Symbiotic species diversity can explain invasion success and stability of the host-parasite system: the case of native and invasive gammarids (Crustacea, Amphipoda)

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Abstract

The major current challenge related to alien species is the absence of a comprehensive theoretical basis to explain the resistance from bioinvasions. One of the factors conferring resistance to invasion is the native species diversity of a recipient ecosystem. We aim to examine patterns of species diversity to understand the mechanisms underlying host-parasite relationships in alien species. The dataset represents 20 host-locality samples, including over 15,067,600 symbiotic organisms counted from 612 host individuals belonging to 7 gammarid species, two native and five invasive, sampled at 16 localities in the Baltic region of Poland. We asked whether diversity patterns depended on locality, habitat and host species, and which factors are deterministic in the variation of diversity. Our results suggest that both locality and host phylogeny are critical factors in determining the composition and abundance of symbiotic communities in gammarids. Out of these two factors, locality appears to be more determinant of the symbiotic community of gammarids than host species, whereas habitat condition was a stronger determinant of the symbiotic communities than geographic distance. The rich and uneven symbiotic communities of native gammarids keep the host-parasite system in equilibrium. Sharply dominated symbiotic species with a high load per host individual stabilize the host population. Simplified symbiotic communities of the invasive hosts showed a relatively even distribution of species with moderate loads per individual, with heavily infected gammarids being rare in the population. The survival rate of invasive gammarids is expected to be relatively high, facilitating their population growth and further spread. Complex communities of the native gammarids constructed from many symbiotic species may prevent host populations from undergoing explosive growth, while such mechanisms may be completely or partially reduced in invasive hosts.

ABSTRACT

The major current challenge related to alien species is the absence of a comprehensive theoretical basis to explain the resistance from bioinvasions. One of the factors conferring resistance to invasion is the native species diversity of a recipient ecosystem. We aim to examine patterns of species diversity to understand the mechanisms underlying host-parasite relationships in alien species. The dataset represents 20 host-locality samples, including over 15,067,600 symbiotic organisms counted from 612 host individuals belonging to 7 gammarid species, two native and five invasive, sampled at 16 localities in the Baltic region of Poland. We asked whether diversity patterns depended on locality, habitat and host species, and which factors are deterministic in the variation of diversity. Our results suggest that both locality and host phylogeny are critical factors in determining the composition and abundance of symbiotic communities in gammarids. Out
of these two factors, locality appears to be more determinant of the symbiotic community of gammarids than host species, whereas habitat condition was a stronger determinant of the symbiotic communities than geographic distance. The rich and uneven symbiotic communities of native gammarids keep the host-parasite system in equilibrium. Sharply dominated symbiotic species with a high load per host individual stabilize the host population. Simplified symbiotic communities of the invasive hosts showed a relatively even distribution of species with moderate loads per individual, with heavily infected gammarids being rare in the population. The survival rate of invasive gammarids is expected to be relatively high, facilitating their population growth and further spread. Complex communities of the native gammarids constructed from many symbiotic species may prevent host populations from undergoing explosive growth, while such mechanisms may be completely or partially reduced in invasive hosts.

Keywords
Gammarus pulex, Gammarus zaddachi, Gammarus roeselii, Gammarus tigrinus, Dikerogammarus villosus, Pontogammarus robustoides, Echinogammarus ischnus

INTRODUCTION

Human activities are changing biological communities and dramatically affecting ecosystem functioning around the world (Dornelas et al. 2014, Bowler et al. 2020). The key issue leading to the development of conservation strategies and sustainable use of ecosystems is to identify the causes behind the underlying changes (Bowler et al. 2020). The most important anthropogenic factors affecting biodiversity are climate and habitat change, nature resources overexploitation, pollution and alien species (Bowler et al. 2020, Jaureguiberry et al. 2022). Designing new systemic policies and action targets should consider a complete set of drivers associated with biodiversity change to achieve sustainability objectives (Jaureguiberry et al. 2022). Pathogens and parasites of invasive species are an important, but so far underexplored step in understanding global patterns of biodiversity change (Chinchio et al. 2020). Since successful invaders may carry multiple symbiotic organisms, the translocation of non-native parasites and pathogens with their host represents an additional threat to ecosystems (Vilcinskas 2015, Young et al. 2017, Bojko et al. 2021). The novel weapons hypothesis proposes that pathogens and parasites that are vectored by invasive species into newly colonized ecosystems can act as biological weapons if they infect and kill native competitors (Vilcinskas 2015).

The main current challenge concerning alien species is the absence of a comprehensive theoretical basis to explain the resistance or recovery from bioinvasions. For instance, antibiotics and bacteriophages are known to be highly effective agents for treating bacterial diseases (Principi et al. 2019), and vaccination can also significantly reduce the risk of new infections. In the case of biological invasions devastating ecosystems, there are no reliable measures to protect environmental health. One of the factors that provide resistance to invasions is the healthy native biodiversity of a recipient ecosystem, via a phenomenon called biotic resistance (Miralles et al. 2016). In tropical marine systems, where natural biodiversity is typically high, the complex biological interactions of native communities often make it difficult for invasive species to gain a foothold (Alidoost Salimi et al. 2021). Although the effect of rich communities is not clear and there are discrepancies between studies, it found that more diverse assemblages are expected to be more resistant to invasions (Fridley et al. 2007). The success of introduced species has frequently been attributed to the escape from parasites (Torchin et al. 2003). Invasive species benefit from parasite-mediated advantages in competition because they are less likely to be infected than their native competitors (Torchin and Lafferty 2009). This phenomenon is known as the enemy release hypothesis (ERH). The ERH is supported by a number of empirical studies but several important mechanisms our understanding of the invasion process. In particular, the role of native diversity in the resistance of recipient ecosystems remains one of the questions in invasion biology (Alidoost Salimi et al. 2021).

Species richness, diversity, prevalence, various measurements of abundance, rarity and aggregation are key components in describing biological communities (Bush et al. 1997, Bock et al. 2007, Krasnov et al. 2015, Llopis-Belenguer et al. 2020, Balbuena et al. 2021, Sarabeev et al. 2022). The species number per sample (richness) is the easiest quantitative descriptor of biodiversity, often used as a characteristic of biological
systems. However, species richness alone is a non-exhaustive indicator as it is just a qualitative characteristic of community diversity and does not contain information on the abundance of species population (Wilsey et al. 2005, Ma 2005, Bock et al. 2007). Here we will study species diversity as the parameter composed of two components, species richness and species population abundance. Depending on the biological system and environment, species richness may or may not be related to species diversity (Wilsey et al. 2005, Ma 2005). Due to the extreme complexity of communities and ecosystems, we are still far from understanding the processes governing the distribution and assembly rules of biological diversity. The most prevailing current view on this issue is that species distributions would be mostly, if not exclusively, constrained by local environmental conditions, historical large-scale events, dispersal capacity of the species, and limiting similarity (Pavoine and Bonsall 2011, Llopis-Belenguer et al. 2020).

Diversity encompasses several levels in the spatial scale of species distribution. Classically, three levels of diversity are defined: α diversity or diversity at the level of the sampling unit; β diversity or the extent of diversity to the sampling unit and γ diversity or total diversity per region and at the larger geographical scale (Pavoine et al. 2016, Llopis-Belenguer et al. 2020). Each diversity level also can include three facets such as taxonomic diversity (TD, richness and abundance of taxa in a community), functional diversity (richness and abundance of functional traits) and phylogenetic diversity (PD, richness and abundance of evolutionary traits). The traditional focus of ecologists on TD leads to a loss of evolutionary and functional information as TD considers that all the species in a community are equally similar and does not take into account the phylogenetic or functional uniqueness of each species (Pavoine and Bonsall 2011, Llopis-Belenguer et al. 2020).

Here we focus on diversity analysis of eukaryotic symbiotic species (parasites and epibiotic commensals) of gammarid species. The host-parasite systems of two native (Gammarus pulex (L.) and Gammarus zaddachi Sexton, 1912) and five invasive gammarids (Gammarus roeselii (Gervais, 1835), Gammarus tigrinus Sexton, 1939, Dikerogammarus villosus (Sovinsky, 1894), Pontogammarus robustoides (G.O. Sars, 1894), Echinogammarus ischnus (Stebbing, 1899)) sampled from 16 localities in the Baltic Region of Poland were used. Since the importance of ecological processes is scale-dependent (Llopis-Belenguer et al. 2020), the analysis was performed at both α and β diversity levels. Moreover, to count both simple taxonomic and evolutionary weighted differences between sampling units, the data were analysed for two facets, TD and PD, at each organisational scale. We asked whether diversity patterns depended on locality, habitat and host species, and which factors are deterministic in the variation of diversity. Based on evidence from free-living organisms (McCann 2000, Proulx et al. 2010), and previous studies on host-parasite systems (Sarabeev 2015, Moss et al. 2020, Llopis-Belenguer et al. 2020, McNew et al. 2021, Sarabeev et al. 2022, 2023a) we hypothesise that: a) both host phylogeny and geographic localities will affect the symbiotic communities, while habitat conditions will be a stronger determinant of the communities than geographic distance; b) stable host-parasite system will be characterized by the rich symbiotic community capable of regulating and rebalancing the entire system, hence invasive host populations will tend to succeed due to poor symbiotic communities. While our previous studies have been concerned with analyzing the richness of symbiotic species of native and invasive hosts (Sarabeev 2015, Sarabeev et al. 2022, 2023a), the present work will examine species diversity to consider species evenness, to understand the mechanisms underlying host-parasite relationships in alien species, and to gain new insights into the factors that determine invasion success.

MATERIALS AND METHODS

Data

Our study is based on a database of eukaryotic symbiotic species from gammarid hosts previously collected and identified as described in Sarabeev et al. (2023a,b). Following the guidelines of Marques and Cabral’s (2007) and Shvydka’s et al. (2018), each sample typically contained 30 dissected gammarids. Overall analysed dataset represents 20 host-locality samples, including over 15,067,600 symbiotic organisms counted from 612 host individuals belonging to 7 gammarid species sampled at 16 localities in the Baltic region of Poland (Fig.1, Supplementary Table S1). The localities cover a range of rivers, streams, deltas and canals with a typical habitat for gammarids. While some of these water sources (such as streams and upper flows of rivers) were almost pristine, others showed varying degrees of anthropogenic influence (e.g. eutrophication, canalization,
and ship traffic). Sixty symbiotic species of nine phyla were identified in all samples: Ciliophora (29 species), Apicomplexa (12), Microsporidia (8), Platyhelminthes (3), Acanthocephala, Nematoda and Rotifera (2 each), and Choanozoa and Nematomorpha (1 each). The highest species richness of symbionts occurred in G. pulex (45 species), followed by D. villosus (33), G. zaddachi (28), P. robustoides, G. roeselii and G. tigrinus (20 in each) and E. ischnus (9) (Sarabeev et al. 2023a).

Data analysis

Unless otherwise stated, all analyses were carried out in the R environment (R Core Team 2022). The analysis of diversity was performed at the two hierarchical levels, infra-community (α diversity) and the component community (β diversity). The α (individual) diversity is the measure of the symbiotic community at the host individual level, while the β (total) diversity describes the symbiotic assemblages at the level of the host populations or communities (Holmes and Price 1986). In turn, the total diversity can be differentiated within a level of an organisational scale (e.g. host population), β1 and between levels of an organisational scale (e.g. host species or host community), β2. Regardless of the α or β level considered, the diversity was studied under two facets, TD and PPD. Because we did not have a complete phylogeny of the symbiotic species within communities, a Proxy for Phylogenetic Diversity (PPD) was used to estimate the phylogenetic pairwise distances between species. The PPD can be viewed as a measure of the path connecting two species traced through a Linnaean classification of the whole species of the community (Clarke and Warwick 1998). Euclidean pairwise distance matrix between species was computed with the “taxa2dist” function of package “vegan” package v. 2.6-2 (https://cran.r-project.org/web/packages/vegan/index.html) applied to a classification table (Supplementary Table S1) with symbiotic species and taxa of these species at higher levels (genus, family, order, class, phylum, in our case). The resulting distance matrix was normalized to rescale its values between 0 and 1.

The diversity of symbiotic communities was analysed with the Double Principal Coordinate Analysis (DP-CoA) (Pavoine et al. 2004). This analysis combines a version of the Rao index of diversity (Rao 1982) and the Weighted Principal Coordinate Analysis (Gower and Legendre 1986). It is based on the abundance matrix of species in a community and requires data on pairwise phylogenetic distances between species in the case of PPD analysis. In the TD analysis, all cells of the distance matrix are considered equally and maximally distant, and Rao’s index becomes equal to the Simpson’s index of diversity (Pavoine et al. 2004). We used the “dpeca” function of ade4 package v. 1.7-19 (https://cran.r-project.org/web/packages/ade4/index.html) to study the relationship between the diversity of symbiotic communities and the factors of host species, locality or habitat. For the β diversity, the result of DP-CoA was visualized using the function of “adegraphicsLoaded” which ordnates species according to the pairwise distances and abundance assembling species in a multivariate space related to the decomposition of diversity. In the case of α diversity, we used “lm.rpp” function of package RRPP v. 1.3.0 (https://cran.r-project.org/web/packages/RRPP/index.html) that performs a linear model by residual randomisation and provides empirical sampling distributions for further ANOVAs. ANOVAs were performed using random distributions of the F-statistics for TD and PPD, independently (Collyer and Adams 2018). Subsequent pairwise comparisons of α TD and PPD between host species and localities were performed using the “pairwise” function of RRPP when differences between samples from different host species or localities were significant. Matrixes with results of posterior pairwise comparisons of α TD and PPD were visualized with package corrplot V. 0.92 (https://cran.r-project.org/web/packages/corrplot/index.html).

Crossed-DPCoA was used to assess the effects of crossed factors on the α diversity (Pavoine et al. 2013) of the co-habiting G. zaddachi, G. tigrinus and D. villosus in Dziewirzyno Regoujście and Rowy Canal (note that the last species was found only in the Rowy Canal). We simultaneously analyzed the impact of two crossed factors: host species and locality. Crossed-DPCoA allowed distinguishing the proportional contribution of the sampling unit, each factor individually and the effect of the interaction of both factors on the community diversity. Three consecutive analyses of the crossed-DPCoA were run: main, the first and the second versions with functions “crossdpeca_maineffect”, “crossdpeca_version1” and “crossdpeca_version2”, respectively, in adiv package v. 2.1.2 (https://cran.r-project.org/web/packages/adiv/index.html). The main version of the...
crossed-DPCoA plots the symbiotic species, the sampling units, and the variables of the host species as the main factor, without taking into account locality differences, in a DPCoA space. Then, the first version of the crossed-DPCoA removes the amount of diversity among sampling units due to the sole effect of locality but retains the combined effects of both factors the host species and locality. Finally, the second version of the crossed-DPCoA eliminates any influence of the factor locality on the factor host species. So it provides diversity exclusively under the light of the main crossed-factor, host species.

The DPCoA and Crossed-DPCoA at the α level were run following the code to R provided by Llopis-Belenguer et al. (2020b), which is available on Zenodo (http://doi.org/10.5281/zenodo.3970614).

**RESULTS**

**Characteristics of symbiotic communities**

The abundance of symbiotic organisms was highest in native host, *G. pulex*, with an average of over 66,500 individuals per host, followed by *E. ischnus, D. villosus, G. zaddachi, G. roeselii, P. robustoides* and *G. tigrinus* (963, 573, 441, 157, 123 and 87 individuals, respectively). The communities were dominated by ciliate epibionts and parasites. Ciliate species accounted for 99.96% of the symbiotic specimens collected in *E. ischnus*, followed by 99.46% in *G. pulex*, 98.76% in *G. zaddachi*, 92.95% in *D. villosus*, 90.54% in *G. tigrinus*, 88.56% in *P. robustoides*, and 79.21% in *G. roeselii*. Out of these, by far the most common (48.2% of all symbiotic individuals) was the specific endoparasitic ciliate, *Paracollinia branchiachium*, which infects the hemocoeol and gills of *G. pulex*, while in other hosts epibiotic ciliates of *Epistylis*, *Zoothamnium* and *Pseudocarchesium* dominated in community, where the proportion of symbiotic species can range from 35.2% to 72.1% of all individuals recorded (Supplementary Table S1.). Eugregarinorida (Apicomplexa), Choanozoa, Microsporidia and Rotifera also may contribute substantially to the number of symbiotic organisms inhabiting the gammarid hosts, while helmints parasites (Platyhelminthes, Nematoda, Acanthocephala and Nematomorpha) were the rarest taxa of host assemblages. The evenest distribution of symbiotic taxa was found for *G. roeselii* and the opposite the highest dominance of ciliates was in *G. zaddachi* and *E. ischnus*.

Ελληνικά η πατηματική ιατρική ημίιτις στηριγμένη ελληνική διάταξη γνώσης και διαπρεπούς

The graphical representation of the DPCoA displayed similar patterns for the β2 TD and PPD when comparing symbiotic communities among host species (Fig. 2A and D). The first axis separated well the diversity of symbiotic communities of the native *G. pulex* from other host species. The β2 diversity of symbiotic communities of invasive hosts broadly overlapped in species TD and PPD. The separate position of *G. pulex* in the DPCoA plot is largely related to the high infection load of host-specific endoparasitic *P. branchiachium* and non-specific epibiotic *Epistylis gammari* and *E. kolbi* (Fig. 2C and F). The Rao index of the diversity of symbiotic communities tends to be lower for the two native gammarids, *G. pulex* and *G. zaddachi*, compared to the invasive hosts, i.e. *D. villosus*, *G. roeselii*, *G. tigrinus* and *P. robustoides* (Fig. 6B and D). The DPCoA did not reveal clear differences in the diversity of symbiotic communities of *D. villosus* and *P. robustoides* in Wisła Sobieszewska where these hosts occurred in mixed populations (Fig. S1). The same analysis for TD of symbiotic communities from Dźwirzyno Regoujście and Rowy Canal, where *G. zaddachi*, *G. tigrinus* and *D. villosus* co-occurred, revealed a stronger separation by spatial gradient and then by host species (Fig. 3A). The species diversity of *G. zaddachi* and *G. tigrinus* overlapped considerably within the localities, while the symbiotic community of *D. villosus* was well separated along both axes, despite co-occurring with the two hosts mentioned above in the same locality. A similar tendency was observed in the species PPD for this case of species co-occurrence but with a substantial overlap of localities for *G. zaddachi* and *G. tigrinus* (Fig. 7B). The distinctions between Dźwirzyno Regoujście and Rowy Canal were largely determined by the local variation of epibiotic ciliates (*Carchesium duplicatum*, *Zoothamnium gammari* and *Zoothamnium hyalinum*). The Rao index of the diversity of symbiotic communities in the native host, *G. zaddachi*, was lower compared to the two invasive species, *G. tigrinus* and *D. villosus* from Dźwirzyno Regoujście and the Rowy Canal.

The TD and PPD of symbiotic communities within populations of *D. villosus* across localities agreed in
showing the separate position of the symbiont community sampled from the Rowy Canal, which was characterized by the lowest diversity index and high infection of ciliates, *Zoothamnium hyalinum* (Fig. S2). All three populations of *G. tigrinus* were well separated on the DPCoA plot based on data inferred from symbiotic organisms (Fig. S3). The first axis separated the diversity of symbiotic communities of *G. pulex* in the Wodnica Slupia and Debki Piasnicy from the other five localities: Orzechowa, Smolędzino Lupawa, Słupsk Stream, Lesny Dwor and Krepa Słupska (Fig. S4). All these five populations of *G. pulex* were heavily infected with *P. branchiarum*. The symbiotic community of the freshwater habitat was more diverse than that of the brackish water and well separated by the first axis from the latter on the DPCoA plot of species TD and PPD (Fig. 4).

Τεστίνγ ηοστ, ηαβιτατ ανδ λοςαλιτψ εφφεςτς ον α διvερσιτψ

At the α level, we found significant differences in both terms of TD and PPD of microsymbionts hosted by the different gammarid species (Table S2), mainly because the parasite community of the invasive Balkan crustaceans, *G. roeselii*, was much more diverse than that of other hosts (Fig. 5). In addition, the PPD analysis showed the lowest diversity of symbiotic communities in *E. ischnus* and *G. pulex*. Examination of the two gammarids *D. villosus* and *P. robustoides* in the Wisła Sobieszewska locality did not reveal significant differences in α diversity of symbiotic communities between these hosts (Table S3 and Fig. S5).

The crossed-DPCoA applied to the survey of symbiotic communities from Dźwirzyno Regoujście and the Rowy Canal, where *G. zaddachi*, *G. tigrinus* and *D. villosus* co-occurred, showed that diversity within each locality constituted the highest proportion of symbiotic diversity in the community (50.3% and 39.7% for TD and PPD, respectively), followed by host species and host individuals (Table 1). The graphical representations of the first version of the crossed-DPCoA considering TD and PPD were congruent (Fig. 6). In both cases, the first axis separated the symbiotic communities of the two localities. The symbiotic communities of the three host species showed a broad overlap in both terms TD and PPD.

The effect of locality on α diversity of symbiotic communities of *G. tigrinus* and *G. pulex* was significant in both terms TD and PPD and only in the PPD for *D. villosus* (Table S4-S6). According to the PPD, symbiotic communities of *D. villosus* were significantly less diverse in the Rowy Canal and Jamienisky Nurt than in the other three localities (Fig. S6). The symbiotic community of *G. tigrinus* from Martwa Wisła was significantly different from Dźwirzyno Regoujście and the Rowy Canal (Fig. S7). The TD and PPD consistently showed the highest α diversity of symbiotic communities of *G. pulex* from the Słupsk stream (Fig. S8). In addition, a higher TD index of symbiotic communities of *G. pulex* was observed from Debki Piasnicy than in the other five localities, while PPD revealed less diverse symbiotic communities from Smolędzino Lupawa and Krepa Słupska compared to the other five localities. Similarly to β diversity, the symbiotic community in freshwater habitats was more diverse than that of brackish waters at the α level (Fig. 7, Table S7).

**DISCUSSION**

**Hypotheses evaluation**

We examined mechanisms driving the symbiotic communities of native and invasive gammarid hosts in the Baltic region. The analysis performed at two organization levels (α and β) and two facets (TD and PPD) of diversity shows largely congruent results and conforms with previous studies of both free-living and parasitic organisms (Devictor et al. 2010, Llopis-Belenguer et al. 2020). Thus, the conclusions drawn in the present study on the factors determining the diversity of symbiotic communities can be interpreted as robust.

Our results showed that both host species and localities affect the diversity of symbiotic organisms but in the case of host co-occurrence, the diversity of different species may broadly overlap. These findings are in accordance with our initial expectation assumed that both the locality and host species are important drivers of the symbiotic community structure of gammarids and generally agree with previous studies of other host-parasite systems (Llopis-Belenguer et al., 2020b; McNew et al., 2021; Moss et al., 2020; Sasal et al., 1997). Out of these two factors, locality appears to be more crucial for the symbiotic community of
ganmarids than host species. For example, the broad overlap at the β1 diversity level and the absence of significant distinctions at the α diversity level of symbiotic communities were observed for *D. villosus* and *P. robustoides* from Wisla Sobieszewska. The analysis of the sympatric hosts *G. tigrinus* and *G. zaddachi* in the Rowy Canal and Dźwirzyno Regoujście revealed a grouping of the symbiotic communities by localities at the β1 diversity level. Similarly, at the α level, the diversity of symbionts differed between localities but not between host species. Furthermore, the percentage of diversity associated with each factor in the crossed-DPCoA was slightly higher for localities than for host species. Although there was no apparent effect of host species on the diversity of symbiotic communities in the described cases, the ANOVA tests applied to the overall data revealed a significant influence of host species on diversity. The pairwise analysis showed that the differences were largely related to the highest richness of the symbiotic community (Sarabeev et al. 2023a) and simultaneously its low diversity of *G. pulex*, as well as to the most diverse community of *G. roselii*. These results are consistent with evidence from parasite communities of amphibian hosts in California ponds (Moss et al. 2020), but differ from the host-parasite system of mugilid hosts in the Western Mediterranean (Llopis-Belenguer et al. 2020). The helminth community of mugilids is predominantly composed of host-specific (stenoxenic or oioxenic) species (Sarabeev et al. 2013, Sarabeev 2015, Sarabeev and Tkach 2019, Llopis-Belenguer et al. 2020), while in gammarids the symbiotic community tends to be nonspecific (euryxenic). Considering the “filters” theory proposed by (Combes 2001) to account for the ability of parasites to colonize hosts, the host specificity filter is a less important driver of symbiotic communities in gammarids than in mugilid hosts. In other words, “the compatibility filter” is more broadly open for symbiotic species in gammarid hosts.

The habitat condition was another important factor in determining species diversity. Differences between freshwater and brackish environments were significant for both levels and facets of data. Geographical distance proved to be less significant than local habitat characteristics in determining the diversity of symbiotic communities of gammarids at the regional scale of Pomerania of the Baltic Sea. The most obvious example is an extremely poor symbiotic community with only 5 species and the lowest load per host individual in 21 specimens in the Martwa Wisla sample characterized by the highest salinity level (Mendeley data, DOI: 10.17632/c9fzyzr52.1, File 3) and the closely situated freshwater localities of Wisla Sobieszewska and Port Lodolamaczcy with 16-20 species and load in 123-171 symbiotic individuals per host. This finding points to the filtering of parasite diversity by the environment, which is in line with earlier research on the impact of habitat on symbiotic communities in both aquatic and terrestrial realms (e.g. Krasnov et al., 2015; Kołodziej-Sobocińska, 2019; Levy et al., 2019; Llopis-Belenguer et al., 2020b). At both levels and facets of diversity, the symbiotic communities of two native hosts, *G. pulex* and *G. zaddachi*, showed a general tendency to be less diverse compared with that of invasive gammarids. Out of these hosts, symbiotic communities of *G. pulex* were well separated on the DPCoA plot presenting the highest richness and relatively low diversity. Thus, the rich symbiotic species community with low diversity indices apparently indicates suggests that the native systems of symbiotic organisms and their gammarid hosts rest in equilibrium where sharply dominated symbiotic species with high load per host individual can regulate the host population. The rich and complex communities of the native gammarid *G. pulex*, constructed from many symbiotic species, may prevent explosive growth of host populations, whereas such a mechanism may be partially reduced in invasive hosts. Many symbiotic organisms are known to alter the behaviour, affect fitness conditions, reduce growth and increase the mortality rate of their crustacean hosts and thus may play the role of effective regulators of the host population dynamics and even shape an entire ecosystem (Gómez-Gutierrez et al. 2003, Prokopowicz et al. 2010). Microsporidians, helminths, apostomatid ciliates and gregarines exhibit various degrees of virulence (Jiménez et al. 2002, Gómez-Gutierrez et al. 2003, Kaldonski et al. 2007, Bacela-Spychalska et al. 2012, Chen et al. 2015). The diversity analysis performed here suggests that most likely candidate that can act as effective regulator of dramatic population explosions of *G. pulex* in the studied area is *P. branchiurum*. This apostomatid ciliate infects the host hemocoel and gills in extremely high abundance and may significantly reduce the viability of its host (our unpublished data). Moreover, apostomatid ciliates are well known pathogens of crustaceans that can substantially increase the mortality rate of infected individuals (e.g. Gómez-Gutiérrez et al., 2012; Metz and Hechinger, 2021).
In contrast to native gammarids, the simplified symbiotic communities of invasive hosts showed relative evenness (high diversity indices) in the species distribution. Because symbiotic species are fairly evenly distributed among hosts with moderate load per individual, heavily infected gammarids are rare in the population. The survival rate of the invasive gammarids is therefore likely to be high facilitating their population success. These findings corroborate the basic ecological ideas of Miller & Odum (1954) and more recent studies by McCann, (2000), Proulx et al. (2010), who suggested that greatly simplified communities are characterized by more violent fluctuations in population density than diverse biological assemblages. In this context, it is interesting to note the symbiotic community of the Balkan species G. roeselii, which has relatively high α richness and the highest diversity at both levels and facets. This pattern of biodiversity contrasts with that of the native G. pulex which is characterized by the highest richness and lowest diversity. This suggest that the symbiotic diversity of G. roeselii has a low regulation ability to control a host’s explosive growth. The high density of G. roeselii at the sampled locality of the Wieprza River (Mendeley data, DOI: 10.17632/c9fzyyjzr52.1, File 3) is an additional argument supporting our assumption about a low regulation ability of the symbiotic community to control the host population growth. Marcogliese and Cone (1997) suggested that every biological system should have certain keystone parasites that can determine its stability in time and space. According to the ERH (Torchin et al. 2003, Torchin and Lafferty 2009), G. roeselii has probably lost a subset of natural pathogens during its invasion history which can control host population growth. This may have facilitated the invasion success of this gammarid crustacean across European waters (e.g. Hesselschwerdt et al., 2009; Paganelli et al., 2015). In fact, Piscart et al. (2010) and Paganelli et al. (2016) considered G. roeselii as a well-naturalised species with a long invasion history in Eastern and Central Europe. At the same time, the host-parasite system of G. roeselii could not be considered as an established self-regulated system and thus the population of this host can further succeed and occupy new geographic areas.

**New findings**

The Rao’s index of diversity captures both the number of species in a community and the relative abundance of each (Pavoine et al. 2004), it is closely related to measurements of aggregation in communities (Ma 2015, Lang et al. 2017, Sarabeev et al. 2017). Thus, our results seem to confirm previous findings showing that parasites of invasive hosts are less aggregated in communities compared to those of native hosts (Sarabeev et al., 2017, 2022). Low aggregation of symbiotic communities entails a short distribution tail of parasite abundance (i.e. lower number of heavily infected hosts). Since host mortality and morbidity are often dose-dependent, parasites have the greatest effect on individuals in the tail of the parasite distribution (Wilson et al. 2002). Thus the present research on the symbiotic system of gammarids and organisms in the Baltic region suggests that parasites have limited ability to regulate host populations of invasive gammarid species in the absence of heavily infected individuals. Although the Ponto-Kaspiian migrants have largely rescued their parasites in the new distribution range (Wattier et al. 2007, Bojko et al. 2013, Sarabeev et al. 2023a), their eukaryotic pathogens have apparently been partially or completely lost in European waters.

An interesting finding of the present study is the occurrence of invasive gammarid species only in canals or river deltas, while in the slightly upper parts of even the same water system only native G. pulex was found. Felten et al. (2008) showed that D. villosus and G. pulex seem to have a wide niche defined by temperature, substrate, salinity and flow velocity, although D. villosus prefers coarser substrates (stones, gravel) and deeper waters than G. pulex. The authors suggested that water depth is the stronger factor for the spatial distribution of D. villosus, as this species has not yet colonize the tributaries of the large rivers where it has rapidly invaded (Beckmann et al. 2005, Josens et al. 2005). At the same time, during our observation sampling in the Lupawa River system, we were able to detect D. villosus in the shallow littoral zone of the Rowy Canal among stones and gravel which were laid out there as elements of strengthening the shore. G. pulex was sampled in the gravel bed from a depth of 0.5-1 m of the same river about 10 km upstream. The two sampling sites are thus characterized by the same depth and substrate, but differ slightly in terms of flow, salinity, eutrophication level and anthropogenic influences (Rowy canal is shipping and partially canalized). The most probable scenario is that a relative “virgin” or undisturbed ecosystem of the Lupawa River characterised by high natural biodiversity and complex biotic interactions between symbionts,
pathogens and hosts may prevent the spread of invaders upstream of the river system. In other words, we assume that a relatively pristine ecosystem characterized by diverse native symbiotic community is resilient to invaders. A similar case was recently described for the invasive so-iuy mullet fish in the Aegean Sea (Sarabeev et al. 2022). Moreover, Alidoost Salimi et al. (2021) in their review of research on non-native species in tropical marine ecosystems, highlighted the difficulty for invaders to get a foothold in a new area when the recipient ecosystem has a high natural biodiversity with complex biotic interactions of the native communities. Thus preserving and maintaining the natural ecosystems with the complex and diverse native communities may play an essential role in hampering the introduction and subsequent spread of invaders in new areas.

CONCLUSIONS

We believe that this is the first analysis of the diversity of eukaryotic symbiotic organisms of native and invasive gammarid hosts in European waters based on original field data and a broad range of taxa providing insights into the biology of host-parasite interactions. Furthermore, this work is the first attempt to study the symbiotic systems of gammarid hosts in the context of a macroecological framework, as it covers multispecies interactions of both hosts and their symbionts at the regional scale of the Baltic basin to study broad-scale ecological patterns and processes. Our results showed that both host phylogeny and locality are key drivers shaping assembly composition. Out of these two factors, locality appears to be a stronger determinant of the symbiotic community of gammarids than host species, whereas habitat conditions were a stronger determinant of the symbiotic communities than geographic distance. The rich and uneven symbiotic communities of the native gammarids keep the host-parasite system in equilibrium, where sharply dominated by symbiotic species with a high load per host individual able to regulate the host population. Complex communities of the native gammarids constructed from many symbiotic species may prevent host populations from undergoing explosive growth, while such mechanisms may be partially reduced in invasive hosts. The simplified symbiotic communities of invasive hosts showed relative evenness in the species distribution with moderate load per individual where heavily infected gammarids are rare in the population. Thus, the survival rate of invasive gammarids is expected to be relatively high, facilitating their population growth and further spread. This study expands our understanding of how native diversity maintains community balance and protects ecosystems from bioinvasions.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

ETHICS STATEMENT

The study does not require any ethical approval.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

DATA ACCESSIBILITY

Associated raw data are available via Mendeley Data, doi: 10.17632/c9fzyzyr52.1 and were explained in Sarabeev et al. (2023). Taxonomy and abundance data of symbiotic species for each sample are provided in the Supplementary Table S1.

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AUTHOR CONTRIBUTIONS
V.S. and J.A.B. conceived the ideas. V.S. collected the data, performed the analyses and drafted the manuscript. M.Ov. supervised and helped to collect the data and identify of microsporidians. V.D. and M.Or. contributed to the interpretation of the data and writing of the manuscript. R.A.S. and J.M.L. Molecular identification of species. All authors approved the final version of the manuscript.

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Biosketch

Volodimir Sarabeev is a professor at Zaporizhzhia National University, Ukraine and an invited researcher at the Institute of Parasitology, Slovak Academy of Sciences, Slovak Republic. He works on parasites of aquatic alien hosts and is broadly interested in understanding the role of parasites in the invasion process. He develops a new macroecological framework for analyzing complex host-parasite relationships of alien species. The framework provides mechanistic explanations for the enemy release hypothesis and has the potential to contribute to biodiversity conservation and the control of invasive species.

Table 1. Percentage of diversity associated with each factor. Taxonomic (TD) and proxy for phylogenetic (PPD) diversity of symbiotic communities of three host species (Dicrogammarus villosus, Gammarus tigrinus and G. zaddachi) from two localities in the Baltic region of Poland (Dźwirzyno Regoujście and Rowy Canal), where these hosts co-occurred.

<table>
<thead>
<tr>
<th></th>
<th>TD</th>
<th>PPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host individual</td>
<td>26.7</td>
<td>37.4</td>
</tr>
<tr>
<td>Locality</td>
<td>50.3</td>
<td>39.7</td>
</tr>
<tr>
<td>Host species</td>
<td>48.1</td>
<td>37.3</td>
</tr>
<tr>
<td>Locality × Host species*</td>
<td>-25.1</td>
<td>-14.3</td>
</tr>
</tbody>
</table>

*A negative interaction coefficient means that the effect of the combined action of two predictors is less than
the sum of the individual effects.

Figure captions:

Fig. 1. Sixteen sample localities of gammarid hosts in the Baltic region of Poland with geographic coordinates in brackets (Adapted from Google Earth (2023)): DP, Debi Piasnicy (54.832288, 18.061855); DR, Dźwirzyno Regoujście (54.153051, 15.390354); DW, Darlowko Wieprza (54.433499, 16.389061); JN, Janiennsky Nurt (54.281658, 16.135581); KS, Krepa Słupsk (54.403371, 17.047010); LD, Lesny Dwor (54.358931, 17.155713); MP, Mrzeżyno Pera (54.140988, 15.284668); MW, Martwa Wisła (54.310727, 18.867225); O, Orzechowa (54.598841, 16.918841); PL, Port Lodolamaczy (54.308968, 18.925215); RC, Rowy Canal (54.667288, 17.056982); SL, Smolędzino Lupawa (54.662085, 17.212266); SS, Slupsk Stream (54.475260, 17.042841); SW, Wodnica Slupia (54.556568, 16.875233); WD, Wolin Dziwna (53.840308, 14.621854); WS, Wisła Sobieszewska (54.314119, 18.931805).

Fig. 2. Double Principal Coordinate Analysis (DPCoA) biplot ordination, describing $\beta^2$ taxonomic (A-C) and proxy for phylogenetic (D-F) differences in symbiotic communities between gammarid hosts. Black circles indicate each host species, and their relative positions reflect their taxonomic and proxy for phylogenetic dissimilarities (A and D). Hosts are one of seven species: DV, Dikerogammarus villosus; EI, Echinogammarus ischnus; GP, Gammarus pulex; GR, G. roeselii; GT, G. tigrinus; GZ, G. zaddachi; PR, Pontogammarus robustoides. Rao’s index of diversity (B and E) and symbiotic species (C and F) are plotted in the same ordination space as the host species. Symbiotic species abbreviations and their taxonomic position are presented in Supplementary Table S1. “d” indicates the length of the side of the grey squares of the background grid.

Fig. 3. DPCoA biplot ordination, describing $\beta^1$ taxonomic (A-C) and proxy for phylogenetic (D-F) differences in symbiotic communities between samples of Gammarus zaddachi (GZ), G. tigrinus (GT) and Dikerogammarus villosus (DV) from two localities Dźwirzyno Regoujście (DR) and Rowy Canal (RC), where these hosts occurred sympatrically. Black circles indicate each locality, and their relative positions reflect their taxonomic and proxy for phylogenetic dissimilarities (A and D). Rao’s index of diversity (B and E) and symbiotic species (C and F) are plotted in the same ordination space as the samples. Symbols are the same as on Fig. 2.

Fig. 4. DPCoA biplot ordination, describing $\beta^1$ taxonomic (A-C) and proxy for phylogenetic (D-F) differences in symbiotic communities between freshwater (FW) and brackish (BW) habitats. Black circles indicate each locality, and their relative positions reflect their taxonomic and proxy for phylogenetic dissimilarities (A and D). Rao’s index of diversity (B and E) and symbiotic species (C and F) are plotted in the same ordination space as the localities. The locality abbreviations are defined in Fig. 1. Symbols are the same as on Fig. 2.

Fig. 5. Symbionts $\alpha$ diversity in terms of taxonomic (TD) and proxy for the phylogenetic (PPD) diversity for each host individual of each gammarus species (A and C). Posteriori pairwise comparisons of $\alpha$ TD (B) and PPD (D). Distances between host species are marked with digits, the size and the colour intensity of circles. Insignificant differences between localities are marked with a cross (P < 0.05). Hosts are one of seven species: DV, Dikerogammarus villosus; EI, Echinogammarus ischnus; GP, Gammarus pulex; GR, G. roeselii; GT, G. tigrinus; GZ, G. zaddachi; PR, Pontogammarus robustoides.

Fig. 6. Position of all communities (host individuals) in the crossed-DPCoA version 1 TD (A and C) and PPD (B and D) space. The $\beta$ diversity grouped by two localities (A and B) and three host species (C and D). Abbreviation for host species: DV, Dikerogammarus villosus; GT, G. tigrinus; GZ, G. zaddachi; abbreviations for localities DR, Dźwirzyno Regoujście and RC, Rowy Canal. d (top-right) provides the scale (length of a square side in the grey grid).

Fig. 7. Symbionts $\alpha$ diversity in terms of taxonomic (TD) and proxy for the phylogenetic (PPD) diversity for each host individual of gammarids in each habitat (BW, brackish and FW, freshwater). Differences between habitats were significant (P > 0.01) (Supplementary Table S7).
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