs with natural thermal regime, could limit food availability and quality for the benthic chironomid larvae. It has been also shown that chironomids living in the mantle cavity of mussels were larger than those living in the ambient environment (Riccardi, 1994). This confirms that bivalve hosts provide better feeding opportunities, increased mobility, protection from disturbance and reduced predation risk (Tokeshi, 1993). *Unionicola ypsilophora* and *U. intermedia* are 2 species commonly observed in mussels from Europe (Davids, 1973; Baker *et al.*, 1992). In our study, *U. ypsilophora* was noted in *S. woodiana* collected from fish ponds only. The presence of the water mites in fish ponds, where the high number of chironomids was noted, is not surprising, as unionicolids use the chironomid larvae as hosts in the life cycle (Baker, 1991). The adult forms of unionicolid water mites are attached to the gills of mussels and cause displacement, rupture, and erosion of the gill epithelium (Baker, 1976; Edwards and Vidrine, 2006; Fisher *et al.*, 2000; Gangloff *et al.*, 2008).

As a large-sized bivalve species expanding its range of distribution, *S. woodiana* can be an attractive microhabitat for various symbionts associated with freshwater reservoirs. This is possible due to both the biological characteristics of the species (large body size and biomass) and its ecological features (tolerance to water pollution and oxygen deficits) (Dudgeon and Morton, 1983; Du *et al.*, 2011). Some authors emphasize that host density is a key parameter determining the prevalence of hosts (Arneberg *et al.*, 1998; Voutilainen *et al.*, 2009). In our study, higher density of *S. woodiana* was noted in heated lakes (Kraszewski and Zdanowski, 2001; Spyra *et al.*, 2012), but higher prevalence of mussels was observed in fish ponds. This result suggests that high host density can decrease the prevalence of inhabited individuals due to

the *dilution effect* as the probability of an individual host being infested by symbionts decreases with increasing host density (Mooring and Hart, 1992; Côté and Poulin, 1995; Samsing *et al.*, 2014).

## CONCLUSIONS

Our research has shown that *S. woodiana*, an exotic mollusc in Europe, is inhabited by native symbionts, including oligochaetes, chironomids, water mites and digenetic trematodes. The acquisition of symbionts by exotic hosts may differ between various types of habitat. Particular attention should be paid to the relationship established between exotic hosts and native symbionts having complex life cycles (*e.g.*, Digenea), which can significantly affect the indirect impact of exotic taxa on native biota.

## ACKNOWLEDGMENTS

We would like to thank Prof. Eugeniusz Biesiadka for help in identifying water mites and Dr Małgorzata Poznańska for help in identifying chironomids, Prof. Piotr Tryjanowski for the support and comments and Sylwia Ufnalska, MSc, MA, for English revision of the manuscript. We are also grateful to both anonymous reviewers for their constructive comments.

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