

## Research

### Native and invasive hosts play different roles in host–parasite networks

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Parasites are often key players in biological invasions since they can mediate the impact of host invasions or can themselves become invasive species. However, the nature and extent of parasite-mediated invasions are often difficult to delineate. Here, we used individual-based, weighted bipartite networks to study the roles (degrees of interactions of individuals in a modular network according to their within- and among-module connections) played by native and invasive host individuals to their parasite communities. We studied two phylogenetically and ecologically close fish species, *Mugil cephalus* s.l. and *Planiliza haematocheilus* (Teleostei: Mugilidae). *Planiliza haematocheilus* is native to the Sea of Japan and invasive in the Sea of Azov whereas, *M. cephalus* s.l. is native to both seas. Based on the common evolutionary history that drives native host–parasite networks, we hypothesised that 1) native networks have higher modularity than invaded ones; and 2) invasive hosts in the invaded area play a peripheral role to structure parasite communities. We analysed the whole parasite community and subsets based on transmission strategy and host specificity of the parasite species to establish whether modularity and host roles are related to these features in the native and invaded areas. All networks were found to be modular. However, modularity tended to be higher in networks of the native area rather than those of the invaded area. Host individuals of both fish species played similar roles in the native area, whereas invasive hosts played a peripheral role in the networks of the invaded area. We propose that long-term monitoring of the roles of invasive hosts in parasite communities can be a useful proxy for estimating the maturity of the establishment of the invasive hosts in an ecosystem.

Keywords: biological roles, host individual–parasite species networks, invasion, modularity, transmission strategy

## Introduction

Biological invasions are human-mediated introductions of species outside their original distribution, which manage to establish viable populations throughout space and time (Richardson et al. 2000). Invasive species represent a major threat to ecosystems, as they do not allow enough time to elapse for gradual evolutionary adjustments of the native species to their presence (Poulin 2017). When species are introduced into a new range, different scenarios can alter ecosystem functioning (Lymbery et al. 2014). Among those, biological invasions are of concern because of their potential to disrupt host–parasite dynamics (Chalkowski et al. 2018). As a result of the invasion (Fig. 1): invasive hosts can lose their parasites (enemy release); parasites can be introduced as free-living stages; invasive hosts can introduce parasites from their native range (or from an intermediate location, Fig. 1b) and transmit parasites to native species (spillover,

co-invasion) or, contrary, do not transmit these parasites (co-introduction). Likewise, the invasive hosts can acquire parasites from native species (acquisition), favouring an increase in the abundance of the native parasite species and, thus, increasing the likelihood of native hosts becoming infected (spillover); or can act as an ecological sink, because they are not fully competent hosts when they become infected with a parasite from the native species (dilution effect) (Kelly et al. 2009, Lymbery et al. 2014, Goedknecht et al. 2016, Chalkowski et al. 2018) (Fig. 1).

Under these scenarios, native and invasive hosts and their native and/or acquired parasite communities can interact in different ways with subsequent outcomes for the ecosystem (Chalkowski et al. 2018). The study of host–parasite associations in an invaded community has been addressed by different types of analyses. For example, Sarabeev et al. (2017a) found support for the enemy release hypothesis, i.e. the invasive species would be benefited by a reduction of natural

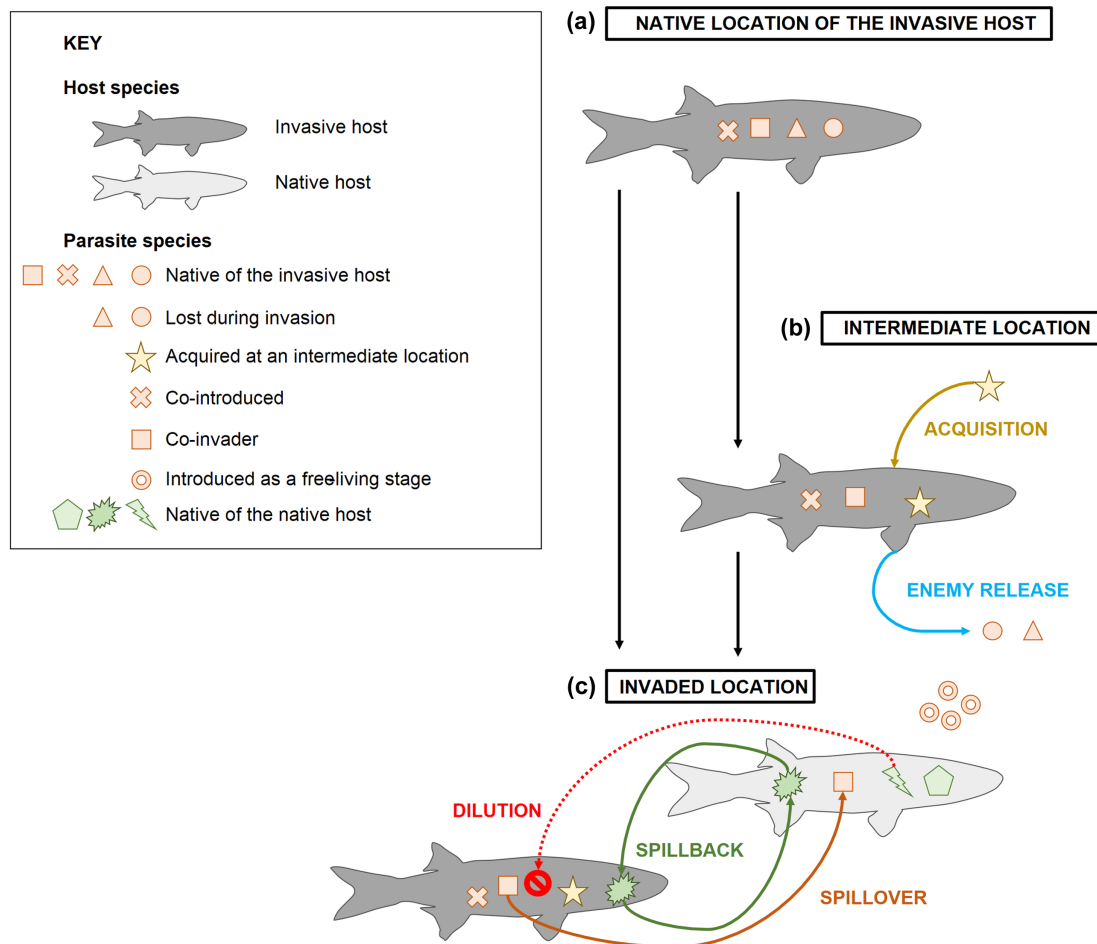


Figure 1. Processes and key concepts to define parasite species during invasions. (a) Invasive host at its native area with its own parasite community. (b) Potentially, the invasive host can lose part of its parasite community (enemy release) and be colonised by new species (acquisition) during the invasion. (c) The invasive host arrives at the invaded location with some of its parasite species (co-introduced). It can acquire new parasite species from the native host. As a result, the probability of the native host to be infected increases (spillover). If the native host acquires co-introduced parasite species these become co-invaders (spillover). The invasive host can be a sink for native parasites if it gets infected, but it is not a competent host (dilution effect). Invasive parasites can arrive at the invaded location as free-living stages.

enemies, such as parasites, in the invaded area (Torchin and Lafferty 2009), by comparing the abundance and aggregation of the parasite community in native and invasive areas. Likewise, Poulin and Mouillot (2003) found that the amount of parasite species in invasive hosts over a short (ecological) period of time results in parasite assemblages becoming more taxonomically diverse than those developed over much longer (evolutionary) time periods in the native range of the host species. This fact highlights that ecological drivers are at least as important as evolutionary processes to model parasite communities (Poulin and Mouillot 2003).

Interactions between hosts and parasites, or any other sets of individuals from two different taxa or ecological guilds (e.g. plant–pollinator), can be explored with bipartite network analysis. The analysis of such biological networks is particularly relevant for parasite ecology since it can illuminate the way in which host individuals and parasites are associated in a community (Poulin 2010). Bipartite networks are usually characterised by non-random associations between individuals or taxa (Fortuna et al. 2010), and one of the patterns that describes this non-randomness is modularity (Newman and Girvan 2004). In modular networks, subsets (i.e. modules) of individuals are expected to interact more frequently among themselves than with individuals from other modules. Thus, modularity measures how well interacting pairs can be separated into different modules, and higher values of modularity indicate better segregation of modules (Newman and Girvan 2004). This may imply barriers to parasite dispersion between hosts from different modules that, for example, differ in behavioural or diet preferences (Pilosof et al. 2015) or are phylogenetically distant (Bellay et al. 2011, Krasnov et al. 2012, Poulin et al. 2013). In consequence, module drivers, such as taxonomic affiliation, have been proposed as predictors of the performance of invasive species in a network through its position in a module (Poulin et al. 2013). Beyond the ubiquity of modularity as a network pattern (Fortuna et al. 2010), we can classify the functional role of each individual in a modular network according to its number of both within-module (z-score) and among-module (c-score) links into four role categories (Guimerà and Amaral 2005, Olesen et al. 2007):

1. Module hubs, or individuals linked to many individuals within their own module (high z, low c).
2. Connectors, or individuals linking several modules (low z, high c).
3. Network hubs, or individuals acting as both connectors and module hubs (high z, high c).
4. Peripherals, or individuals that have a few links within its own module and to other modules (low z, low c).

Thus, we can graphically represent the position of each host individual by values of its cz-scores (see Fig. 1D in Olesen et al. 2007) and expect that individuals with the same role play similar functions in determining the structure of the parasite community. In fact, these cz-scores have been used to explain the specificity of host–endoparasite networks

(Bellay et al. 2011, 2013) or to determine the role of native and invasive plants and pollinators (Traveset et al. 2013).

Network analysis has arguably been under-exploited in studies of host–parasite invasions (Médoc et al. 2017), although it has been more widely applied to understand invasions of other biological systems. For example, Traveset et al. (2013) found that invaders made the Galápagos Archipelago resistant to species loss but vulnerable to disease spread. Regarding host–parasite invasions, one of the few examples is the study of Amundsen et al. (2013) in which the authors evaluated how the introduction of two fish species, followed by the co-introduction of five parasite species and four predatory bird species, altered the topology of a native food web.

To fill the gap in the understanding of host–parasite interactions during invasions, we study the host individual–parasite species associations of two, native and invasive, host species by bipartite network analysis. Furthermore, we characterise networks of the native and the invaded distributions of both host species. To our knowledge, this represents the first study that evaluates and compares real (i.e. not simulated computationally) native and invaded networks. Furthermore, the position of an individual in a host–parasite network gives us an idea of its relative importance in the flow of parasites (Godfrey 2013). So, we downscale analyses to host-individual level to control for intraspecific host variation. This is necessary because parasite distribution across hosts can be patchy depending on the individual capacity of each host to prevent infection, and it will ultimately determine the ability of a parasite species to invade a new area (Morand and Deter 2009). Furthermore, the success of an invasive parasite can be greatly facilitated by the high abundance of the suitable host or even the number of invasive hosts can also determine amplification or dilution effects of native parasites (Telfer and Bown 2012). Finally, we will also implement role analysis (modularity and cz analyses) to understand the structure of native and invaded host–parasite communities, and the ecological impact of invasive hosts and parasites in an existing (native) community.

The grey mullets (Teleostei: Mugilidae) and their helminth parasites represent excellent systems to study and compare the role variation of individuals of a host species depending on its distribution (native or invasive) (Sarabeev et al. 2017a). They also provide a benchmark to control for such variation. Here we study the roles played by individuals of *Planiliza haematocheilus* and *Mugil cephalus* s.l. for their parasite communities in the native area for both hosts (Sea of Japan) and in an area where *P. haematocheilus* is invasive and *M. cephalus* is native (Sea of Azov). Since 1972, *P. haematocheilus* was repeatedly introduced into the Black Sea and the Sea of Azov for commercial purposes. In the early 80s, it established a reproductive population in these seas (Sabodash and Semenenko 1998, Occhipinti-Ambrogi and Savini 2003). The arrival of *P. haematocheilus* at its new habitat entailed a deep structural change in its parasite community: it lost native parasite species with complex life cycles, acquired new ones from the invaded area, and co-introduced some of its

ectoparasite species (simple life cycle) to the invaded area (Sarabeev et al. 2017a). Although *M. cephalus* is now considered to represent a complex of sibling species (Whitfield et al. 2012), we assume that *M. cephalus* entities from the Sea of Azov and Japan are phylogenetically and ecologically equivalent (i.e. equally close) in their relationships with *P. haematocheilus* (Sarabeev et al. 2017a) and in the function performed in ecosystems.

We specifically employ modularity and *cz* analyses to determine the role of individuals of both host species for the whole parasite community and for parasites exploiting certain transmission strategies. Based on previous studies (Sarabeev 2015, Sarabeev et al. 2017a, b, 2018, 2019), we first expect that modularity will be higher in native than in invaded networks. Native generalist parasites will parasitise the invasive host, and the acquisition of such parasites should connect existing distinct modules and decrease modularity. In contrast, the modularity signal should be similar in native and invaded communities when the analysis of networks includes highly host-specific parasites (i.e. carried species, Fig. 1) as these species should enhance modular structure. Second, we hypothesise that hosts of both species will play similar roles in the native area whereas they will display different roles in the invaded area. In the latter, the invasive hosts will mainly perform a peripheral role (low *c* and *z* scores) since they do not share a common evolutionary history with local parasite species (most of them are acquired, Sarabeev 2015) and will have low relevance in maintaining within- and among-module cohesion. Third, the role of both host species could also vary regarding the parasite transmission strategy considered. We expect that *P. haematocheilus* individuals will play a peripheral role for passively/trophically transmitted parasites in the invaded sea since all of them have been acquired from this sea. In contrast, we expect hosts of both species to play similar roles for their actively transmitted parasites in the Sea of Azov for two reasons. First, some of the actively transmitted parasites are host-generalists (able to infect a new host species by its own means). So, they will parasitise native and invasive host species equally. Second, the remainder of the actively transmitted parasites are highly host-specific and exclusively parasitise one host species (those of *P. haematocheilus* were carried from the Sea of Japan). So, they share a well-established evolutionary history with their hosts.

## Material and methods

### Data

Our study is based on a database of fish and helminth parasites previously collected and identified as described in Sarabeev (2015) by standardised sampling methods across sites, seasons and years. We considered 872 fish individuals from 11 localities in the Azov-Black (hereafter Azov) and Japan seas, during three seasons (spring, summer and autumn) and seven years (1998, 1999, 2004, 2005, 2009, 2011, 2013) (Supplementary material Appendix 1 Table

A1). We aggregated data from different samplings because the analyses of short periods of time possibly misrepresent the real dynamic of the network structure at a macroecological scale (Poulin 2010). These two fish species differ in their migration periods and paths for wintering and spawning (Sarabeev et al. 2017b). As a consequence, fish from both seas were not always collected at the same localities (Sarabeev 2015) (Supplementary material Appendix 1 Table A1). In total, our database includes 52 helminth parasite species of Acanthocephala, Nematoda and Platyhelminthes in adult and larval stages. Six of these species were co-introduced monogeneans (Fig. 1) from the Sea of Japan into the Sea of Azov, so they occur in both seas.

By means of bipartite network analyses, we asked about the roles of the host individuals for (see summary of databases in Table 1):

- 1) The whole helminth parasite community.
- 2) Actively transmitted parasites (Monogenea and metacercaria of Trematoda), i.e. species with larval stages that actively swim to reach the fish.
- 3) Passively/trophically transmitted parasites (adults of Trematoda and Acanthocephala and larva and adults of Nematoda), i.e. transmitted via the food web.
- 4) Ectoparasites (Monogenea). This is the only group with species introduced into the Sea of Azov. With the exception of one species, these monogeneans are highly host-specific and are not able to infect both host species (Sarabeev 2015). Note also that this group is a subset of 2) above.

Besides, different life stages of parasites were analysed as different nodes because they belonged to different species. In other words, parasite individuals of a species were always found in the same developmental stage in the analysed hosts.

For each of these four subsets, we constructed infection networks as incidence matrices where rows represented fish individual hosts and columns represented parasite species for each location (native versus invasive). Each cell contained the abundance of a parasite species in each host individual (i.e. edges values were the number of individuals of a particular parasite species in a single infected host). Across our two locations, we therefore ended up with eight different networks to analyse.

## Network analyses

### Modularity

Modularity analyses were run for each of the eight networks under study (Table 1). We used the Beckett (2016) algorithm because it considers quantitative information (i.e. weighted networks). This algorithm was implemented with function `computeModules` from package `bipartite` (Dormann 2011) in R (<[www.r-project.org](http://www.r-project.org)>). The algorithm assigns fish individuals and parasite species to modules to compute a modularity value (*Q*) that is higher when links (i.e. interactions) within modules are more prevalent and/or stronger than links between modules. To account for modularity dependence on



Table 1. Sample sizes of the eight studied networks. The number of modules found in each modularity analysis is given in parentheses.

|                                             | Sea of Azov                                           | Sea of Japan |
|---------------------------------------------|-------------------------------------------------------|--------------|
| Whole parasite community                    | 612 (fish hosts) × 31 (parasite species) (modules: 9) | 260 × 27 (5) |
| Actively transmitted community              | 565 × 16 (8)                                          | 251 × 16 (4) |
| Passively/Trophically transmitted community | 462 × 15 (8)                                          | 240 × 11 (5) |
| Ectoparasites (Monogenea)                   | 525 × 10 (5)                                          | 241 × 13 (5) |

network size, we transformed the observed  $Q$  value into a standardised score (z-score sensu Dorman and Strauss 2014; we did not call it z-score to avoid possible confusion between z-scores in Olesen et al. 2007 and Dormann and Strauss 2014) by using the mean ( $\bar{Q}_{\text{null}}$ ) and standard deviation ( $\sigma Q_{\text{null}}$ ) of  $Q$ s from 1000 randomly generated bipartite networks (see description below):

$$\text{standardised } Q = \frac{Q_{\text{observed}} - \bar{Q}_{\text{null}}}{\sigma Q_{\text{null}}}$$

To test the significance of our  $Q$  values, we compared them with those of 1000 bipartite networks generated randomly with the function nullmodel from bipartite. We assumed the null hypothesis that the eventual organisation of host–parasite interactions into modules, or symmetry of the strength of the interactions, is driven by relative abundance of species in a sample, thus interactions are random between individuals. To validate our results of modularity, we carried out analyses in two different ways that work with quantitative link information. First, we tested modularity with the less constrained method described by Vázquez et al. (2007), that randomises the total number of host–parasite interactions observed in the original interaction matrix, constrains the connectance, but not the marginal totals. So, the number of observed infections is the same as in the original interaction matrix. The method relies on the reciprocal relative frequency of interactions ( $s$ ) of one actor (e.g. a parasite species,  $i$ ) over the another (e.g. host individual,  $j$ ). The difference between the two reciprocal coefficients of  $s$ ,  $d_{ij} = s_{ij} - s_{ji}$ , measures the symmetry of the strength of an interaction. Then, if we focus on a parasite species,  $i$ ,  $A_i$  is the sum of all  $d_{ij}$  divided by its number of links ( $k_i$ ). Under the abundance–symmetry null hypothesis, we expect a positive correlation between species abundance ( $N_i$ ) and  $A_i$ . Second, we tested modularity with the swap.web algorithm (Dormann et al. 2009). In addition to connectance as in the previous method, it also constrains marginal totals that are taken from the original interaction matrix. The procedure starts with a Patefield-generated matrix. Then, it randomly selects  $2 \times 2$  submatrices without zeros and subtracts the minimum values of the diagonal from the diagonal (thereby it generates an empty cell) and adds this value to the values on the minor diagonal. The marginal totals are maintained while the number of links is reduced. This procedure is repeated until the number of links (i.e. connectance) is equal to that of the real network (Dormann et al. 2009).

Finally, we bootstrapped with replacement the eight networks to compare and assess the overlap of the standardised

$Q$ s across networks. The number of individuals of each species in the bootstrapped matrices was fixed.

## c and z scores

The  $c$  and  $z$  scores were calculated with function cz values from bipartite. We performed these analyses for each of the eight networks (Table 1). Following Guimerà and Amaral (2005) and Olesen et al. (2007), we classified host fish as ‘peripherals’ ( $z \leq 2.5$  and  $c \leq 0.62$ ), ‘connectors’ ( $z \leq 2.5$  and  $c > 0.62$ ), ‘module hubs’ ( $z > 2.5$  and  $c \leq 0.62$ ) and ‘network hubs’ ( $z > 2.5$  and  $c > 0.62$ ).

## Statistical analyses

We used Fisher’s exact tests (fisher.test function from R package stats) to assess whether individuals of both fish species were similarly distributed among the four role categories in each of the eight networks (Supplementary material Appendix 2 Tables A1–A8). When significant differences were revealed, we tested whether the number of peripherals of *P. haematocheilus* or *M. cephalus* was higher or lower than expected by chance in comparison to the observed proportion of peripherals of *M. cephalus* and *P. haematocheilus*, respectively. Since we hypothesised that individuals of *P. haematocheilus* would tend to play peripheral roles (i.e. less connected with other members of the network) in the parasite community, we predict more peripheral individuals of *P. haematocheilus* in the invaded area than expected by chance. Similarly, since we expected that both species would play similar roles in the parasite community in the native area, the observed proportion of peripheral individuals of both species should be similar to the proportion expected by chance. To test this, we simulated 10 000 replicates of the number of peripheral individuals observed in each of the original cz analyses, independently. The number of individuals of each species in each replicate was set as the number of peripherals of each species observed in the cz analysis and the proportion of peripheral individuals of each species in each replicate were calculated. Finally, we established whether the observed proportion of peripherals of each species in our sample fell within the 95% confidence interval of the simulated proportions.

Furthermore, we performed season-specific modularity and cz analyses to evaluate the existence of a seasonal effect on species roles. Due to the reduced size of season-specific networks, we only tested the seasonal effect on the whole network (database (1)). As for the global analyses, we tested whether individuals of both fish species were similarly distributed within each of the four role categories for each

season and sea, independently, by means of Fisher's exact test. Then, we assessed if the number of peripherals of *P. haematocheilus* and *M. cephalus* differed from the expected numbers, independently, by simulating 10 000 replicates as described above.

## Results

The eight networks (Table 1) were all significantly modular ( $p < 0.05$ ), regardless of the null model used. The number of modules found for each network is reported in Table 1 and ranged from 9 to 4. The standardised  $Q$  scores of the whole and the trophically transmitted parasite communities were higher in networks from the Sea of Japan than those from the Sea of Azov (first hypothesis in the Introduction). Although, the networks that involved monogeneans (actively transmitted and ectoparasite community) had higher standardised  $Q$  scores (i.e. higher modularity) in the invaded area than in the native one, their confidence intervals (bootstrap results) overlapped. This suggests that their modularities are not significantly different between the two areas at least for these two networks (Fig. 2: actively transmitted and ectoparasites).

The cz analyses revealed that individuals of both fish species were distributed among the four role categories. However, individuals most frequently played peripheral roles, and only a few individuals were hubs (Fig. 3). Regarding our second and third hypotheses, we did not find significant differences between the role played by individuals of the two fish species in the Sea of Japan (native area). We also found no significant differences in analyses involving ectoparasites (actively transmitted and ectoparasite communities), regardless of the area studied (Fig. 4, Table 2). When significant differences were found, the proportion of peripherals of *Planiliza haematocheilus* in the Sea of

Azov (invaded area) was always larger than the proportion of peripherals of *Mugil cephalus* (Fig. 4).

The seasonal distribution of both fish species among the four roles was consistent with the results obtained from the combined analyses, except for spring at the Sea of Azov. In this case, we found no significant differences in the roles played by both host species (Supplementary material Appendix 3 Table A1–A5, Fig. A1–A5).

## Discussion

We have compared field data (i.e. not simulated computationally) on invaded and native networks, which provides direct insight into the post-invasion changes in host–parasite associations. A study of this nature has been repeatedly called for to unveil well-grounded macroecological patterns and to avoid biases in the conclusions, such as overestimates of enemy release (Roy and Lawson Handley 2012). To our knowledge, such a study had not been implemented yet to date. Our case study provides a clear example on how to compare quantitatively the roles of invaders in the native and invaded areas.

A second innovative aspect of the present study is that we have downscaled network analyses from species to individuals. Most previous studies that work with bipartite networks have been carried out at species level. However, different authors have recently proposed to implement network analyses at the individual level in order to determine the properties that emerge at this scale (Dupont et al. 2014, Tur et al. 2015). This is important because, in a species-based network analysis, individuals of *Mugil cephalus* and *Planiliza haematocheilus* would strictly belong to one module or another (Tur et al. 2015). However, in a study like the one presented here, modules of the whole parasite communities included individuals of both host species, regardless of the area considered. This allows the identification of individuals of different species that overlap in some traits or niche preferences that make them to hold a similar role in the parasite community (Dallas et al. 2019). In other words, the heterogeneous partitioning in the use of resources by a single population would be missed at a higher-level analysis (Tur et al. 2015) and this distinction at individual level can be especially important during the host–parasite invasion process. For example, parasite distributions across hosts are usually highly aggregated, in which most host individuals harbour few or no parasites, whereas a few hosts harbour the majority of the parasite population (Poulin 2013). Even in an invasion, the individual ability of hosts to avoid parasites can be determined by such ability of the conspecifics that also arrived at the invaded locality (Ugelvig and Cremer 2012), which might lead to different roles played by host individuals in their parasite communities. Then, if we pool host individuals together, we focus on parasite mean abundance in a host species and will miss within-species variation which, eventually, carries information about host individual role in the transmission of the parasites (Telfer and

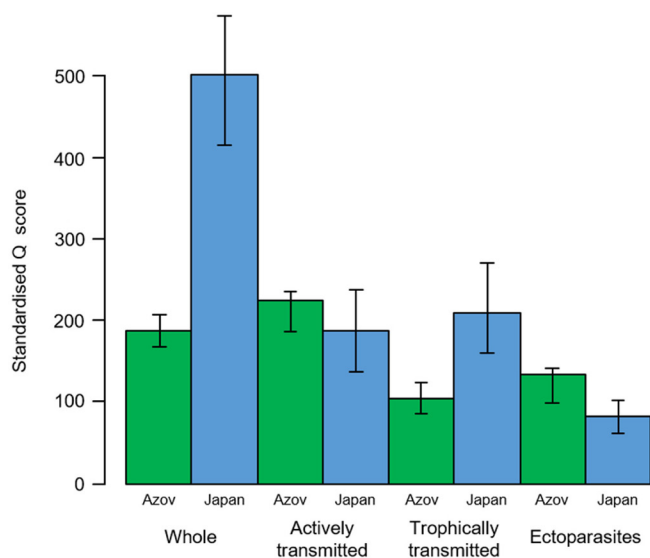


Figure 2. Standardised modularity ( $Q$ ) scores. The error bars indicate the standardised  $Q$  scores derived from 1000 bootstrapped matrices.

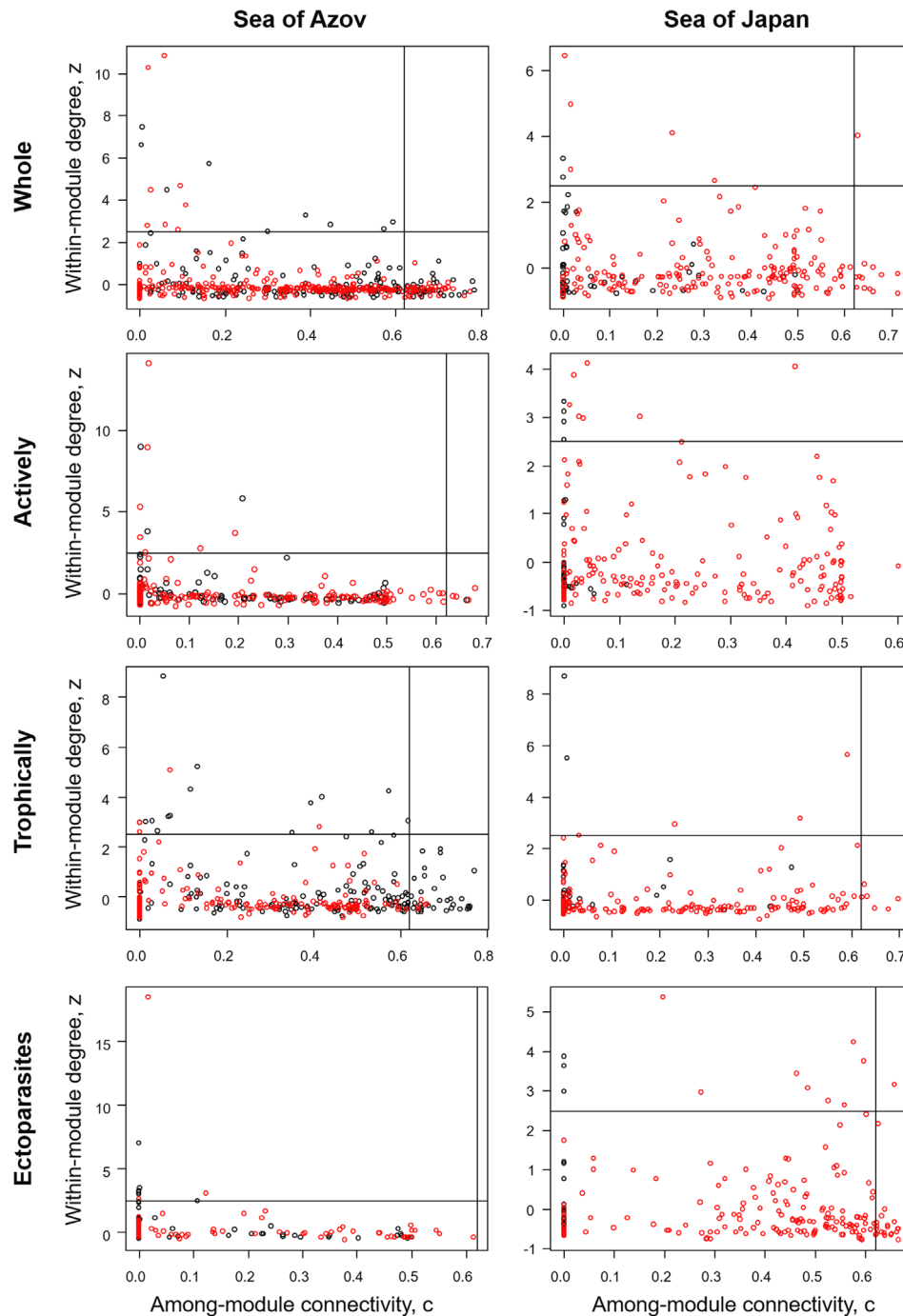


Figure 3. Distribution of *Mugil cephalus* (black) and *Planiliza haematocheilus* (red) individuals from the Sea of Azov and the Sea of Japan among the four network roles considered by Olesen et al. (2007): Peripherals:  $z \leq 2.5$  and  $c \leq 0.62$ ; connectors:  $z \leq 2.5$  and  $c > 0.62$ ; Module hub:  $z > 2.5$  and  $c \leq 0.62$ ; Network hubs:  $z > 2.5$  and  $c > 0.62$ .

Bown 2012). Furthermore, the subsetting of the networks into parasite infracommunities (i.e. at the host individual level) affords evaluating the change in the performance of the fish individual depending on the parasite characteristics. This can be especially relevant for researchers who want to predict changes in host–parasite dynamics.

Our modularity analyses revealed that networks are composed of subsets of host individuals that interact more frequently with certain parasite species than others. Our predictions of modularity (i.e. higher modular signal in native networks than in invaded networks) were partly supported by the results. As for the roles played by *M. cephalus* and

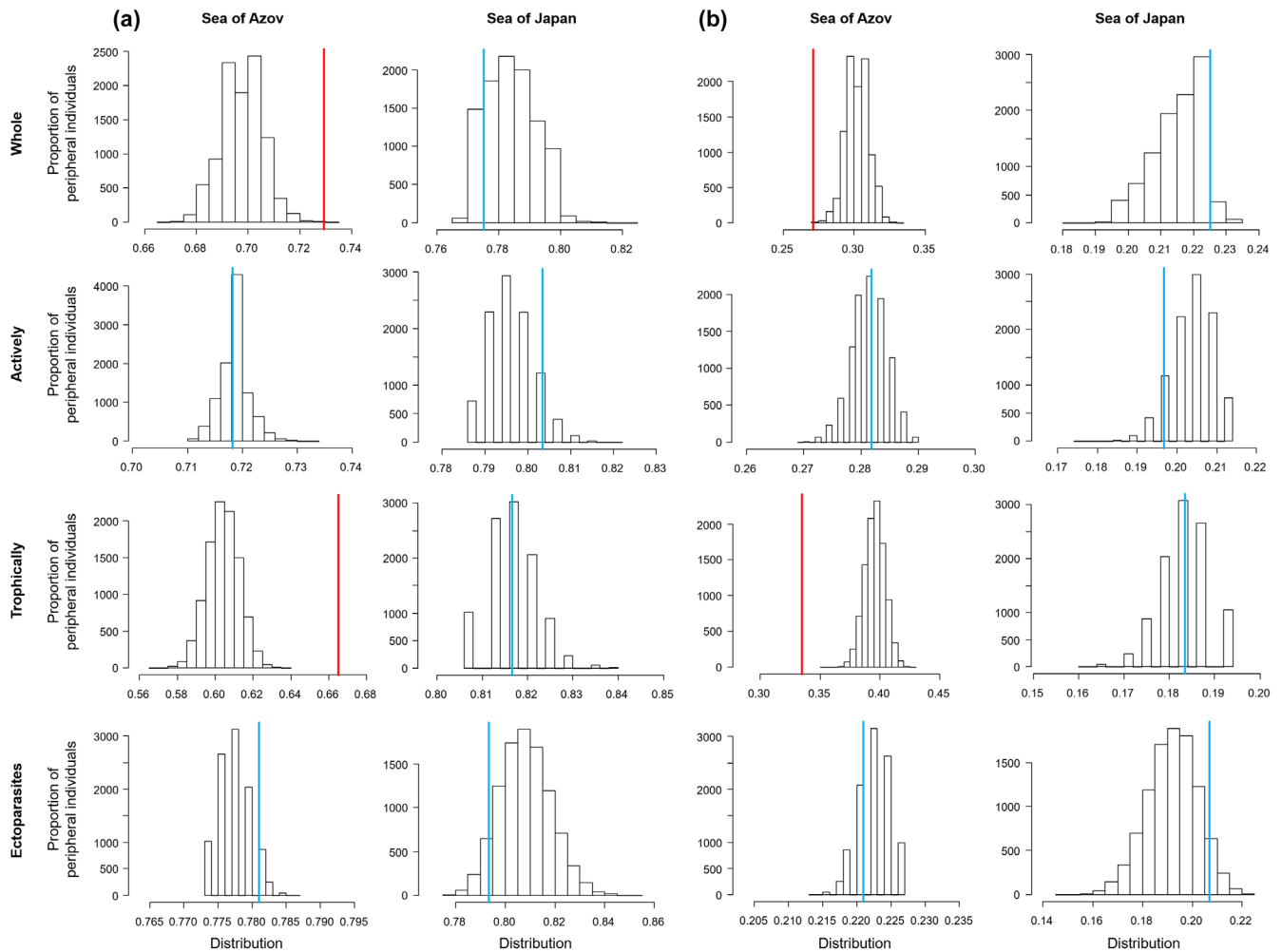


Figure 4. Observed proportion of host individuals as peripherals (red and blue lines) and expected proportions in an ensemble of 10 000 random replicates (white bars) from the Sea of Azov and the Sea of Japan. (a) *Planiliza haematocheilus*; and (b) *Mugil cephalus*. Red lines indicate that the observed proportions significantly differ from the expected by the null models, whereas blue lines indicate non-significant results.

*P. haematocheilus*, the results also supported our hypothesis. Individuals of both host species had similar roles in the native area (Table 2). Finally, the results did not provide evidence for a strong seasonal effect on the roles of both host species regardless of the native or invaded area condition. Although all eight networks were modular, modularity was higher in the native area than in the invaded area for the whole parasite

community and the passively/trophically transmitted parasites (Fig. 2). The higher modularity in these two native communities may indicate that associations between host and parasites are well grounded. In contrast, modularity may not be so well defined in the invaded area because accidental associations would blur the structure of the community. Furthermore, the network subsetting allowed us to unveil mechanisms that would be neglected otherwise. Analyses of the actively transmitted parasites and ectoparasites (both of them involved ectoparasites carried from the native area to the invaded one), displayed similar results in native and invaded communities. In addition to a previous study showing that *P. haematocheilus* co-introduced part of its ectoparasite fauna (Sarabeev 2015), our results suggest that it also maintained its community structure with the co-introduction. This is especially true for the monogeneans *Ligophorus* spp., as they were not able to colonise host species from the invaded area (Sarabeev 2015), which probably results from their high host specificity (Sarabeev et al. 2013).

Table 2. Results of the Fisher's exact test for differences in the proportion of individuals of the two host species among the four role categories (i.e. peripheral, connector, module hub, network hub).

| Parasite community                | Sea of Azov | Sea of Japan |
|-----------------------------------|-------------|--------------|
| Whole                             | *           | NS           |
| Actively transmitted              | NS          | NS           |
| Passively/trophically transmitted | *           | NS           |
| Ectoparasites (Monogenea)         | NS          | NS           |

NS, non-significant at 0.05 level; \* p-value <0.001.



This contention also gains strength from results of the role analyses (i.e. peripheral, connector, module hub and network hub host categories established by *cz*-scores) (Fig. 4). When actively transmitted (partially including ectoparasite) and ectoparasite communities were considered, individuals of *M. cephalus* and *P. haematocheilus* played similar roles in both the native and invaded areas. In contrast, individuals of *P. haematocheilus* mostly played a peripheral role in the invaded sea when considering the trophically transmitted parasite community. This community is mainly formed by parasites of *M. cephalus* and other sympatric grey mullets (Sarabev 2015), which implies that *P. haematocheilus* partially shares similar prey items in the trophic network to the native grey mullet *M. cephalus*. Thus, our results suggest a lack of a shared evolutionary and ecological history between invasive hosts and native parasites, which conforms with the enemy release hypothesis (Torchin and Lafferty 2009). In addition to the native versus invasive status of the hosts, we acknowledge that the ecological properties of the regions could also generate differences between the parasite composition of the fish species and, consequently, be a confounding factor. However, if the effect of the invasion is important, we will detect changes in the roles of the fish species as time passes. In this scenario, more individuals of the introduced species could play connector or hub roles and change the structure of the community over time (Traveset et al. 2013), which concurs with the colonisation time hypothesis, i.e. the longer an invader is established, the more native parasites it should have acquired (Gendron et al. 2012). Eventually, *P. haematocheilus* might adopt a more central role in this community (Médoc et al. 2017) and the benefit of parasite release would be finally suppressed (Gendron et al. 2012). Therefore, long-term monitoring of the distribution of invasive individuals for the acquired parasites between the four categories of the *c* and *z* scores (Olesen et al. 2007) should be encouraged because it can be used as a proxy of maturity of the establishment of the invasive species in a community.

We consider that this role approach can stimulate future research despite being limited to modular networks (Guimerà and Amaral 2005). For example, it represents a means to assess the capacity of invasive individuals to act as ecosystem disruptors by determining their roles in transmitting parasites in the new community. Furthermore, it is well known that host–parasite associations are driven by host traits and/or phylogenetic determinants (Kamiya et al. 2014). In consequence, future studies could take into account traits/taxonomic position of the most connected native hosts to predict the effect of invasive hosts to maintain or spread diseases across communities. Finally, on the parasite side, their impact on invasion processes depends on their life-history traits that can influence host invasion by aiding or limiting expansion (Roy and Lawson Handley 2012). Future research could hence be aimed at explaining or predicting the roles of parasites in terms of traits. For instance, do connector species/individuals have the same traits and peripherals never possess them? Also, we could test whether the enemy/parasite release hypothesis can still be verified in terms of trait diversity of

enemies rather than species diversity of enemies. In sum, role analyses similar to those performed here would illuminate the mechanisms by which host–parasite interactions change during the biological invasion process.

## Data availability statement

Data available from the Harvard Dataverse Repository: <<https://dataverse.harvard.edu/>> (Llopis-Belenguer et al. 2019).

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**Author contributions** – CLB, IBC, JAB and DBS conceived the ideas and designed methodology. VS collected the data. CLB arranged the databases and analysed the data. CLB led the writing and IBC, JAB, VS and DBS contributed to the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Supplementary material (available online as Appendix ecog-04963 at <[www.ecography.org/appendix/ecog-04963](http://www.ecography.org/appendix/ecog-04963)>). Appendix 1–3.