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Tangled communities: links between predation and parasitism through trophically transmitted digeneans in aquatic communities ☆

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ABSTRACT

Many trophically transmitted digeneans (Platyhelminthes: Trematoda) benefit from prey–predator interactions to complete the last step of their life cycles. These parasites use prey as second intermediate hosts and predators as definitive hosts. We expect a relationship between predation and life-history and community aspects of trophically transmitted digeneans. We hypothesise at species level: (1) host ranges of metacercaria and adult digeneans are positively related; (2) second-intermediate-host range of a digenean relies on prey breadth of its definitive hosts. At community level: (3) digenean species that share second intermediate hosts share definitive hosts; (4) modularity (i.e., community composed of subsets of frequently interacting species) and module affiliation (i.e., tendency of species to share interacting partners across different ecological networks) is high between predator–prey, second intermediate host-metacercaria and definitive host-adult parasite networks. We used data from metacercaria and adult digeneans, their hosts and predation among hosts from nine aquatic locations or replicates. We tested hypotheses (1–3) with richness and Rao diversity dissimilarities (Spearman and Mantel correlations, respectively). For hypothesis (4), we combined the three types of networks to evaluate the multilayer network modularity and module affiliation of the species that participate in different ecological process (predation, metacercaria and/ or adult infection). We found: (1) significant positive correlations between host richness of metacercariae and adults in seven out of nine communities (Spearman correlations $p < 0.05$). (2) The relationship between the second-intermediate-host range of metacercariae and the diet breadth of their definitive hosts varied between communities and depended on the specialisation degree of the digeneans. (3) Metacercariae sharing second intermediate hosts also shared definitive hosts as adults in seven communities (Mantel correlations $p \leq 0.05$). (4) Communities were modular. Module affiliation varied from high to intermediate. Trophic ecology of definitive hosts provides a mechanistic understanding for the second-intermediate-host range and transmission pathways of trophically transmitted parasites.

1. Introduction

Species have multiple roles in ecosystems. For example, a species may act as a predator for its prey and as a host for its parasites (Park, 2019). Such species link ecological processes (e.g., predation and parasitism). The link between ecological processes increases ecological complexity, for instance by changing patterns of energy flow (Lafferty et al., 2008; Sato et al., 2019), and triggers evolutionary mechanisms, such as diffuse coevolution (Urban et al., 2008). The combined study of several ecological processes may provide a mechanistic understanding for diversity patterns of disparate biological groups, such as predators, prey, hosts and parasites, or community assembly (Locke et al., 2014; Frainer et al., 2018; Johnson et al., 2019; Liu and Chen, 2022).

Complex-life-cycle parasites rely on a series of hosts to complete a generation. In these parasite species, the larval stages occur in the intermediate hosts (they may require one or more successive intermediate hosts), whereas the adult form infects the definitive host. Often, the last transmission step in these complex life cycles

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requires the last intermediate host to be consumed by the definitive host, which are prey and predator, respectively. In this last step, the juvenile parasites mature into adults (Shoop, 1988; Benesh et al., 2014; Buck, 2019). In the case of parasite species, stable predator–prey interactions favour the evolution of their complex life cycles by trophic transmission (Parker et al., 2003; Benesh et al., 2021). At a broader ecological scale, interactions between species of different guilds compose tangled networks, for example predator–prey or host–parasite networks. Network structure, or how interactions are distributed among ecological guilds, is seldom random. Usually, eco-evolutionary factors, such as matching traits or shared evolutionary history between interacting species, explain the observed network structure (Dehling, 2018). Additionally, species involved in different ecological processes can make the structure of different types of networks (e.g., predator–prey and host–parasite networks) dependent on each other (Pilosof et al., 2017). For instance, a prey and its predator can function as the intermediate and definitive hosts of a trophically transmitted parasite (Park, 2019). Therefore, at community level, food web (network of who eats whom) and network of hosts and trophically transmitted parasites may feedback to shape similar network structures (Locke et al., 2014; Pilosof et al., 2017; Park, 2019).

Predator-prey interactions and diet of hosts may then be related to some life-history aspects of trophically transmitted parasites. We make four predictions, two at species level and two at community level. First, without other major constraints limiting a parasite species, we foresee that host richness (i.e., number of hosts a parasite species infects) of juveniles and adults of a trophically transmitted parasite species will be positively related. If the juvenile stage of a parasite species has the potential to infect many intermediate hosts, it will likely be exposed to a wider range of definitive hosts than a juvenile with a narrow intermediate host range (Locke et al., 2014). Second, diet breadth or prey richness of predators (definitive hosts) and last-intermediate-host range of juveniles of trophically transmitted parasite species will be related. For example, a generalist predator feeds on many prey species. Hence, a parasite can virtually use the broad range of prey species as last intermediate hosts during its juvenile stage to reach the predator (or definitive host). However, a specialist predator has a narrow diet breadth, with one or few prey species. A parasite will only succeed in reaching the specialist predator if it infects the limited number of prey species as last intermediate hosts. A positive correlation was found between the diet breadth of definitive hosts and the intermediate host range of larvae of Acanthocephala (Rotifera), Cestoda (Platyhelminthes) and parasitic Nematoda (Park, 2019). This finding suggests that the diet breadth of the definitive hosts and the last-intermediate-host range of juvenile parasites may be strongly related, for example, by generalist predators posing relatively low costs of parasite generalism at the last intermediate host (Park, 2019).

host–parasite network structure has been linked with predation properties (Amundsen et al., 2009). For example, it has been associated with prey “vulnerability”, or the number of predators of an intermediate host species. Intermediate hosts usually carried more juvenile parasites when they were more vulnerable (Chen et al., 2008; Chen et al., 2011; Locke et al., 2014). In this regard, we expect host–parasite network structure and prey–predator network structure to be at least partially related upon each other. Our third prediction foresees that, at community level, juveniles of different trophically transmitted parasite species that share intermediate hosts will tend to share definitive hosts as adults, as well. Food webs usually have modular structures. That is, they are composed of compartments or modules of prey and predators that interact more frequently among themselves than with other members of the community (Stouffer and Bascompte, 2011). Trophically transmitted parasites will pass from prey to predators of the same module more frequently than from/ to species in other modules. Hence, trophically transmitted parasites of different species that share intermediate hosts will tend to infect the same definitive host species, too. Fourth, we predict that species affiliation to modules will be preserved. In other words, a species can, for instance, function as a predator in a trophic network, and as a definitive host in an infection network. Modularity is then calculated for both the trophic and the infection networks. Module affiliation is preserved when the species belongs to the same module in both network types, regardless of its role as a predator or definitive host. Module affiliation will be preserved if species share interacting partners in networks of different ecological processes (Pilosof et al., 2017; Farage et al., 2021).

However, some processes may act against these predictions. Consequently, in some cases, the last-intermediate-host range of the parasite and the diet breadth of the definitive host may have evolved independently. Hence, the prey–predator and host–parasite network structures may not be related. We introduce a few examples to support these alternatives, this list should not be considered as closed, though. First, concomitant predation occurs when the parasite is eaten along with the prey by a predator, and the predator is not a suitable definitive host. Specialisation degree of trophically transmitted parasites on definitive host has been pointed as the driver of concomitant predation, since predators sharing the same prey breadth may get infected by different trophically transmitted parasites (Thieltges et al., 2013). Therefore, a wide prey range may not imply a higher infection rate. Second, manipulative parasites alter prey–predator interactions to maximise their chances to reach or avoid certain definitive hosts, thus suppressing or creating trophic links (Sato et al., 2019). Hence, differences in the structure of similar food webs might be attributed to the presence of manipulative parasites. Third, paratenic hosts can be ecologically relevant for parasite transmission but are not necessary for undergoing any ontogenetic changes to complete the parasite’s life cycle (Möhl et al., 2009). For example, *Alaria* spp. (Platyhelminthes: Trematoda) infect amphibians as last intermediate hosts and carnivores (mainly canids) as definitive hosts. Amphibians are not common prey of carnivores, hence *Alaria* individuals a have little chance to reach the definitive host through trophic transmission from the last intermediate host. Instead, amphibians are frequently eaten by a broad range of terrestrial vertebrates, which are more common prey of carnivores and the paratenic hosts of *Alaria* (Möhl et al., 2009). As a result, although the parasite exploits the trophic transmission, ranges and network structures of last intermediate and definitive hosts may have evolved independently.

Digeneans (Platyhelminthes: Trematoda) offer an excellent model system for studying the relationship between predation and trophic transmission of parasites. First, digeneans are obligate parasites with a great diversity of complex life cycles. However, in general, many digenean species take advantage of the trophic interaction between their last intermediate host (i.e., second intermediate host) and their definitive host, prey and predator respectively, to complete their life cycles. Thus, digeneans develop from the metacercaria (juvenile) stage in the second intermediate host into the adult stage in the definitive host (Shoop, 1988). Second, digeneans are closely dependent on water to complete their life cycle. Such dependence on aquatic habitats is reflected on the higher diversity of adult digeneans in aquatic vertebrates than in their non-aquatic counterparts (Cribb, 1998).

In our study, we assess the relationship between predation and the last transmission step of trophically transmitted digeneans at species and community levels. To address our objective, we combine three types of ecological interaction networks: one prey–predator trophic network (PPT) and two digenean infection networks (second intermediate host-metacercaria parasite, 2IHMP; and definitive host-adult parasite, DHAP) (Fig. 1). The study of the links between ecological processes, such as predation and parasitism, is challenging because large amounts of data are required (Hutchinson et al., 2019). Furthermore, data from different network types must be consistently sampled to make the effects of different interaction types comparable (Cagnolo, 2018). Our unique data consist of nine ecological communities from aquatic environments for which presence/ absence of predation and parasitic interactions were well-resolved, and that were systematically recorded for the three types of networks (Supplementary Table S1). Digenean individuals are resolved to species, although not all the species were identified. Resolution to species is generally accurate for free-living organisms, but all communities suffer from exceptional taxa aggregation for some organisms. Taxa aggregation may affect diversity and network analyses (Llopis-Belenguer et al., 2023). Despite this limitation, up to our knowledge, these are the most precise datasets worldwide that allow us to test our hypotheses. Therefore, analysing these datasets as replicates will enable us to confidently decipher overall patterns between predation and parasitism.

Earlier studies have used literature-based trait databases to analyse the cost of parasite species generalism, by relating prey richness of predator species and the phylogenetic distance between intermediate hosts of juvenile parasites different from digeneans (Park, 2019). Additionally, by means of food web field data, prey position in food web structure was related to parasite load of prey (Locke et al., 2014). Here, we use field data of digenean-inclusive food webs to infer the relationship between predation and trophic transmission of digeneans at species and community levels. Based on the findings of earlier studies (Locke et al., 2014; Park, 2019; Farage et al., 2021) and our four predictions at species and community levels, we aim to answer the following questions:

1. Are the host ranges of metacercaria and adults of the same digenean species positively related?
2. Is the second-intermediate-host range of a metacercaria related to prey breadth of its definitive hosts? In this regard, do metacercariae with a wide range of second intermediate hosts end up as adults in definitive hosts with a wide prey breadth? Do metacercariae with narrow range of second intermediate hosts infect, as adults, definitive hosts with a narrow prey breadth?
3. When trophically transmitted digeneans share second intermediate hosts at the metacercaria stage, do they also share definitive hosts at the adult stage?
4. Are the communities modular when different ecological process (i.e., predation, metacercaria parasite infection and adult parasite infection) are accounted for? Is module affiliation preserved across the trophic and both infection networks?

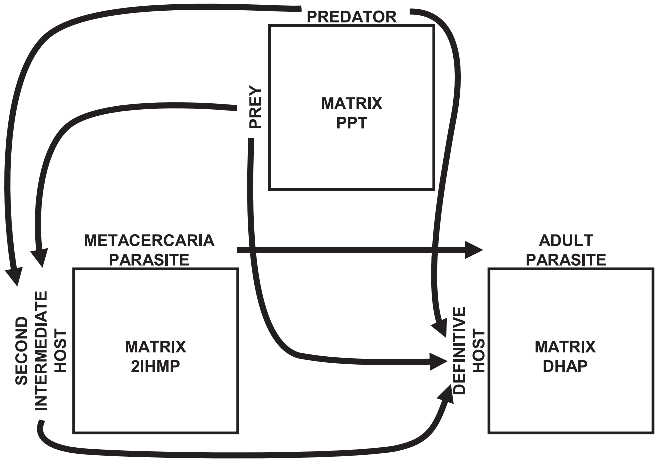

Fig. 1. Three types of bipartite networks in an ecological community. Matrix PPT: prey–predator Trophic network. Matrix 2IHMP: second Intermediate Host-Metacercaria Parasite network. Matrix DHAP: Definitive Host-Adult Parasite network. Matrix cells contain the presence/ absence of interactions. Arrows connect the same species that appear in different networks with different roles. Note that we only represented matrices and arrows that were relevant to our study. A species can play several roles in a community that are not accounted in this figure (e.g., a parasite can appear in a community as a metacercaria, adult or prey).

2. Material and methods

2.1. Ecological communities

We used nine ecological datasets from aquatic environments as independent replicates, namely (location – country code): Otago – NZ (Mouritsen et al., 2011), Takvatn – NO (Moore et al., 2024), Flensburg – DE, DK (Zander et al., 2011), Sylt – DE, DK (Thieltges et al., 2011), Ythan – GB (Huxham et al., 1996), Bahía San Quintín – MX, Estero de Punta Banda – MX and Carpinteria Salt Marsh – US (Hechinger et al., 2011) and Quick – US (Preston et al., 2012) (Supplementary Table S1). We subset each dataset into three bipartite matrices, that is matrices of interactions between organisms of two guilds (Runghen et al., 2021). Each matrix comprised resource-consumer interactions. Cells in the matrices contained the presence/ absence of either trophic or parasitic interactions. Matrix PPT consisted of prey–predator trophic interactions. Matrix 2IHMP included second intermediate host-metacercaria parasite interactions. Matrix DHAP comprised definitive host-adult parasite interactions. Since some prey and predator species from each location were the hosts of the digenean species, matrices of different interaction types (i.e., predation Matrix PPT, and parasitism Matrices 2IHMP and DHAP) are connected through species that play different roles as prey, predators or hosts in the ecosystem. Moreover, Matrices 2IHMP and DHAP are connected through trophically transmitted digenean species as metacercaria and adult stages (Fig. 1).

We restricted our analyses to digenean species for which both metacercaria and adult stages are known. We excluded other digenean species due to the impossibility to track their trophic transmission, and other metazoan parasite groups dependent on trophic transmission (i.e., Acanthocephala, Cestoda, Nematoda) because the limited number of species precluded our analysis from being robust within groups. Finally, some communities split interactions of a few free-living species by developmental stage. Since not all free-living species were split by developmental stage, we aggregated interactions of free-living species regardless of their developmental stage to homogenise free-living species data within and between datasets. All analyses were carried out independently for each ecological community in R (R Core Team, 2024).

2.1.1. Data accessibility

Data used in this study are available for Otago – NZ (Mouritsen et al., 2011), Flensburg – DE, DK (Zander et al., 2011), Sylt – DE, DK (Thieltges et al., 2011), Ythan – GB (Huxham et al., 1996), Bahía San Quintín – MX, Estero de Punta Banda – MX and Carpinteria Salt Marsh – US (Hechinger et al., 2011) and Quick – US (Preston et al., 2012); and available upon request for Takvatn – NO (Moore et al., 2024). Code to reproduce our analyses is also available (Llopis-Belenguer and Blasco-Costa, 2025).

2.2. Reporting sex- and gender-based analyses

This study is not affected by gender aspects since these organisms do not have a sociocultural organisation. Data do not have a sex influence for all digeneans and some other invertebrates since they are hermaphrodites. Data may have a sex influence for dioicous free living species. Infection or predation data in our study was not split by sexes, hence we could not account for the effect of hosts' sex on our results. This could be a limitation to the generalisation of our results. First, hosts of the same population may differ in their parasitic burden and prevalence depending on their sex (Shaw et al., 1998). Second, sex-selective predation may be a widespread phenomenon when species show sexual dimorphism (Boukal et al., 2008). Nonetheless, sexual dimorphism is not common for most of the species in our datasets.

2.3. Diversity analysis

Host and prey richness were measured with *speciesdiv* function in *adiv* package (Pavoine, 2020). To answer whether host ranges of metacercariae and adults of the same digenean species are positively related (first question), we assessed the significance of the correlations of the host richness between metacercariae and adults by means of a Spearman correlation (*cor.test* function in *stats* package).

Similarly, to evaluate whether the second-intermediate-host range of a metacercaria species is related to prey richness of its definitive hosts (second question), we evaluated the significance of the Spearman correlations between the second-intermediate-host richness of metacercariae and the prey richness of definitive hosts. Our results showed weak relationships between the second-intermediate-host richness of metacercariae and the prey richness of their definitive hosts (Otago – NZ: rho = –0.05, p-value = 0.7; Takvatn – NO: rho = 0.2, p-value = 0.05; Flensburg – DE, DK: rho = –0.09, p-value = 0.38; Sylt – DE, DK: rho = –0.00, p-value = 0.95; Ythan – GB: rho = 0.14, p-value = 0.28; Bahía San Quintín – MX: rho = –0.14, p-value = 0.00; Estero de Punta Banda – MX: rho = –0.03, p-value = 0.55; Carpinteria Salt Marsh – US: rho = –0.02, p-value = 0.67; Quick – US: rho = 0.11, p-value = 0.65). The lack of correlation in these analyses made us search for additional explanations. We hypothesised that generalist predators could host parasites that are both generalist and specialist of their second intermediate hosts at the metacercaria stage, whereas specialist predators would only host specialist metacercaria parasites of their second intermediate hosts. To check this prediction, we carried out additional Spearman correlations. On the one hand, we correlated prey richness of each definitive host species (which also function as predator species) with the second-intermediate-host richness of its metacercaria parasite species with the highest second-intermediate-host richness. We expected a significant positive trend between the prey richness of each predator and the second-intermediate-host richness of its metacercaria parasite with the highest second-intermediate-host richness. On the other hand, we correlated prey richness of each definitive host species with the second-intermediate-host richness of its metacercaria with the lowest second-intermediate-host richness. We expected a non-significant flat trend between the prey richness of each predator and the second-intermediate-host richness of its metacercaria with the lowest second-intermediate-host richness.

To know if metacercariae that share second intermediate hosts also tend to share definitive hosts as adults (third question), we first measured host diversity (dis)similarities among digenea species at metacercaria and adult stages, independently. We used the Rao dissimilarities to measure the similarity in the host range composition among parasite species at each developmental stage with the *dpcoa* function in *ade4* package (Thioulouse et al., 2018). This function produces a matrix of pairwise dissimilarities among either metacercariae or adults. Low dissimilarities among two digenea species indicate a shared host range (Pavoine et al., 2004). Second, we used a Mantel test for each locality to assess the correlation between the pairwise dissimilarity matrices of metacercariae and adults with *mantel* function in *vegan* package (Oksanen et al., 2019). Positive and significant Mantel correlations indicate that parasite species that tend to share second intermediate hosts at their metacercaria stage also share definitive hosts at their adult stage.

2.4. Multilayer network analysis

We employed the multilayer network framework to address whether the three ecological process (i.e., predation, metacercaria parasite infection and adult parasite infection) are organised in modular structures and if module affiliation is preserved across the different networks (fourth question). In the multilayer network framework, different ecological processes are accounted in layers, that is matrices or bipartite networks of interactions (Fig. 1). The matrices are connected through common species (Pilosof et al., 2017) (arrows in Fig. 1). We used the Infomap modularity algorithm to detect modules of species within a matrix while accounting for the amount of shared species (i.e., flow) between matrices (Farage et al., 2021). The algorithm first assigns species to modules and measures the cost or bits of such modular structure. A random walker selects a new species and produces a small change in the modular organisation, that is moving a species from one module to another module. The algorithm then tests whether the change minimises the bits of the modular structure. Infomap finally selects the modular structure that minimises the bits after 100 trials. In the multilayer network extension, the random walker selects species within and across matrices, and allows the same species to be assigned to the same or different modules in different matrices (Farage et al., 2021). The algorithm does not allow a species to be a resource and a consumer simultaneously in the same community. In our case study, some species (vertebrates and invertebrates) functioned as prey and predators in Matrix PPT of the nine ecological communities. We removed duplicated species in Matrix PPT (Fig. 1) from predators (i.e., columns). We left them as prey species because they usually occupied low or intermediate trophic positions, as opposed to those that only functioned as predators that usually occupied higher trophic positions. After the removal of double-role species from columns, only two predator species were retained in columns of the Matrix PPT from Quick – US. Since it was not realistic to test the fourth question and run the multilayer network analysis on the Quick – US community with only two predators, we excluded this community from this analysis. We ran the Infomap algorithm on the remaining eight communities with the *run_infomap_multilayer* function in *infomapecology* package (Farage et al., 2021).

To test the significance of the multilayer modularity of each locality, we created 100 null communities for each ecological community. That is, we created 100 null networks for Matrix PPT, 100 null networks for Matrix 2IHMP and 100 null networks for Matrix DHAP of each ecological community. Each set of 100 null networks was created with the method "quasiswap" in the *permatswap* function in *vegan*. This method randomises the presence/ absence of interactions while the total number of interactions for each consumer and resource species was kept as the same as in the ecological networks (Miklós and Podani, 2004). Afterwards, we compared modularity (i.e., bits) of each ecological community with the respective modularity, or bits, of the 100 null communities. We used a z-test, when the distribution of the null modularities was normal (i.e., Flensburg – DE, DK; Ythan – GB), and Wilcoxon tests, when the distribution of the null modularities differed from normality (i.e., Otago – NZ; Takvatn – NO; Sylt – DE, DK; Bahía San Quintín – MX, Estero de Punta Banda – MX; Carpinteria Salt Marsh – US), to assess whether the observed modularity differs from the null modularities. We performed the statistical analyses with *z.test* function in *BSDA* package (Arnholt, 2023) and *wilcox.test* in *stats* package. The lower was the number of bits, the higher was the modularity of a community (Farage et al., 2021). Finally, we calculated the percentage of species that changed their module affiliation between Matrices PPT-2IHMP, 2IHMP-DHAP and DHAP-PPT out of the total number of species common to two layers.

3. Results

A summary for the richness diversity analyses is in Table 1. At parasite species level (first question), significant and positive trends in Otago – NZ (rho = 0.78, p-value = 0.01), Flensburg – DE, DK (rho = 0.45, p-value = 0.04), Sylt – DE, DK (rho = 0.54, p-value = 0.01), Bahía San Quintín – MX (rho = 0.57, p-value = 0.00), Estero de Punta Banda – MX (rho = 0.42, p-value = 0.02), Carpinteria Salt Marsh – US (rho = 0.73, p-value = 0.00) and Quick – US (rho = 0.77, p-value = 0.04) indicated that the higher was the second-intermediate-host richness of metacercaria, the higher was the definitive hosts richness of adult parasites. The Spearman correlations were non-significant in Ythan – GB (rho = 0.13, p-value = 0.69), and non-significant and negative in Takvatn – NO (rho = -0.17, p-value = 0.56) (Fig. 2).

The Spearman correlation showed that second-intermediate-host richness of the most generalist metacercariae significantly increased with prey richness of their definitive hosts that they use as adults (second question) in Sylt – DE, DK (rho = 0.68, p-value = 0.00) (Fig. 3). This trend was also positive but not significant in Otago – NZ (rho = 0.41, p-value = 0.08), Flensburg – DE, DK (rho = 0.14, p-value = 0.6), Ythan – GB (rho = 0.34, p-value = 0.07), Estero de Punta Banda – MX (rho = 0.19, p-value = 0.22), Carpinteria Salt Marsh – US (rho = 0.1, p-value = 0.5) and Quick – US (rho = 0.58, p-value = 0.11) (Fig. 3). We found a negative significant trend in Takvatn – NO (rho = -0.72, p-value = 0.00) and negative but non-significant trend in Bahía San Quintín – MX (rho = -0.13, p-value = 0.4), since the higher the prey richness of the definitive host (or predators) was, the lower the second-intermediate-host richness of its most generalist metacercariae was (Fig. 3). In other words, the more generalist a predator was, the fewer second intermediate hosts were used by its most generalist metacercaria.

The most specialist metacercariae appeared as adults in definitive hosts (or predators) with the highest prey richness in Sylt – DE, DK (Spearman correlation: rho = –0.47, p-value = 0.00) (second question) (Fig. 4). Spearman correlations displayed this negative trend but they were non-significant in Otago – NZ (rho = -0.28, p-value = 0.28), Flensburg – DE, DK (rho = -0.41, p-value = 0.07) and Carpinteria Salt Marsh – US (rho = –0.02, p-value = 0.89) (Fig. 4). Conversely, the Spearman correlations were positive and significant in Takvatn – NO (rho = 0.72, p-value = 0.00), and positive but non-significant in Ythan – GB (rho = 0.15, p-value = 0.45), Bahía San Quintín – MX (rho = 0.08, p-value = 0.58), Estero de Punta Banda – MX (rho = 0.11, p-value = 0.47) and Quick – US (rho = 0.09, p-value = 0.82) (Fig. 4). The positive trends showed that as prey richness of the definitive host (or predators) increased, second-intermediate-host richness of its most specialist metacercaria increased.

At parasite community level (third question), the Mantel tests showed that when parasite species tended to share hosts as metacercariae, they also tended to share hosts as adults (Otago – NZ: statistic = 0.53, p-value = 0.00; Takvatn – NO: statistic = 0.17, p-value = 0.19; Flensburg – DE, DK: statistic = 0.2, p-value = 0.05; Sylt – DE, DK: statistic = 0.42, p-value = 0.00; Ythan – GB: statistic = 0.36, p-value = 0.01; Bahía San Quintín – MX: statistic = 0.26, p-value = 0.03; Estero de Punta Banda – MX: statistic = 0.27, p-value = 0.04; Carpinteria Salt Marsh – US: statistic = 0.41, p-value = 0.00; Quick – US: statistic = 0.48, p-value = 0.49).

The z-tests and the Wilcoxon tests of multilayer network modularity showed that the nine ecological communities were significantly more modular than their null assemblages (fourth question) (Table 2; Fig. 5): Otago – NZ: V = 5050, p-value = 0.00; Takvatn – NO: V = 5050, p-value = 0.00; Flensburg – DE, DK: z = 232.73, p-value = 0.00; Sylt – DE, DK: V = 5050, p-value = 0.00; Ythan – GB: z = 81.32, p-value = 0.00; Bahía San Quintín – MX: V = 5050, p-value = 0.00; Estero de Punta Banda – MX: V = 5050, p-value = 0.00; Carpinteria Salt Marsh – US: V = 5050, p-value = 0.00. Fig. 5 shows the multilayer network modularity. In Fig. 5, the number of species in each matrix is depicted by each column height. Columns are divided in stacked boxes or modules. The numbers on each box of the columns indicate the module number (total number of modules: Otago – NZ = 9; Takvatn – NO = 7; Flensburg – DE, DK = 7; Sylt – DE, DK = 15; Ythan – GB = 11; Bahía San Quintín – MX = 6; Estero de Punta Banda – MX = 9; Carpinteria Salt Marsh – US = 8). The box or module height represents the number of species in each module. Lines connect the same species in different matrices and show the species flow between matrices and modules. Some modules are maintained between matrices, although they change their number of species (e.g., Module 1 of each locality appears on Matrices PPT, 2IHMP and DHAP). Other modules only appear on one or two matrices. For instance, in Fig. 5B, species on Module 1 of Matrix PPT are distributed between Modules 1, 3 and 5 on Matrix 2IHMP. This result suggests that although many species frequently interact as prey and predators, they do not so frequently share metacercariae as second intermediate hosts.

Module affiliation was variable within and between ecological communities (Table 3; Fig. 5; Supplementary Fig. S1-Supplementary Fig. S8). The highest module affiliations were in Bahía San Quintín – MX and Carpinteria Salt Marsh – US (Table 3; Supplementary Fig. S6, Supplementary Fig. S8), followed by Otago – NZ, Flensburg – DE, DK, Ythan – GB and Estero de Punta Banda – MX (Table 3; Supplementary Fig. S1, Supplementary Fig. S3, Sup-

Table 1
Summary of richness diversity analyses. Resources are second intermediate host for metacercariae, definitive host for adult digeneans and prey for species with both predator and definitive host roles.

	Metacercariae	Adult digeneans	Species with both predator and definitive host roles
	n° of species; mean n° of resources ± standard deviation		
Otago – NZ	11; 4 ± 3.44	11; 6.55 ± 1.63	19; 17.58 ± 16.26
Takvatn – NO	14; 6 ± 6.48	14; 6.43 ± 3.03	14; 30.64 ± 21.35
Flensburg – DE, DK	21; 3.14 ± 1.77	21; 4.19 ± 2.09	20; 17 ± 8.8
Sylt – DE, DK	25; 5.52 ± 6.28	25; 15.32 ± 4.79	43; 14.35 ± 9
Ythan – GB	12; 1.75 ± 1.22	12; 5.17 ± 2.04	29; 8.66 ± 7.92
Bahía San Quintín – MX	32; 3.81 ± 2.71	32; 15 ± 7.89	45; 15.8 ± 11.77
Estero de Punta Banda – MX	32; 4.19 ± 3.45	32; 11.91 ± 6.09	45; 19.64 ± 10.42
Carpinteria Salt Marsh – US	27; 4.63 ± 3.09	27; 15.41 ± 6.61	44; 12.91 ± 5.95
Quick – US	7; 4.71 ± 2.21	7; 3 ± 1.41	9; 21 ± 10.7

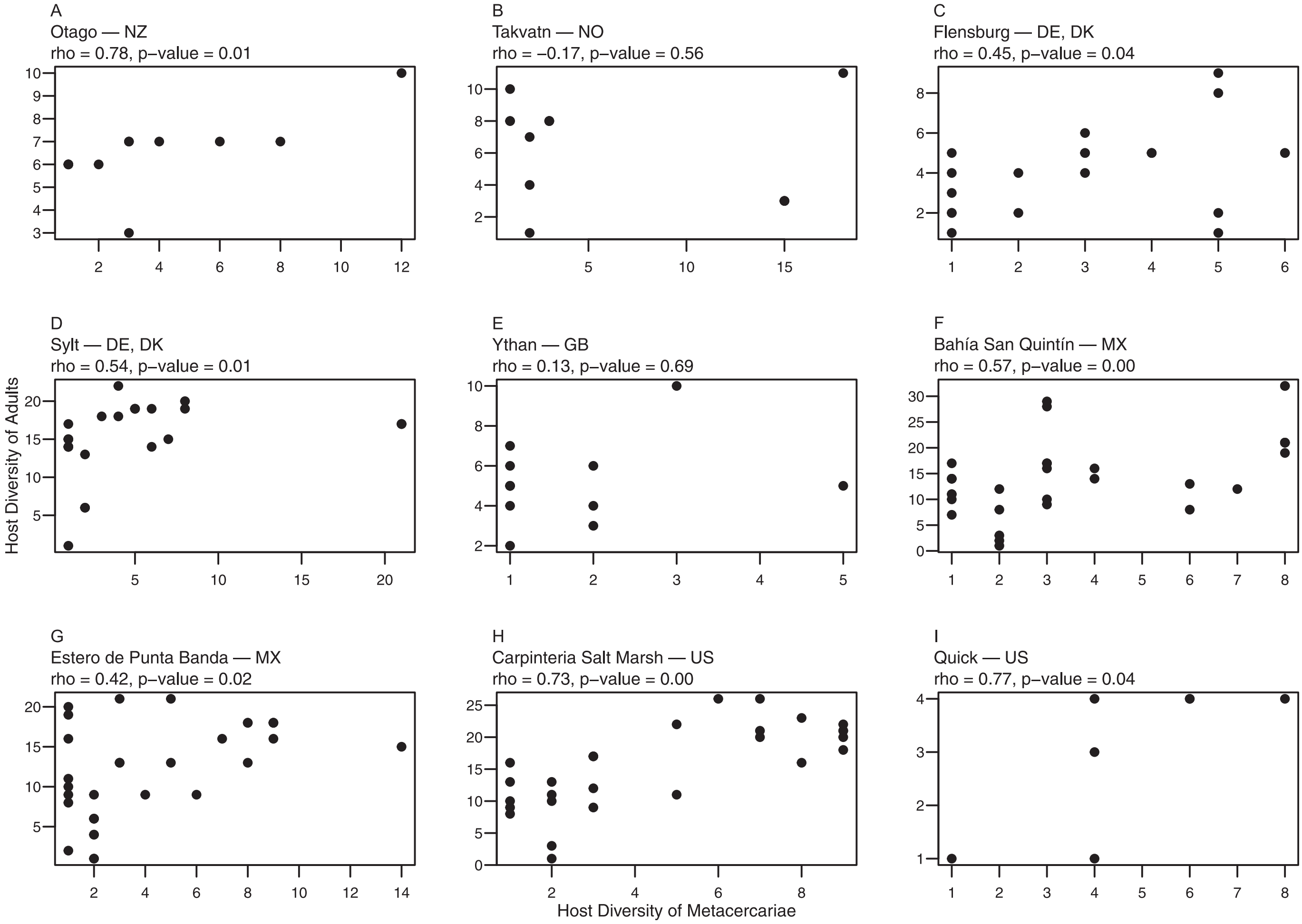

Fig. 2. Host richness of metacercaria and adult digeneans. Spearman correlation between second-intermediate-host richness and definitive host richness of metacercaria and adult digeneans of the same species in (A) Otago – NZ, (B) Takvatn – NO, (C) Flensburg – DE, DK, (D) Sylt – DE, DK, (E) Ythan – GB, (F) Bahía San Quintín – MX, (G) Estero de Punta Banda – MX, (H) Carpinteria Salt Marsh – US and (I) Quick – US.

plementary Fig. S5, Supplementary Fig. S7). Species in Sylt – DE, DK presented an intermediate level of module affiliation (Table 3; Supplementary Fig. S4). The lowest module affiliation was in Takvatn – NO (Table 3; Supplementary Fig. S2). The species that changed their module between Matrix PPT (prey) and Matrix 2IHMP (second intermediate hosts) were two molluscs and 16 arthropods in Takvatn – NO. In Flensburg – DE, DK, one mollusc species changed its module between Matrix PPT (prey) and Matrix 2IHMP (second intermediate hosts). In Sylt – DE, DK, a fish, a mollusc and five arthropods changed their modules. They played predator (Matrix PPT) and intermediate host (Matrix 2IHMP) roles. Between Matrices 2IHMP-DHAP, all the communities had species that changed modules. All the species that changed modules were parasites (metacercaria and adult roles of digeneans), except for two fish species, one in Ythan – GB and one in Bahía San Quintín – MX (second intermediate and definitive host roles). Between Matrices PPT-DHAP, all the species that changed modules were birds except for one mammal in Sylt – DE, DK (predator and definitive host roles) and one fish in Bahía San Quintín – MX (prey and definitive host roles).

4. Discussion

We showed a connection between predation and life-history aspects of trophically transmitted digeneans in aquatic communities. First, host richness of metacercaria and adult parasites of the same species tended to be correlated. This is probably because the more generalist for the second intermediate hosts the metacercariae are, the more definitive hosts they are exposed to (Locke et al., 2014). Second, the relationship between second-intermediate-host range of metacercariae and prey richness of the definitive hosts that they use as adults seemed to rely on location and degree of specialisation of the digenean species. Finally, parasite species that shared second intermediate hosts as metacercariae, also tended to share definitive hosts as adults. Similarly, module affiliation was usually high or intermediate. The modular structure of the ecological process “predation” probably enhances the patterns of host sharing and module affiliation of the ecological process “infection”. Overall, our results help us to better understand the functioning of ecosystems and the links between the ecological processes predation and parasitism.

Takvatn – NO discrepancies from our hypothesis may be explained by different non-exclusive factors. First, mean prey richness of predators (definitive hosts) in Takvatn is about two-times higher than mean prey richness of predators in the other localities (Table 1; x-axis of Fig. 3 and Fig. 4). This may produce concomitant predation on non-generalist parasites by predators (Amundsen et al., 2009). As a result, the structure of infection networks would differ with respect to the predation network. Second, a phylogenetic constraint may disrupt the expected patterns. The digeneans of the genus *Crepidostomum* (three species) use 15 second intermediate host species and infect three fish species as definitive hosts.

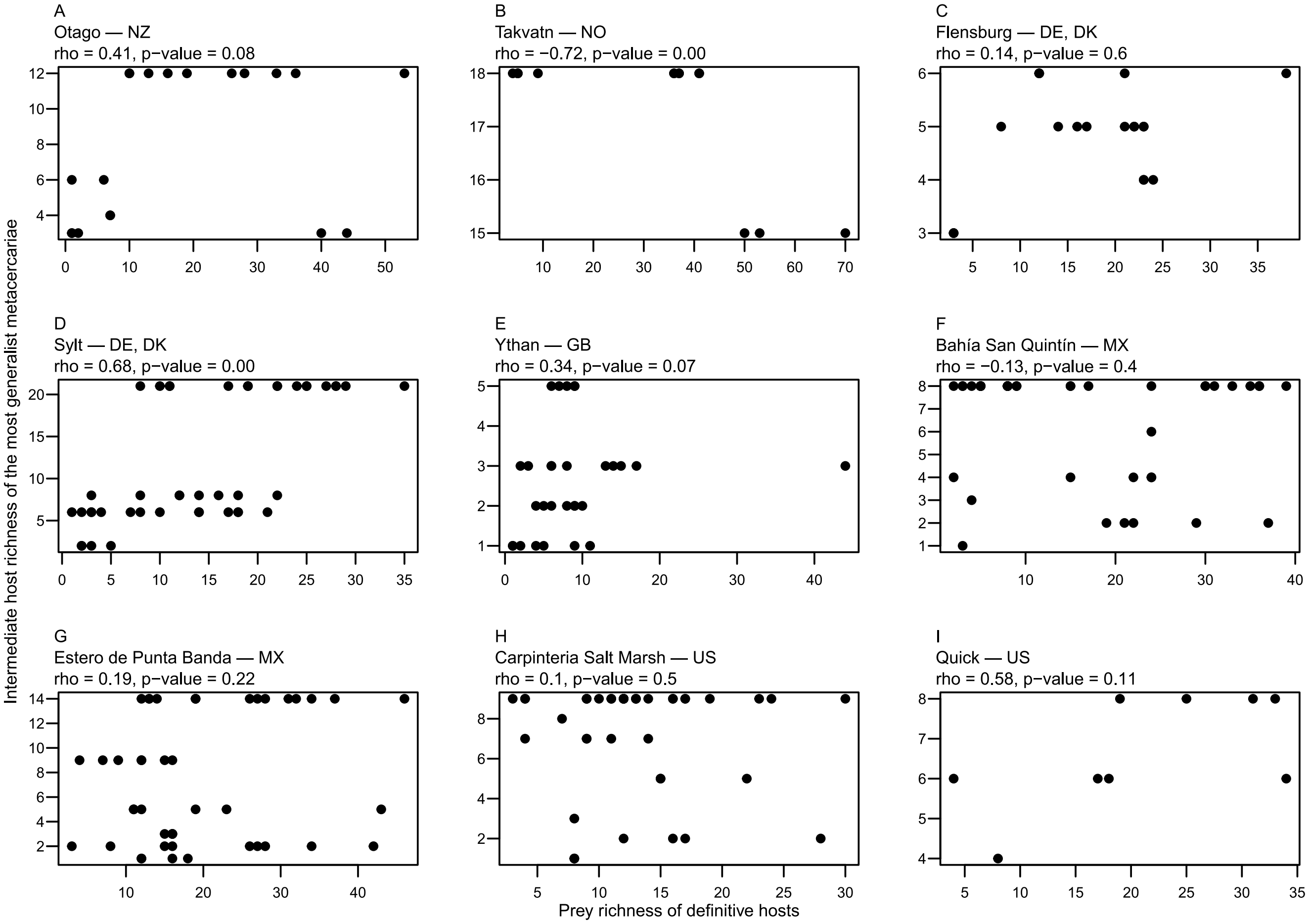

Fig. 3. Prey richness of definitive hosts and second-intermediate-host richness of their most generalist metacercariae. Spearman correlations between prey richness of each definitive host (predator) and second-intermediate-host richness of their most generalist metacercaria in (A) Otago – NZ, (B) Takvatn – NO, (C) Flensburg – DE, DK, (D) Sylt – DE, DK, (E) Ythan – GB, (F) Bahía San Quintín – MX, (G) Estero de Punta Banda – MX, (H) Carpinteria Salt Marsh – US and (I) Quick – US.

The rest of the 11 digenean species infect from one to 18 second intermediate hosts and use birds as definitive hosts. These patterns create a cluster of three points on Fig. 3B and Fig. 4B that correspond to the three *Crepidostomum* spp. Since all predators are rather generalists and fish are not infected by other adult digeneans, our predictions especially the second question (relationship between prey richness of definitive host and second-intermediate-host richness of metacercaria), may be difficult to be accomplished. Third, Lake Takvatn has distinctive conditions and recent history in comparison to the other localities. Takvatn is the only system located in the subarctic region. The diversity and network structure of Takvatn has been altered due to various successive events since the first decades of the 20th Century. The overexploitation of the brown trout (*Salmo trutta*) was followed by deliberated introductions of arctic charr (*Salvelinus alpinus*) and three-spined stickleback (*Gasterosteus aculeatus*), that were as well followed by unintentional arrival of nine new parasites and predators to the lake (Amundsen et al., 2007; Amundsen et al., 2013). After management strategies during the last-half of the 20th Century, brown trout population notably increased, while arctic charr population decreased. Nowadays, both fish populations remain stable and the community is at a new stable state (Persson et al., 2007). As the fish dynamics changed, the parasite community responded rapidly to the successive disturbances and management events. Although the parasite diversity and parasite interactions were altered as well, they appear to have also reached a new stable state (Amundsen et al., 2019). Finally, amongst the nine datasets analysed, only previous studies from Takvatn explicitly mentioned the use of molecular techniques to identify digeneans (Supplementary Table S1). It is worthwhile to mention this difference between Takvatn and the other datasets. The morphological identification methods could miss the presence of cryptic species and bias the recorded interaction patterns if a substantial number of cryptic species were present and undetected.

We first observed that host richness (first question) and host sharing (third question) of metacercaria and adult digeneans tended to be positively correlated. These findings agree with previous studies that showed links between trophic interactions and parasite distribution between hosts (Locke et al., 2014; Park, 2019), and suggests that host diet may exert a great selective pressure on trophically transmitted parasites (Park, 2019). That is, if predation links between hosts affect the number (first question) and composition (third question) of hosts of trophically transmitted digeneans, trophic associations between hosts will eventually select for many of the parasite traits (Llopis-Belenguer et al., 2019). Therefore, studies on host range of digeneans or digeneans distribution across hosts should consider trophic interactions between hosts as an explanatory variable and, perhaps more widely, the transmission strategy.

Our expectation of a relationship between the second-intermediate-host range of metacercaria and prey breadth of its definitive host was partially satisfied (second question). Indeed, it was only fully fulfilled in Sylt – DE, DK. The definitive hosts (predators) with the richest prey diversity tended to get infected

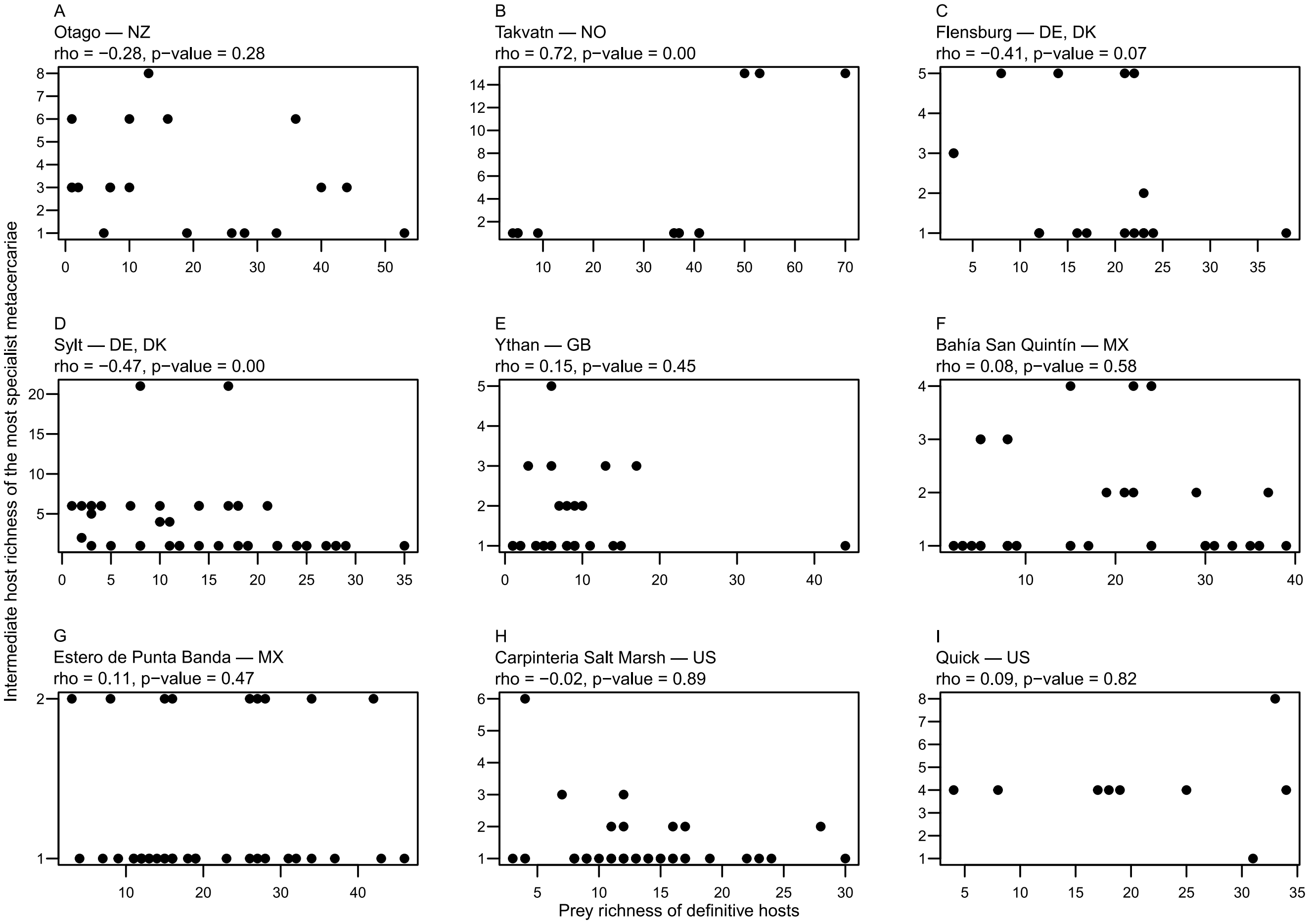

Fig. 4. Prey richness of definitive hosts and second-intermediate-host richness of their most specialist metacercariae. Spearman correlations between prey richness of each definitive host (predator) and second-intermediate-host richness of their most specialist metacercaria in (A) Otago – NZ, (B) Takvatn – NO, (C) Flensburg – DE, DK, (D) Sylt – DE, DK, (E) Ythan – GB, (F) Bahía San Quintín – MX, (G) Estero de Punta Banda – MX, (H) Carpinteria Salt Marsh – US and (I) Quick – US.

Table 2
Summary of each network type by ecological community in the multilayer network analyses. Matrix PPT: prey–predator Trophic network. Matrix 2IHMP: second Intermediate Host-Metacercaria Parasite network. Matrix DHAP: Definitive Host-Adult Parasite network.

	Matrix PPT	Matrix 2IHMP	Matrix DHAP
	n° of resource species × n° of consumer species; n° of interactions		
Otago – NZ	88 × 32; 430	26 × 11; 44	19 × 11; 72
Takvatn – NO	65 × 13; 309	24 × 14; 84	14 × 14; 90
Flensburg – DE, DK	42 × 9; 146	25 × 21; 66	20 × 21; 88
Sylt – DE, DK	93 × 26; 214	44 × 25; 138	43 × 25; 383
Ythan – GB	30 × 29; 145	14 × 12; 21	29 × 12; 62
Bahía San Quintín – MX	63 × 46; 609	30 × 32; 122	45 × 32; 480
Estero de Punta Banda – MX	79 × 45; 702	38 × 32; 134	45 × 32; 381
Carpinteria Salt Marsh – US	58 × 37; 442	30 × 27; 125	44 × 27; 416

by parasite species whose metacercaria had the richest second-intermediate-host range (Fig. 3D). Whereas, the host range of the most specialist metacercaria of all definitive host species was similar, regardless of the diet range of the definitive host (Fig. 4D: close to flat trend if we exclude the parasite species with second-intermediate-host range > 20). A potential explanation is that even when predators have wide diet breadth, they might not acquire digeneans through many of those hosts. For example, by feeding on arthropods they would avoid metacercariae (since arthropods are less often intermediate hosts for digeneans), while their diet breadth could be still wide. Moreover, parasites might be able to expand their second intermediate and definitive host ranges without losing their ability to infect their original intermediate and definitive hosts. Therefore, their second-intermediate-host range would not be restricted by their original-definitive-host diet breadth. Finally, conversely to our results, intermediate-host phylogenetic diversity was positively related to prey richness of definitive hosts in helminths different from digeneans (Park, 2019). Therefore, second-intermediate-host phylogenetic distance could be a relevant factor in explaining host richness of metacercaria, which we did not account for in our study.

Communities were modular when we accounted for predation, metacercaria infection and adult infection networks all together (fourth question). This is an innovative aspect of our research, since ecological processes have scarcely been studied in combination (Melián et al., 2009). While network analysis is a consolidated tool in parasite community ecology (Runghen et al., 2021), the multilayer network analysis is only starting to grow in this field. We used the multilayer network analysis to study trophic transmission of parasites between prey and predators. Diverse factors have explained modularity in host–parasite networks (Runghen et al., 2021). Here, we found evidence for modularity of prey–predator networks and modularity of host-trophically transmitted parasite networks to be related, since module affiliation was generally high for free-living species and high to intermediate for parasite species. The combined analysis of predation and parasitism does not allow us to establish a direction of causality between the two processes, that is, we cannot assume trophic interactions to be the driver of parasitic interactions. Indeed, in some instances, parasite traits

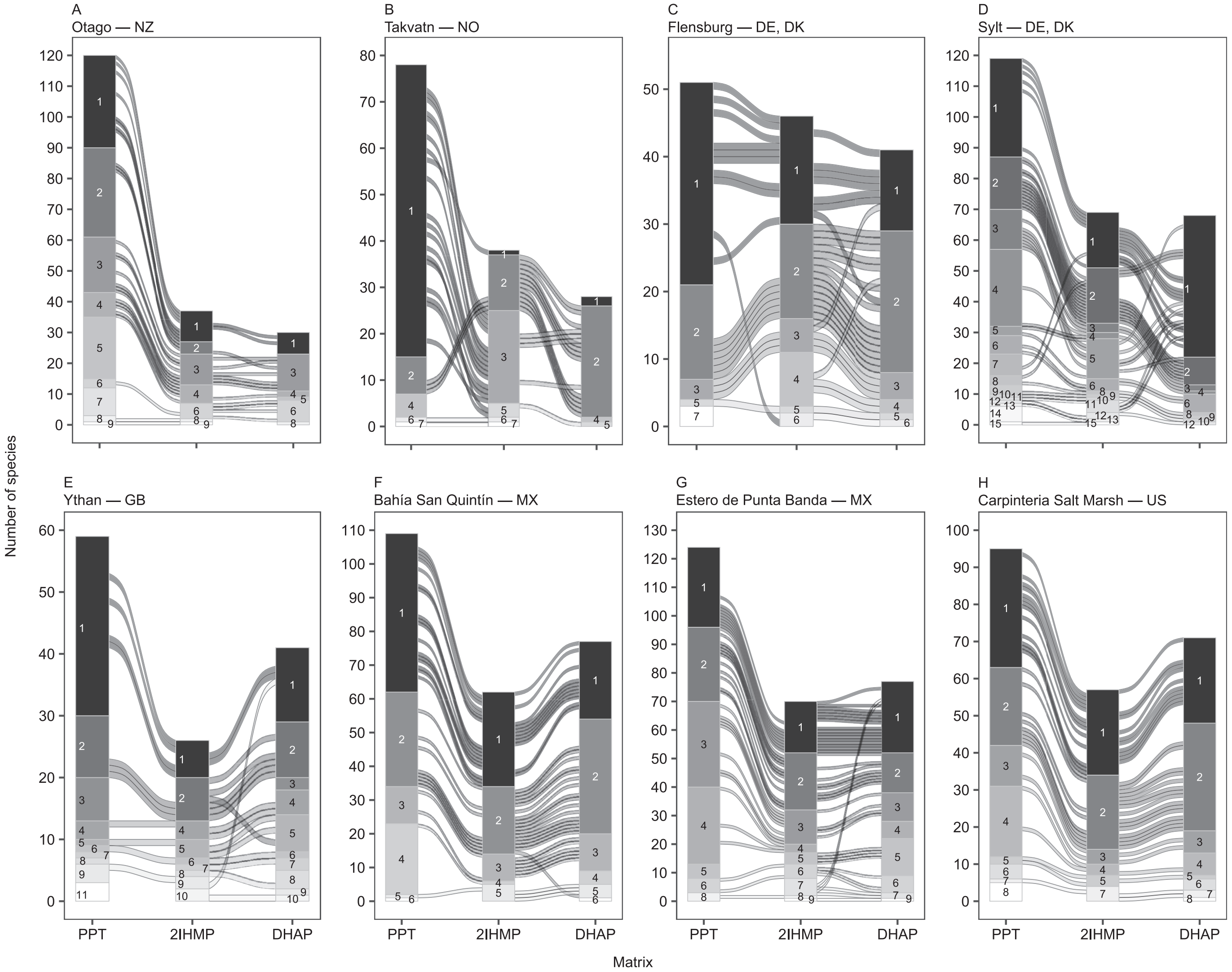

Fig. 5. Multilayer modular structure. (A) Otago – NZ, (B) Takvatn – NO, (C) Flensburg – DE, DK, (D) Sylt – DE, DK, (E) Ythan – GB, (F) Bahía San Quintín – MX, (G) Estero de Punta Banda – MX, and (H) Carpinteria Salt Marsh – US. Each column in the x-axis represents a matrix or network of interactions (Matrix PPT: prey–predator Trophic network. Matrix 2IHMP: second Intermediate Host-Metacercaria Parasite network. Matrix DHAP: Definitive Host-Adult Parasite network). The y-axis indicates de number of species in each matrix. Columns are divided by boxes. Boxes represents modules, which are numbered from 1 to the total number of modules in a community. The height of a box indicates the number of species contained in the module. Lines connect the same species in different matrices, and show species flow between matrices.

Table 3
Module affiliation. Number of species that mantained its module affiliation between matrices in each ecological community. In brackets, percentage of species that kept its modules out of the total number of species common to two matrices. Matrix PPT: prey–predator Trophic network. Matrix 2IHMP: second Intermediate Host-Metacercaria Parasite network. Matrix DHAP: Definitive Host-Adult Parasite network.

	Matrix PPT – Matrix 2IHMP	Matrix 2IHMP – Matrix DHAP	Matrix PPT – Matrix DHAP
Otago – NZ	26 (100%)	13 (92.86%)	17 (89.47%)
Takvatn – NO	6 (25%)	13 (76.47%)	10 (71.43%)
Flensburg – DE, DK	19 (95%)	28 (90.32%)	20 (100%)
Sylt – DE, DK	36 (83.72%)	39 (71.8%)	38 (88.37%)
Ythan – GB	11 (100%)	15 (88.24%)	24 (85.71%)
Bahía San Quintín – MX	29 (100%)	36 (97.3%)	44 (97.78%)
Estero de Punta Banda – MX	35 (100%)	38 (95%)	40 (89%)
Carpinteria Salt Marsh – US	29 (100%)	31 (100%)	41 (93.18%)

may drive trophic interactions, e.g., manipulative parasites. However, the most likely explanation is that parasites will exploit existing trophic interactions rather than create new ones (Parker et al., 2003). In this regard, our results might indicate that modularity in host-trophically transmitted parasite networks could mainly be an ecologically driven process. Especially, if trophically transmitted parasite species are generalists and being generalist has a low fitness cost, no other factors (other than predator–prey interactions) would limit their distribution across hosts (Park, 2019). This idea is based on the expectation that parasites have the potential to infect other hosts in addition to their actual hosts, analogous to the fundamental and realised niches (Nylin et al., 2018). For example, a parasite may be able to infect several definitive hosts. However, if only a few definitive hosts prey on its second intermediate hosts, the parasite will only infect those few definitive hosts, despite their potential ability to infect many other definitive hosts in the community. In such a case, modularity in host–parasite networks will arise as a consequence of predation, and not because of parasite specialisation in definitive hosts. Indeed, recent evidence at the interaction level of individuals (definitive host individual-adult parasite individual) points towards host trophic ecology, rather than host genetic differentiation, to be the most relevant driver of incipient differentiation (and speciation in the long term) of parasites across hosts (Brabec et al., 2024). This ecological process at the level of individuals would scale-up at community level and produce patterns such as modularity.

Intermediate module affiliation of parasites in Sylt – DE, DK could be attributed to fusion of a few modules from Matrix 2IHMP into Module 1 in Matrix DHAP (Fig. 5D). This would indicate that a few of these digeneans differ in their second-intermediate-host range but infect common definitive hosts. All the species that changed modules between Matrices PPT and 2IHMP in Takvatn – NO were arthropods (except two bivalve species). This could denote their role as connectors between terrestrial and aquatic environments, being eaten by/ feeding on terrestrial and aquatic species, but mainly being infected by aquatic parasites.

Finally, previous studies used the multilayer network analysis to, for instance, track parasite persistence or infection preference across time (Farage et al., 2021), and to follow parasite spread through interdependent transmission paths (i.e., protozoans spread through trophic and vectorial networks) and to detect which hosts facilitate parasite spread (Stella et al., 2017; Stella et al., 2018). Ours and these applications show that the multilayer network analysis is a promising avenue in parasite ecology. For example, spatial (e.g., biological invasions) or temporal (e.g., phenological changes) disturbances to the distributions of prey and predators alter the original trophic network structure by modifying the frequency of, or adding or suppressing feeding interactions (Thompson et al., 2012; Valiente-Banuet et al., 2015). Changes in trophic interactions can potentially spread cascading effects on the transmission of trophically transmitted parasites in contrasting ways (Strona, 2022). On the one hand, parasites with complex life cycles are prone to secondary extinctions due to their dependence on several hosts (Chen et al., 2011; Cizauskas et al., 2017). That is, when a host becomes rare or disappears from a community, the parasite diversity is likely affected (Chen et al., 2011). Our results showed a tight relationship between modularity of host–parasite networks and modularity of predator–prey networks. Hence, the alteration of modularity in predator–prey networks can be an early warning for alterations in the host–parasite networks and parasite diversity, even prior to species extinction. In this regard, monitoring species that connect ecological process can be useful to quantitatively anticipate knock-on effects from the trophic network to the infection networks (Strona, 2022). On the other hand, new transmission paths become available to parasites when trophic interactions are altered. For example, a prey or a predator can change its temporal or spatial distribution, and it will then be consumed by/ consume new species with which it did not interact before. This may cause that a previously inaccessible host becomes more common to a parasite. Such trophic changes grant the conditions for emerging infectious diseases to thrive, especially if naive hosts become infected (Brooks and Boeger, 2019), or on the contrary, if they are not suitable hosts for the parasite, they will become epidemiological sinks (Johnson et al., 2019).

To sum up, interspecific interactions and the relationship between different types of interactions are crucial drivers of ecosystem functioning (Frainer et al., 2018). We have shown how trophic interactions between hosts and predator–prey network structure should be taken into account to achieve a thorough understanding of the distribution of trophically transmitted parasites. This is because trophic interactions directly relate observed infections with their transmission pathways for trophically transmitted parasites (Locke et al., 2014).

Data statement

Data used in this study are available for Otago – NZ (Mouritsen et al., 2011), Flensburg – DE, DK (Zander et al., 2011), Sylt – DE, DK (Thieltges et al., 2011), Ythan – GB (Huxham et al., 1996), Bahía San Quintín – MX, Estero de Punta Banda – MX and Carpinteria Salt Marsh – US (Hechinger et al., 2011) and Quick – US (Preston et al., 2012); and available upon request for Takvatn – NO (Moore et al., 2024). Code to reproduce our analyses is also available (Llopis-Belenguer and Blasco-Costa, 2025).

CRediT authorship contribution statement

Cristina Llopis-Belenguer: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Isabel Blasco-Costa:** Writing – review & editing, Conceptualization.

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Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not used any AI or AI-assisted technology during the preparation of this work.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2025.04.007.

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