



Changes in native and introduced host–parasite networks

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Abstract Introduced species can alter the dynamics and structure of a native community. Network analysis provides a tool to study host–parasite interactions that can help to predict the possible impact of biological invasions or other disturbances. In this study, we used weighted bipartite networks to assess differences in the interaction patterns between hosts and helminth parasites of native (Sea of Japan) and invasive (Black Sea and Sea of Azov) populations of *Planiliza haematocheilus* (Teleostei: Mugilidae). We employed three quantitative network descriptors, connectance,

weighted nestedness and modularity, to gain insight into the structure of the host–parasite networks in the native and invaded areas. The role of parasite species in the networks was assessed using the betweenness centrality index. We analyzed networks encompassing the whole helminth community and subsets of species classified by their transmission strategy. The analyses were downscaled to host individual-level to consider intraspecific variation in parasite communities. We found significant differences between networks in the native and invaded areas. The latter presented a higher value of nestedness, which may indicate a co-occurrence between parasite species with many connections in the network and species with fewer interactions within the same individual-host. In addition, modularity was higher in the native area's networks than those of the invaded area, with subgroups of host individuals that interact more frequently with certain parasite species than with others. Only the networks composed of actively transmitted parasites and ectoparasites did not show significant differences in modularity between the Sea of Azov and the Sea of Japan, which could be due to the introduction of a part of the native community into the invaded environment, with a lower diversity and abundance of species. We show that network analysis provides a valuable tool to illuminate the changes that occur in host–parasite interactions when an invasive species and its parasite community are introduced into a new area.

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Introduction

Parasites play an important role in the functioning, structure and stability of ecological communities (Timi and Poulin 2020). Currently it is recognized that parasite communities are a fundamental component in ecological systems, since they represent a large proportion of its diversity and biomass (Poulin and Morand 2000; Kuris et al. 2008; Timi and Poulin 2020). In addition, they have the ability to directly or indirectly disrupt their host community dynamics (Timi and Poulin 2020). Invasive species represent a threat to communities because their introduction does not allow enough time to elapse for gradual evolutionary adjustments of native species to their presence (Poulin 2017; Llopis-Belenguer et al. 2020). Hence, parasites can deeply modify different levels of ecological organization (Tompkins et al. 2011). However, an often neglected aspect of biological invasions is that invasive hosts can influence the processes of ecosystems through the parasites they harbor (Tompkins et al. 2011; Chalkowski et al. 2018). In fact, parasites can play a critical role in biological invasions (Chalkowski et al. 2018). For instance, invasive hosts can disrupt native host–parasite interactions and co-introduced parasites can emerge as new pathogens and establish new interactions with native hosts. Despite this realization, research on parasitism is lagging behind investigations of free-living organisms in the context of biological invasions (Poulin 2017; Timi and Poulin 2020).

Traditionally, the study of the impact of parasites in communities has been based on mathematical models or experiments that only have the ability to address interactions between a few species at a time and often rest on simplifying assumptions (Poulin 2010). However, the influence of parasites on ecological systems can be better understood if the parasite community is analyzed in its entirety, that is, as a biological network of interactions (Poulin et al. 2013). For instance, the parasite community can modulate host responses and adaptations to infection (Ashby and King 2017; Betts et al. 2018). Several studies have focused on the possible scenarios and effects caused by parasite

species in the new environment and the dynamics of their communities (Lima Jr et al. 2012; Amundsen et al. 2013; Goedknecht et al. 2016; Campião and Dáttilo 2020). However, the impact of host invasions on the whole parasite community, especially how invasive parasites alter the interactions between native parasites and their native hosts, remains a largely unstudied issue (Telfer and Bown 2012; Amundsen et al. 2013; Llopis-Belenguer et al. 2020).

Bipartite networks offer an effective tool to examine these host–parasite interactions. In bipartite networks, only host–parasite interactions are considered, which allows knowing how host individuals and parasite species are associated in a community (Dormann et al. 2008; Poulin 2010). So, parasites and hosts represent nodes of the network, and the interactions between nodes (i.e., presence–absence or abundance of parasites in one host) become links (i.e., edges) in the network (Poulin 2010). Whereas most studies to date have focused on the characterization of the host–parasite network (Runghen et al. 2021), few have attempted to compare different networks, and much less within the context of a biological invasion (e.g. Traveset et al. 2013; Bufford et al. 2020).

Here, we study the interaction networks composed of populations of the so-iuy mullet *Planiliza haematocheilus* (Temminck and Schlegel 1845) and its community of helminth parasites in two geographic areas, the Sea of Japan and the Black Sea—Sea of Azov. *Planiliza haematocheilus* is native to the Sea of Japan and Amur River Estuary. It was introduced in the Sea of Azov and Black Sea for commercial purposes in the 1970s, where it established and originated a viable population, becoming an invasive species (Sarabeev 2015). Currently, *P. haematocheilus* outgrows and displaces other local fish, notably sympatric grey mullets (Mugilidae). Up to five grey mullets are known to be distributed in the Sea of Azov (Froese and Pauly 2021). The abundance, distribution and biodiversity of helminth parasites of this species in both its native and invaded area is well known (Sarabeev 2015; Sarabeev et al. 2017a, b, 2018, 2019), which provides a unique system to study and compare the host intraspecific distribution of parasites and the disturbances caused in a community that has been invaded.

In this study, we use three network indices, connectance, weighted nestedness and modularity, to assess the changes in the interaction patterns of *P.*

haematocheilus with its parasites and assess the effect of its introduction using a host individual-based network analysis (Llopis-Belenguer et al. 2020). These three indices are deeply affected when a disturbance occurs in the system (Landi et al. 2018) and have been widely used as indicators of network properties (Olesen et al. 2007; Traveset et al. 2013; Dáttilo et al. 2014; Hernández-Castellano et al. 2020), including those involving host–parasite interactions (Lima Jr et al. 2012; Amundsen et al. 2013; Bellay et al. 2020; Campião and Dáttilo 2020; Valverde et al. 2020).

Each index is informative about a different aspect of the nature of the network. Connectance is the proportion of links made out of all possible links on the network (Dormann 2020), i.e., the actual observed interactions of all possible interactions between host individuals and parasite species. In an ecological network, it is unlikely that all possible interactions occur, that is, all parasite species infect all host individuals. So connectance informs about the severity of biological (immune system) and ecological (exposure to parasites and behavior) constraints that impede the complete infection of the individual (Bellay et al. 2013). Nestedness measures the hierarchical organization of the community (Almeida-Neto and Ulrich 2011). It describes the extent to which a group of parasite species with few interactions co-occurs in a subset of host individuals infected with parasites with many interactions. In other words, in a nested network, the former interacts with host individuals with high parasite diversity, whereas the latter interact with host individuals with rich and poor parasite communities (McQuaid and Britton 2013; Runghen et al. 2021). Modularity assesses the division of the network into different modules or compartments that are made up of a set of species or individuals that interact more frequently with each other than with others belonging to another module (Newman and Girvan 2004). That is, in an individual-based network, higher modularity would mean that there are subsets (i.e., modules) of host individuals that tend to interact more frequently with certain parasite species than with others in the community (D’Bastiani et al. 2020; Llopis-Belenguer et al. 2020). Additionally, the betweenness centrality index (*BC*) was calculated. This index quantifies the importance of a node (i.e., parasite species) as intermediaries between the different nodes of the network (Chen et al. 2008; Martín González et al.

2010; Gómez et al. 2013). Thus, *BC* allows us to quantify parasite species that are important for the cohesion of the network, or, in other words, to describe the role of a parasite species as a connector (Martín González et al. 2010).

Since helminth species of grey mullets differ in their transmission strategies and thus in the way they colonize their hosts, we also examined networks with different subsets of parasite species according to their transmission strategy. We expect that comparing results from the different subsets between areas would enable us to better assess how host invasion affects the structure and dynamics of the host–parasite networks (Sarabeev et al. 2018; Llopis-Belenguer et al. 2020).

The objective of the present study is to compare the structure of the networks of *P. haematocheilus* individuals and their parasite communities between two locations (native and invaded area). In addition, we determine whether the introduction of *P. haematocheilus* into a new area altered the structure of its helminth parasite community. Antagonistic networks, such as a host–parasite networks, are characterized by being significantly modular and antinested (Fortuna et al. 2010; Thébault and Fontaine 2010; Fontaine et al. 2011; Morris et al. 2014; Morrison and Dirzo 2020). When a biological invasion occurs, the host–parasite interaction structure is expected to be disrupted (Runghen et al. 2021), and hence, we foresee a loss of its antagonistic “identity” or original structure. Previous studies indicate that, when *P. haematocheilus* arrived at the invaded area, it lost part of its native parasites (enemy release), while it acquired other parasite species native from the invaded area, especially trophically transmitted parasites (Sarabeev 2015; Sarabeev et al. 2017a, 2017b, 2018, 2019). Therefore, we expect the structure of the host–parasite network from the invaded area to be modified that will depend on nature of parasite origin and host specificity.

Material and methods

Data

The present study is based on a database of helminth parasites (Acanthocephala, Platyhelminthes and Nematoda) occurring as larval or adult stages from grey mullets (Mugilidae) collected in two areas, the

Sea of Japan (thereafter the native area) and Sea of Azov and Black Sea (thereafter the invaded area) (see Sarabeev 2015 and Availability of data and material). All fish and parasites were collected according to a standardized protocol (Kostadinova et al. 2004). We built abundance matrices of *P. haematocheilus* individuals (rows) by helminth species (columns) for the two areas. Data was collected at 11 locations between the native and invaded area, during three seasons (winter excluded) in 1998, 1999, 2004, 2005, 2009, 2011 and 2013 (Sarabeev 2015). Samples were aggregated by area because the analyses of short periods of time possibly misrepresent the real dynamic of the network structure at a macroecological scale (Poulin 2010). Furthermore, previous evidence indicates that seasonal variation among surveys has not a strong effect in the structure of helminth infracommunities and network structure (Sarabeev et al. 2018; Llopis-Belenguer et al. 2020). The present *P. haematocheilus* dataset comprises information of 204 and 427 fish, and 21 and 25 helminth species in the native and invaded areas, respectively. Six species of Monogenea were co-introduced with *P. haematocheilus* in the invaded area and are the only ones shared in both areas (Sarabeev et al. 2017a).

Based on previous studies (Sarabeev et al. 2017a), to analyze networks formed by different subsets of parasite species differing in transmission strategy, we considered different subsets of all matrices (Supplementary Information, Table S1). Thus, analyses were performed for the following sets:

1. The whole helminth community.
2. Actively transmitted parasites. Adults and larvae of Monogenea, and metacercaria of Trematoda. These forms infect the host actively as free-swimming larval stages.
3. Trophically (passively) transmitted parasites. Adult Trematoda and Acanthocephala, adult and larval Nematoda. Infections occur by ingestion of infected prey.
4. Ectoparasites. Subset of actively transmitted parasites that occur on the host formed exclusively by Monogenea. This group is the only one that presents helminth species co-introduced with *P. haematocheilus* (Sarabeev et al. 2017a).

Thus, eight bipartite matrices (four per area) were analyzed (Supplementary Information, Table S2). Hosts without interaction with the parasite species

concerned were excluded from the corresponding subset. Additionally, data on the prevalence and abundance of the parasites in both locations were determined (Supplementary Information, Tables S3, 4).

Network analysis

Previous studies comparing native and invaded host–parasite networks have generally been performed at the species-level, i.e., considering the interactions between host species and parasite species (e.g., Amundsen et al. 2013; Bufford et al. 2020). However, interactions in ecosystems actually occur between individuals (Tur et al. 2015) and this is especially important in an invasion context because the acquisition and transmission of parasites occur at host–individual level (individuals are infected, rather than species) (Tompkins et al. 2011; Pilosof et al. 2015). In a host–parasite network, a host individual may interact with different species of parasites (co-infection). So, this host individual can play a more important role for the transmission of parasites due to their immunity or behavior status, than a conspecific infected with a single species (Morand and Deter 2009; Pilosof et al. 2015; Runghen et al. 2021). For this reason, we performed the analyses at the host individual-level. This allows representing interactions between parasites and host individuals in the community and thus considering the intraspecific variation of the parasite community within a host species (Godfrey 2013; Llopis-Belenguer et al. 2020).

All the analyses were carried out in R (R Core Team 2020). We used the package `ggbiplot` (Jordano 2021) to plot the eight bipartite networks. The eight matrices were then analyzed independently using package `bipartite` (v. 2.15) (Dormann et al. 2008). Three indices/metrics were computed: (1) connectance (C) (Dunne et al. 2002), (2) weighted nestedness based on overlap and decreasing fill ($WNODF$) (Almeida-Neto and Ulrich 2011), and (3) modularity (Q) (Newman and Girvan 2004).

C and $WNODF$ were calculated using the `networklevel` function. C can vary between 0 (no realized links) and 1 (all realized links) (Fonseca and John 1996) and non-standardized $WNODF$ values can range between 0 (not nested) and 100 (full nested) (Almeida-Neto and Ulrich 2011). Q was estimated with function `computeModules`, and non-

standardized values can vary from 0 (prevalent interactions between modules) to 1 (prevalent interactions within modules) (Miranda et al. 2019). We ran `computeModules` with the Beckett (2016) algorithm that considers quantitative information (i.e., the parasite species abundance in one single host), assigns different parasite species to one module and only allows host–parasite interactions (Supplementary Information, Table S5–7).

As C is a proportion of the possible interactions in the network, its value is not influenced by the size of the network (Dunne et al. 2002). In contrast, $WNODF$ and Q values are influenced by network size, and thus the initial estimates were standardized for comparison between the areas. For this purpose, 1000 null model replicates of the real matrices were generated with function `null.model` and the `swap.web` algorithm (Dormann et al. 2009). Since the standardized values of $WNODF$ are known to be highly dependent on the algorithm used for the null model (Miranda et al. 2019), we initially standardized the estimates with both the `swap.web` and the `vaznull` algorithms (Vázquez et al. 2007). The latter randomizes the total number of individual interactions observed in the original interaction matrix, being more restrictive than `swap.web`. Since results were similar (Supplementary Information, Table S8–10), we report here standardized values obtained with `swap.web`. Standardized values were then obtained as

$$\text{standardised } X = \frac{X_{\text{observed}} - \bar{X}_{\text{null}}}{\sigma \bar{X}_{\text{null}}}$$

where X_{observed} is the original $WNODF$ or Q value, \bar{X}_{null} and $\sigma \bar{X}_{\text{null}}$ are, respectively, the mean and standard deviation of $WNODF$ or Q values obtained in the 1000 null model replicates (Dormann and Strauss 2014). For those communities that show a negative $WNODF$ value (i.e., the real value of $WNODF$ is smaller than what would be expected by chance), the parasite niches are well established in their hosts, which would not promote a nested structure (Almeida-Neto and Ulrich 2011).

We built 95% confidence intervals of the three estimated indices using bootstrap replicates by rows (host individuals) of the original matrices. So, 1000 random networks of each matrix were generated with replacement and a fixed number of individuals, and then the confidence intervals for each index are

calculated (Llopis-Belenguer et al. 2020). Statistical differences of the three indices between the native and invaded areas were tested by means of non-parametric Mann–Whitney–Wilcoxon tests (function `wilcox.test`).

In order to facilitate interpretation of the patterns observed, we computed the betweenness centrality index (BC) (Newman 2010) for each of the parasite species in the eight networks independently. Centrality measures have been widely used to search for species importance to the structure of host–parasite networks. Specifically, the BC index allows us to identify the species that are relevant for the cohesion of the network through its function as a connector or bridge to form links between host–individuals (Martín González et al. 2010; Poulin et al. 2013; Dallas et al. 2019). Parasite species which have a disproportionate number of host interactions or that connect otherwise unconnected groups of parasite species into the network will have a value $BC > 0$ and will be termed connector parasites (Martín González et al. 2010; Dallas et al. 2019). Otherwise, those parasite species with poor or none importance for the connectivity and cohesiveness of the network will have a value $BC = 0$, and will be termed peripheral parasites (Martín González et al. 2010). BC was estimated with function `specieslevel` in `bipartite`.

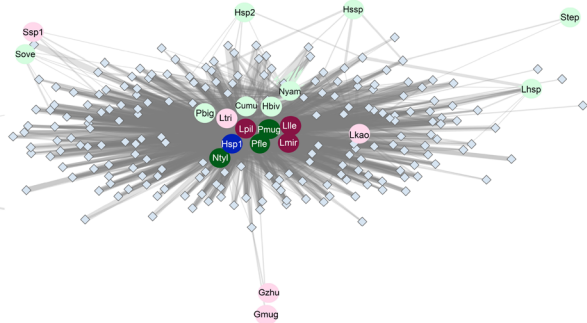
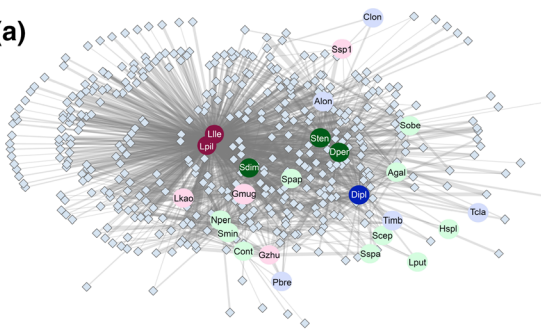
Results

The plots of bipartite networks (Fig. 1) indicate differences between the native and invaded areas and among the different subsets of parasites based on their transmission mode. Only in actively transmitted and ectoparasite networks of the invaded area, the trend was that of a nested structure. Furthermore, we can observe that connector species were lost in the introduction of *P. haemathocheilus*, while others were acquired in the invaded area, occupying peripheral positions. Co-introduced species maintained their role as connectors or peripheral species in the network, except in species of the genus *Gyrodactylus* (Gmug and Gzhu in Fig. 1b, d). Networks of trophically transmitted parasites were similar in both areas, since they tended to be modular. In the invaded area, several parasite species were connectors in the trophically transmitted network and shared host individuals, while others had few connections and remained more

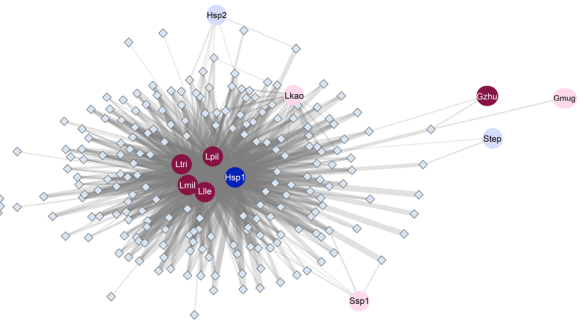
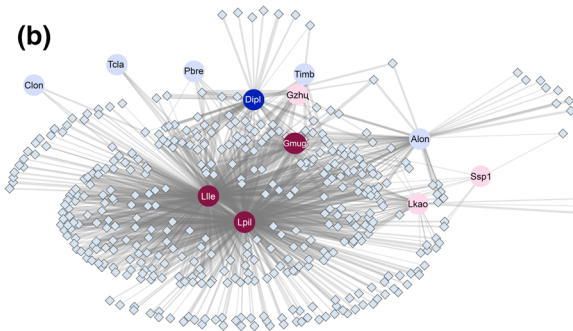
SEA OF AZOV

SEA OF JAPAN

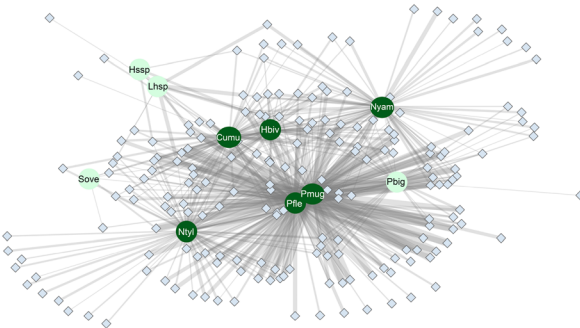
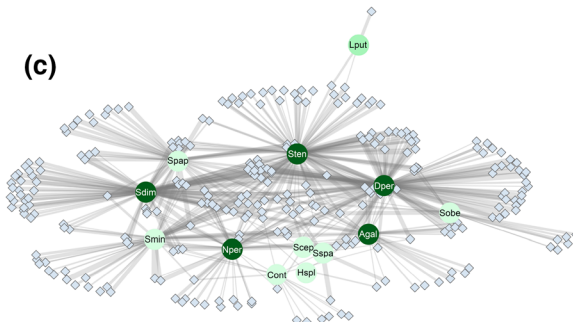
(a)



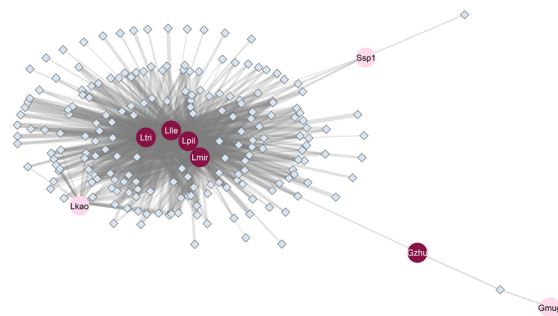
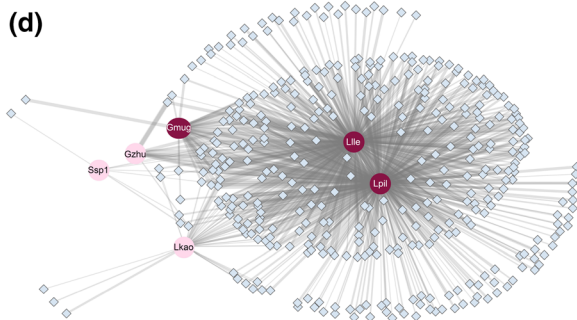
(b)



(c)



(d)



- a) Whole helminth community
- b) Actively transmitted parasites
- c) Trophically transmitted parasites
- d) Ectoparasites

Ectoparasites
 $BC = 0$
 $BC > 0$

Active (not ectoparasites)
 $BC = 0$
 $BC > 0$

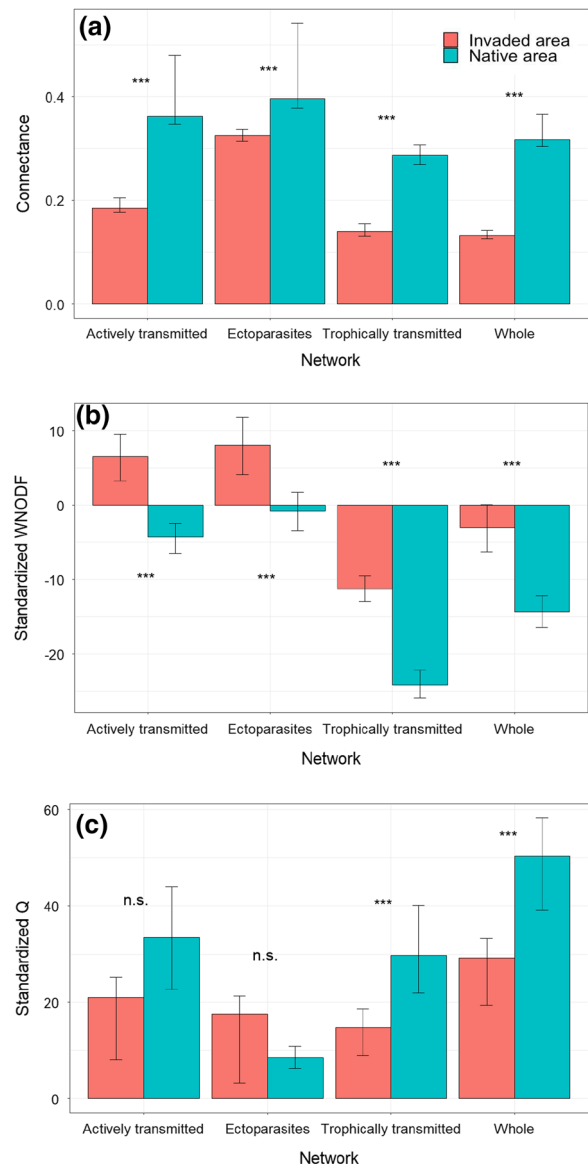
Trophic
 $BC = 0$
 $BC > 0$

◀ **Fig. 1** Diagrams of the bipartite networks of *Planiliza haematocheilus* individuals (diamonds) and their helminth parasite species (circles) in the Black Sea and Sea of Azov (invaded area) and the Sea of Japan (native area). The diagrams were constructed using the force-directed graph drawing algorithm (Jordano 2021), which distributes the nodes to minimize both differences in length and the number of intersections of the links. Parasite species present their corresponding abbreviation in the center of the circle (available in Supplementary Information, Table S1), their transmission strategy determined by color, and their role as peripheral species ($BC = 0$) or connector species ($BC > 0$) in the network. The width of the links represents the number of observations of the parasite species in the host individual. BC betweenness centrality

Fig. 2 Network indices of *Planiliza haematocheilus* individuals and their helminth parasite species in the Black Sea and Sea of Azov (invaded area, in red) and the Sea of Japan (native area, in blue): **a** connectance index, **b** weighted nestedness index and **c** modularity index. In **a** the values obtained with the replacement bootstrap were used, creating the 95% confidence intervals. In **b**, **c** the standardized values were used, and the 95% confidence intervals were built by bootstrap. Statistical differences were established by Mann–Whitney tests. Significance levels: *** $p < 0.001$; n.s., $p > 0.05$. Q : modularity. $WNODF$ weighted nestedness based on overlap and decreasing fill

peripheral (Fig. 1c) (Supplementary Information, Table S11). Networks encompassing the whole parasite community were quite different between sampling areas. In the native area, most interactions concerned a group of connector-parasite species, whereas other peripheral species interacted with fewer hosts, leading to a higher cohesion of the network. In contrast, the interactions in the invaded area did not appear to follow a clear pattern (Fig. 1a).

Although the number of connector species barely differed between all the transmission networks, the species positions in the network did. Such variations



led to a change in the role of the parasite species as a connector and in the structure of the native and invaded networks. In the native area, species of all transmission categories were connectors in the network, whereas only two co-introduced ectoparasites, *Ligophorus pilengas* and *L. lewellyni* (Lpil and Llle in Fig. 1), were connectors in the native and invaded areas. In addition, actively transmitted metacercariae displayed more peripheral positions in the invaded area (Fig. 1).

Figure 2 displays the values of C , and standardized $WNODF$ and Q , and their respective 95% confidence intervals of the simulated networks. C was significantly higher in all native host–parasite networks than in the invaded ones (Fig. 2a). Although nestedness estimates were significantly higher in all invaded networks, nestedness (as evidenced by positive standardized $WNODF$ values) only occurred in the ectoparasite and actively transmitted networks (Fig. 2b). Standardized Q values did not show a congruent pattern across networks. In networks of the whole, actively-transmitted and trophically-transmitted communities, Q was higher in the native than in the invaded area, whereas the opposite trend was observed in the ectoparasite network (Fig. 2c) (Supplementary Information, Tables S5, 8, 10). All differences between areas were significant ($p < 0.001$), except those concerning Q in networks formed by ectoparasites and by actively-transmitted parasites (Supplementary Information, Table S12).

Discussion

We have compared host–parasite networks in native and invaded areas based on parasite abundance in individuals of *P. haematocheilus*. Our results indicate substantial differences in the network structure obtained by resampling methods between both areas. It cannot completely be ruled out that dissimilarities in environmental and physical conditions can account to a greater or lesser extent for the differences observed (Bellay et al. 2018). In fact, the Sea of Japan and the Sea of Azov greatly differ in water conditions, such as temperature or salinity (Sarabeev et al. 2017a). In addition, a real effect of the invasion would only be possible to establish with data from before and after the invasion in the invaded area. Unfortunately, information on local grey mullet parasites in the

invaded area was very scarce before the invasion. So, this approach could not be followed. Therefore, we recognize that we must be careful in the interpretation of our data since we cannot establish a cause-effect relationship, and acknowledge that much remains to be done to reach more precise conclusions. However, it seems likely that the time elapsed since the introduction of *P. haematocheilus* area has been too short for the interactions in the community to be defined and stabilized since most of our results aligned with the initial hypothesis about how host–parasite networks might respond to an invasion.

First, connectance was higher in the native area for all networks than in the invaded counterparts. Through evolutionary time, it is expected that parasites evolve a certain degree of host specialization (Feis et al. 2016). In a native area, all possible interactions between the species that make up the community are much more likely to occur, since parasites and hosts have been interacting longer than in the invaded area (Poulin et al. 2013; Feis et al. 2016). In our case, as the native and invasive hosts belong to the same species, it is possible that some native parasites from the host's native area, especially those transmitted trophically, have more difficulties to establish themselves in the introduced area. This could account for the reduced number of the realized links observed in the invaded zone (Blüthgen et al. 2008; Poulin et al. 2016) (Fig. 1). The highest connectance in the invaded area corresponded to the ectoparasite network. This group includes co-introduced helminths, which share an evolutionary history with their host. However, connectance still was significantly lower than that of the native area. This may reflect that the invasive ectoparasite community is only a fraction of the native one (Sarabeev et al. 2018), because some connector species were lost (Fig. 1b, d). Native networks show higher cohesion with more connector species and, therefore, with more links to host individuals. In contrast, connector species in invaded networks have been lost or become peripheral, leading to a hierarchical structure with fewer links with host individuals.

Second, all networks showed significantly higher nestedness values in the invaded area. Only the ectoparasite and the actively-transmitted networks of the invaded area were nested (positive $WNODF$), which may result from the loss of connector parasite species (Fig. 1b, d). In these networks, only two species, *L. lewellyni* and *L. pilengas* (Llle and Lpil in

Fig. 1b, d) are connectors in both areas, which facilitates that communities with only one of these connector species represent a subset of communities with both species. Both are highly specific to *P. haematocheilus* (Sarabeev et al. 2013). Several studies of the helminth fauna of *P. haematocheilus* in the studied regions indicate that the parasite community in the invaded area is both smaller in terms of abundance and prevalence, and more aggregated in the host population than in the native area (Sarabeev et al. 2018, 2019). The abundance of these co-introduced monogeneans was much lower in the invaded area (Sarabeev et al. 2018). When a host reaches a new environment, it is expected to lose its native trophic parasites (enemy release) and to acquire new ones via consumption of infested prey. In fact, this pattern has been observed in *P. haematocheilus* (Sarabeev et al. 2017a). It can explain the lack of nestedness in trophically transmitted networks, since other native parasite species can exploit the host as a resource. In contrast, some actively transmitted parasites from the invaded area, notably monogeneans tend to be highly host specific which hampers colonization of the introduced *P. haematocheilus*. For instance, at least eight *Ligophorus* spp. occur in native grey mullets in the invaded area (Sarabeev et al. 2013) but so far they have been unable to colonize *P. haematocheilus*. This evidence points to a relatively recent parasite community in which interaction patterns have not emerged, yet.

Third, the whole network and that formed by trophically transmitted parasites showed a significantly higher modularity in the native area (Fig. 1a, c). In other words, these networks are composed of subgroups of host individuals that tend to interact more frequently with certain parasite species, probably connector species, than with others in the community, probably peripheral species. High modularity in host–parasite networks have been interpreted as an indication of the associations between hosts and parasites being well established (Llopis-Belenguer et al. 2020) and suggests a convergence between their adaptations (Traveset et al. 2013; Tur et al. 2015). The modules of a network could be determined by phylogenetic proximity and similarity in characteristics between individuals, such as differences in behavioral or diet preferences (Pilosof et al. 2015). Hosts may vary in traits that determine their encounter with parasites or their susceptibility to

infection that could either favour or constrain the interaction, thereby driving the formation of modules in the native area (Pilosof et al. 2015; Llopis-Belenguer et al. 2020). In the invaded area, the results indicate a network with blurred modules, suggesting many connections between different modules, decreasing the original modularity of the network (Traveset et al. 2013). This may be due to the fact that all trophically transmitted parasites were acquired in the invaded zone. Most acquisitions correspond to species that are able to parasitize several local grey mullets (Sarabeev 2015; Sarabeev et al. 2017a). So, these infections are more generalized (Fortuna et al. 2010; Dupont et al. 2011), with several species attaining the role of network connectors, even without having well-defined interaction patterns.

By contrast, no significant differences between areas in modularity were observed in the ectoparasite networks (Fig. 2c). In the invaded area, the ectoparasite community is mostly a subset of the native community. As a result, fewer and less abundant ectoparasite species are expected at host individual level (Sarabeev et al. 2018) (Fig. 1d). Consequently, the ectoparasite network from the invaded area has fewer connections and greater modularity (albeit not statistically significantly different) than in the native area. This can also explain to some extent the lack of significant differences between areas of the actively transmitted parasite networks. In addition, these networks include metacercariae, which are in general fairly unspecific (Sarabeev 2015). Then, metacercariae could act as network connectors and blur the modules in both areas.

In our study, host individuals were collected following a standardized protocol (Kostadinova et al. 2004), which attempted to minimize biological differences among samples. However, some hosts are infected by one or two species of parasites, while other hosts are highly infected (Fig. 1), perhaps due to the heterogeneity of the population and its individual variables (Sarabeev 2015). In fact, differences in such variables (e.g., age, weight, sex) can account for the structure of host individual-parasite networks (Bellay et al. 2020). Thus, incorporating host variables into future studies, as well as data on host intraspecific trait variation, could improve our understanding of how interactions are distributed among individuals (Tur et al. 2015; Pilosof et al. 2015; Bellay et al. 2018).

In conclusion, the comparison of the interaction patterns of *P. haematocheilus*, native in the Sea of Japan and invasive in the Sea of Azov, has allowed us to identify changes in patterns between two different geographic areas that may be attributed to the invasion. The structure of communities may not change when the functional roles of the species are maintained (Dallas and Poisot 2018). In fact, the roles of co-invasive parasites were maintained in both areas. Hence, the differences in the structure of the networks reflect a profound alteration in other interactions (those that do not involve co-invaders) in the invaded area.

Thus, we believe that our approach is valuable to illuminate the changes that occur in host–parasite interactions when an invasive species and its parasite community are introduced into a new area. Further studies could also consider host traits and phylogenetic determinants of parasites, which are known to dictate the success of host–parasite associations (Wells and Clark 2019; Campião and Dáttilo 2020). Furthermore, the analysis of host individual-parasite species interactions as bipartite networks can be useful in other study contexts, such as in prediction and mitigation of impacts of climate change, human activities, and/or habitat loss or fragmentation. So, there are a number of possible applications of bipartite host–parasite networks that could prove valuable to study ecological communities and host–parasite interactions in depth and provide insight into the dynamics and processes that influence them.

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Authors' contribution MLR, JAB and CLB conceived the ideas and designed methodology. VS collected the data. MLR arranged the databases and analyzed the data. MLR led the writing and JAB, CLB and VS contributed to the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Code availability Code availability from Zenodo (<http://doi.org/10.5281/zenodo.5137368>).

Declarations

Ethics approval Not applicable.

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Conflict of interest All authors declare no competing interests.

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