

Phylogeny determines the role of helminth parasites in intertidal food webs

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Summary

1. Parasites affect interactions among species in food webs and should be considered in any analysis of the structure, dynamics or resilience of trophic networks.
2. However, the roles of individual parasite species, such as their importance as connectors within the network, and what factors determine these roles, are yet to be investigated. Here, we test the hypotheses that the species roles of trematode, cestode and nematode parasites in aquatic food webs are influenced by the type of definitive host they use, and also determined by their phylogenetic affiliations.
3. We quantified the network role of 189 helminth species from six highly resolved intertidal food webs. We focused on four measures of centrality (node degree, closeness centrality, betweenness centrality and eigenvalue centrality), which characterize each parasite's position within the web, and on relative connectedness of a parasite species to taxa in its own module vs. other modules of the web (within-module degree and participation coefficient).
4. All six food webs displayed a significant modular structure, that is, they consisted of subsets of species interacting mostly with each other and less with species from other subsets. We demonstrated that the parasites themselves are not generating this modularity, though they contribute to intermodule connectivity.
5. Mixed-effects models revealed only a modest influence of the type of definitive host used (bird or fish) and of the web of origin on the different measures of parasite species roles. In contrast, the taxonomic affiliations of the parasites, included in the models as nested random factors, accounted for 37–93% of the total variance, depending on the measure of species role.
6. Our findings indicate that parasites are important intermodule connectors and thus contribute to web cohesion. We also uncover a very strong phylogenetic signal in parasite species roles, suggesting that the role of any parasite species in a food web, including new invasive species, is to some extent predictable based solely on its taxonomic affiliations.

Key-words: connectedness, modularity, network centrality, phylogenetic signal, trematodes

Introduction

Several analyses contrasting food webs with and without parasites have demonstrated that inclusion of parasites is necessary for a full assessment of the structure, dynamics and resilience of complex trophic networks (Lafferty *et al.* 2008; Poulin 2010). More specifically, earlier studies have reported that the inclusion of parasites as separate network nodes, that is, as distinct species, within food webs

alters several whole-web metrics, such as connectance or average food chain length (see Huxham, Raffaelli & Pike 1995; Thompson, Mouritsen & Poulin 2005; Lafferty, Dobson & Kuris 2006; Hernandez & Sukhdeo 2008; Amundsen *et al.* 2009). Recently, the focus has shifted from effects on whole-web properties to the species-level (or network) role played by free-living taxa in parasite transmission, or how the role that free-living taxa play in the network affects their use by parasites. Thus, host taxa that occupy central positions within webs, that are highly connected to other taxa at lower or higher trophic levels,

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that are involved in asymmetric predator–prey relationships, and/or that are part of modules of strongly interacting species within the larger network, are used by more parasite species (Chen *et al.* 2008; Anderson & Sukhdeo 2011; Rossiter & Sukhdeo 2011). These patterns may arise because of the special position of these hosts in food webs, but also through sampling artefacts, with well-sampled host taxa having both longer lists of diet items and of parasites.

Surprisingly, the same species-level attention has not been paid to the parasites themselves. Different parasite taxa have different life cycles and different degrees of host specificity. In addition, parasites are involved in diverse trophic relationships. They do not act only as consumers in food webs, but also as resources, through predation on their free-living infective stages or concomitant predation of their host (Johnson *et al.* 2010). The network roles of parasites as connectors within the network may be associated with their life cycle. Thus in the case of trophically transmitted parasites, their roles within the web may reflect the extent of their importance in the food chains they follow towards the top predator they use as definitive host. For instance, in aquatic systems, parasites using birds as definitive hosts may incur different concomitant predation than those using fish, since birds generally occupy higher trophic levels than fish (some birds eat fish, though no fish eat birds). It may also be that the roles played by parasites in food webs are constrained by their phylogenetic affiliations. Through phylogenetic niche conservatism, closely related species should be more ecologically similar to each other than expected based solely on the timing of their phylogenetic divergence (Peterson, Soberon & Sanchez-Cordero 1999). Measured as a phylogenetic signal, that is, the degree of phylogenetic dependence of a given character (Blomberg, Garland & Ives 2003), niche conservatism has been demonstrated in numerous plant and animal taxa, and for several ecological and life-history traits, including niche breadth and geographical range size (e.g. Jablonski 1987; Peterson, Soberon & Sanchez-Cordero 1999; Freckleton, Harvey & Pagel 2002; Qian & Ricklefs 2004). If such traits vary among species in direct proportion to their shared phylogenetic history, then we might expect species roles in food webs to show the same phylogenetic conservatism.

And indeed they do, at least for free-living species. Across several marine, freshwater and terrestrial food webs, phylogenetic relatedness between free-living taxa is generally more important than actual similarity in their body sizes in accounting for similarities in their set of consumers and, to a lesser extent, in their set of resources (Naisbit *et al.* 2012). Using an even larger set of food webs, Stouffer *et al.* (2012) found that the way a (free-living) species is embedded within the trophic network and how it affects network dynamics show strong phylogenetic signals and are thus true species attributes. The only test of evolutionary conservatism in the roles of parasites within networks involved the measurement of phylogenetic

signals in several bipartite host–parasite networks consisting of fleas and their mammalian hosts (Krasnov *et al.* 2012). Overall, a phylogenetic signal was found in the degree to which parasite species in one module of the network are connected to host species from other modules, but not in their degree of within-module connectedness (Krasnov *et al.* 2012). Phylogenetic conservatism of the roles of parasite species with multi-host life cycles within entire food webs, as opposed to one-host parasites in bipartite networks, has not yet been quantified.

In this paper, we test the nonmutually exclusive hypotheses that (i) the role of a trophically transmitted parasite in a food web is associated with the type of definitive host it uses, and (ii) the role of the same parasite in the web is determined mostly by its taxonomic (phylogenetic) affiliations as opposed to being shaped by local web properties. We investigate six intertidal food webs, where the biomass and productivity of parasites attain substantial levels (Kuris *et al.* 2008) and where parasitism is known to impact community structure and diversity (Mouritsen & Poulin 2002). Our investigation is limited to the major helminth groups (trematodes, cestodes and nematodes), because their identity was relatively well resolved and these higher taxa occurred in all webs. The definitive hosts of these helminths are usually either fish or birds, with the former generally occupying lower trophic levels than the latter. Although the parasite species compositions of the six different webs vary, there is consistency among them in the order- and family-level taxonomic make-up of their parasite assemblages, allowing direct measurement of the contribution of taxonomic affiliations to variance in species roles. These roles were measured using several indices that quantify either the centrality of a species' network position based on its connectedness to others, or its pattern of connections to species within and outside its particular module within the broader food web. Our results shift the focus of earlier analyses of overall effects of parasites on whole webs, towards a consideration of phylogenetic constraints limiting the potential influence of individual parasites on web topology and dynamics.

Materials and methods

FOOD WEB DATA SETS

We focused on six highly resolved intertidal food webs, all in the public domain, that include data on metazoan parasites (Table 1). The first three webs (see Hechinger *et al.* 2011) are from estuarine salt marshes along the North American Pacific coast: Carpinteria Salt Marsh (Carp), California, USA, Estero de Punta Banda (Punta), Baja California, Mexico and Bahia Falsa (Falsa) in Bahia San Quintín, Baja California, Mexico. The three other food webs are from Flensburg Fjord (Flens), a brackish shallow water inlet on the Baltic Sea between Germany and Denmark (Zander *et al.* 2011), Sylt Tidal Basin (Sylt), an intertidal bight ecosystem on the North Sea between Germany and Denmark (Thieltges *et al.* 2011a), and Otago Harbor (Otago), an intertidal mudflat ecosystem in New Zealand (Mouritsen *et al.*

Table 1. Basic properties of the six intertidal food webs and the taxonomic composition of their helminth parasites

Food web	Total number of taxa	Total number of links	Total number of parasite taxa	Number of trematodes	Number of cestodes	Number of nematodes	Number of acanthocephalans	Number of other parasites
Carp	154	3350	59	28 (26)	6 (6)	5 (2)	2 (0)	18 (0)
Punta	185	4671	77	39 (34)	8 (7)	7 (4)	4 (0)	19 (0)
Falsa	142	3006	52	33 (27)	4 (4)	4 (3)	2 (0)	9 (0)
Flens	109	1252	46	25 (25)	3 (2)	10 (8)	2 (0)	6 (0)
Sylt	149	2680	35	27 (27)	1 (0)	1 (0)	1 (0)	5 (0)
Otago	118	1354	19	13 (12)	0 (0)	2 (2)	2 (0)	2 (0)

The numbers in parentheses indicate how many taxa were included in the mixed effect models.

2011). The effects of parasite inclusion on food web metrics have recently been investigated in these webs or in earlier, less complete versions of some of them (Thompson, Mouritsen & Poulin 2005; Lafferty, Dobson & Kuris 2006; Rudolf & Lafferty 2011; Dunne *et al.* 2013).

Including all taxa, both free-living and parasitic, the six webs have between 109 and 185 taxa, and between 1252 and 4671 links, that is, trophic connections between pairs of species (Table 1). At a broad taxonomic scale, the composition of these webs is roughly similar, with crustaceans, molluscs and polychaetes dominating intermediate trophic levels, and fish and birds the higher levels (see Appendix S1, Supporting Information). Parasites comprise 16–42% of taxa in those webs; trematodes are by far the most common parasite group, with nematodes, cestodes and acanthocephalans being less common though found in all webs (Table 1). Other parasite taxa found in those webs and not considered here were mostly arthropods and apicomplexans. Note, however, that these other parasites were included in tests of web modularity and in calculations of species-level roles; it is only in the subsequent mixed effects models (see below) testing for the determinants of species roles that only helminths (trematodes, cestodes, nematodes and acanthocephalans) were considered.

Our goal is to determine the network role of parasite species within food webs. Therefore, we resolved parasites to the species level rather than splitting them into ontogenetic life stages representing different phases of their complex life cycles. Aggregating to species level masks developmental shifts in host use, which can be important for analyses of the robustness of food webs to species loss (Lafferty & Kuris 2009; Chen *et al.* 2011; Rudolf & Lafferty 2011). However, we chose to work at the species level because (i) developmental stages are not independent of each other, that is, if one stage of a parasite species is present in a system, then its other stages are present too; (ii) patterns of ontogenetic change in host use are fairly consistent across parasite species, with host specificity typically decreasing from larval/juvenile stages in invertebrates to adult worms in vertebrates; and (iii) previous studies on species roles in interaction networks have focused on species and not ontogenetic stages (Rezende *et al.* 2009; Anderson & Sukhdeo 2011).

We excluded all helminth taxa not identified at least to the family level. However, we retained cestodes of the orders Tetraphyllidea and Trypanorhyncha, even if not identified to the family level, because they represented two well-defined groups. In addition, we chose to exclude (i) acanthocephalans, since only four species from three webs were sufficiently identified, and (ii) two nematodes using mammals as definitive hosts (see Appendix S1, Supporting Information).

The parasite species composition of the different food webs varies across the broad geographical scale of this study, but the same higher taxa, such as families and orders, recur everywhere. Therefore, and in the absence of a complete molecular phylogeny for all parasite taxa included here, each parasite species was placed in a nested taxonomic hierarchy including family, order, class and phylum. For this information, we used the comprehensive systematic classification for trematodes (Gibson, Jones & Bray 2002; Jones, Bray & Gibson 2005; Bray, Gibson & Jones 2008), cestodes (Khalil, Jones & Bray 1994) and nematodes (Anderson 2000). These taxonomic schemes generally match the phylogenetic relationships uncovered using genetic data (Blaxter *et al.* 1998; Olson *et al.* 2001, 2003), strengthening any inference of phylogenetic constraints based on the detection of taxonomic ones.

WEB MODULARITY

Each of the six webs was slightly modified such that the few duplicate links, that is, those involving the same pair of taxa, were collapsed into one. Therefore, if adults of parasite A infect host B, and B feeds on juvenile stages of A, the two are connected by a single undirected link in the web. We did this because there were very few such cases, and our focus was on the general structure of each web, and not on the direction in which energy flows along particular links. The very few cannibalistic links (loops) were also removed, and the final webs consisted of a binary matrix of undirected links with no duplicates, to allow the measurement of species roles and positions (see Rezende *et al.* 2009). Note that the modularity analyses include the full food webs, and do not exclude any sub-webs consisting of specific types of trophic interactions (Lafferty, Dobson & Kuris 2006).

We verified that the food webs consisted of modules, that is, distinct subsets of taxa that interact preferentially among themselves and to a lesser extent with taxa from other modules (Olesen *et al.* 2007; Carstensen & Olesen 2009; Rezende *et al.* 2009). We used a simulated annealing optimization approach (Guimerà & Amaral 2005) for finding the maximization of a function called modularity (Newman & Girvan 2004) for each food web. The equation for this function and its explanation are given elsewhere (e.g. Guimerà & Amaral 2005; Fortuna *et al.* 2010). The algorithm (implemented with the software NETCARTO, provided by R. Guimerà) follows a heuristic procedure for maximizing the number of links within modules and minimizing the number of links between modules. We ran the algorithm 10 times for each food web and used the maximum modularity value obtained as the modularity of that network (Fortuna *et al.* 2010; Krasnov *et al.* 2012). The simulated annealing algorithm is

considered highly accurate for module detection (see Dupont & Olesen 2009 and references therein). We then tested whether observed modularity values departed significantly from random expectations. The significance of the level of modularity for each web was estimated using the algorithm provided within NET-CARTO (Guimerà, Sales-Pardo & Amaral 2004). The program constructed 500 random webs for each real food web and calculated the degree of modularity for each of these webs. The random webs were random graphs with the same overall connectivity as the original network (Guimerà, Sales-Pardo & Amaral 2004). Modularity of an observed food web was considered statistically significant when its observed value fell outside one SD of the average modularity of the random networks.

Having performed these modularity analyses on the full food webs, we repeated them after the removal of parasite taxa. This was done to verify that parasites themselves are not responsible for the modular structure of the whole web. For this, we used the procedures outlined above on each web after the removal of all species for which at least one life stage is involved in a parasitic interaction, variously labelled in the original food webs as macro-parasite, micropredator, trophically transmitted parasite, parasitic castrator, parasitoid or pathogen.

PARASITE SPECIES ROLES

We calculated six species-level measures of the role of each parasite taxon (represented by a network node) in each of the six food webs. These measures, used in combination, capture all relevant facets of a species role or position in a web. They included four centrality indices: (i) Node degree, or the number of other species to which a species is connected; (ii) Closeness centrality, which is the inverse of the average length of the shortest paths from a species to all the other species in the network; (iii) Betweenness centrality, which quantifies the extent to which a focal species lies on the shortest paths between two other species (Freeman 1979); and (iv) Eigenvector centrality, which measures a species' importance in a network based on whether it has connections to other species that are themselves important. Thus, instead of awarding species just one point for each neighbour (as is the simple node degree measure above), eigenvector centrality gives each species a score proportional to the sum of the scores of its neighbours (Bonacich 1987). The four centrality indices were calculated with IGRAPH version 0.5.3 (Csárdi & Nepusz 2006). Together, these indices capture different aspects of the biological significance of each species to the food web (González, Dalsgaard & Olesen 2010). Node degree measures the number of other species with which a focal species interacts directly, by acting as their resource or consumer. Closeness centrality is proportional to how rapidly the indirect effects of the focal species can spread to other species in the web. Betweenness centrality measures the importance of a focal species to the cohesiveness of the web through its role as a 'bridge' for energy transfer between other species. Finally, eigenvector centrality quantifies the extent to which a focal species has direct interactions with other species that are themselves highly connected within the web, that is, influential for energy transfer. To render values for node degree, closeness centrality, betweenness centrality and eigenvector centrality comparable across different food webs, they were rescaled by dividing them by the maximum value observed among helminths in their web. These relative values are used hereafter.

In addition, in a modular network, species interact mostly with other species within their own module but can also interact with

a few species from other modules. Topologically, therefore, a species can be characterized by (i) its standardized within-module degree z , which describes how a species is connected to others in the same module; and (ii) its participation coefficient p , which describes how a species is connected to species in other modules (see Guimerà & Amaral 2005). The within-module degree of a species is calculated as the difference between the within-module number of links of this species and the mean within-module number of links of all species in this module divided by the latter's standard deviation (Guimerà & Amaral 2005). It indicates whether a species is more generalist (positive z values) or more specialist (negative z values) within the module than the other species in that module. The participation coefficient of a species is calculated as 1 minus the across-module sum of the squared quotients of that species' number of within-module links and that species' total number of links. It is therefore close to 1 if the links of a species are uniformly distributed among all the modules and 0 if all its links are within its own module. We calculated z and P values for each species using NETCARTO (see above). Finally, for descriptive purposes only, we assigned 'functional roles' to all parasite species based on the classification of Olesen *et al.* (2007). A *peripheral* species has both low z and P values, that is, few links inside its own module and almost none to other modules. A *connector* has a low z and a high P value, and thus serves to link modules together. A *module hub* has a high z and a low P value and maintains the coherence of its own module. Finally, a *network hub* has both high z and P values, contributing to the coherence of the whole network (see Olesen *et al.* 2007 for details).

STATISTICAL ANALYSES

The six species roles calculated for each parasite, and used as response variables in the following analyses, were strongly positively correlated with each other ($0.279 \leq r \leq 0.973$, all pairwise correlations: $P < 0.0001$). Although considered separately below, they are clearly not independent of one another.

The above response variables (following log transformation in the case of betweenness centrality, closeness centrality and the participation coefficient, to improve data distributions) were analysed using six separate mixed-effects models implemented in JMP version 9.0.2 (SAS Institute, Inc, Cary, NC, USA). Our main goal was to estimate to what extent the role of a parasite in a food web is affected by its definitive host, or determined by its taxonomic or phylogenetic affiliations as opposed to local web properties. Therefore, definitive host type (fish or bird) was included as a main effect. In addition, web identity (Carp, Punta, Falsa, Flens, Sylt or Otago) was included as a random effect in the models. This accounts for the fact that multiple parasite taxa come from the same food web, and that idiosyncrasies of particular webs may influence species roles. In addition to the well-known scale-dependence of network properties resulting from differences among webs in species richness, number of links or number of links per species (Martinez 1993; Dunne, Williams & Martinez 2004; Stouffer *et al.* 2005), local factors associated with the food webs' distinct geographical areas are also part of the random effect. Finally, taxonomy (family, order, class and phylum) was also included in the models, as a set of hierarchically nested random factors. This allowed us to estimate how much of the variance in each response variable was due to either web identity or taxonomic heritability, the latter being an estimate of the phylogenetic signal in the data. We did this by calculating the

proportion of the total variance explained by the fixed effect, and the proportion that could be accounted for by web identity and each of the taxonomic levels (Nakagawa & Schielzeth 2013).

As a separate test of the importance of food web size (total number of species in a web) in determining species roles, we calculated the averages for each of the six roles considered, for each parasite family in each web. Using only families represented in either five or six of the webs, we then regressed these family averages against food web size, to assess how often node-level properties covaried with web size among related parasites.

Results

When all free-living and parasitic taxa were included, all six intertidal food webs had a significantly modular structure (Table 2). There were either three or four modules detected per web, depending on the food web examined. Module composition varied, though there were some consistent elements across modules (see Appendix S1, Supporting Information). For example, all modules except one contained trematodes, and also the vast majority of modules included birds, crustaceans and fish. When parasitic taxa were removed from the webs, all webs still had a significant modular structure, and the same number of modules was detected in each web as in the above analyses including parasites, except for the Sylt web, where one extra module (five instead of four with parasites included) was observed. This additional module was very small and comprised five taxa of cnidarians and polychaetes, which all belonged to the same module in the full web analysis. Apart from this difference, module composition was very similar whether parasites were included or not, with generally 60–80% of free-living taxa found in one module in the full web analysis also found in the same module in the parasite-free analysis.

In total, 189 parasite species were included in the analyses. Of these, based on their z and P values, 88 (46.6%) were categorized as connectors and 99 (52.4%) as peripherals. The remaining two species (about 1% of the total) were the trematodes *Cryptocotyle lingua* (family Heterophyidae) and *Podocotyle atomon* (Opecoelidae), both from the Sylt food web; they were categorized as network hubs, having both high z and P values. For comparison, among

Table 2. Observed modularity of six intertidal food webs compared to that expected from 500 simulated webs in which the size and connectance of the networks are retained but the connections among species are randomized. All six food webs are significantly modular

Food web	Observed modularity	Mean (\pm SD) modularity from randomized webs	Number of detected modules
Carp	0.2099	0.110 (0.002)	4
Punta	0.2105	0.100 (0.001)	4
Falsa	0.2196	0.110 (0.002)	3
Flens	0.2538	0.145 (0.002)	4
Sylt	0.1821	0.115 (0.002)	4
Otago	0.2990	0.155 (0.003)	3

the free-living taxa from the same six food webs (not analysed further here), 32.3% were connectors, 65.9% were peripherals, 1.2% were module hubs and 0.6% were network hubs.

The mixed-effects model revealed that of the six parasite species roles investigated, the type of definitive host used by a parasite only had a significant influence on eigenvalue centrality ($F = 7.364$, $P = 0.0112$); the only other measure for which definitive host type came close to having an influence was closeness centrality ($F = 3.677$, $P = 0.0702$). In general, values for both closeness and eigenvalue centrality were higher in parasites using birds as definitive hosts than in those using fish, although the magnitude of this effect was not consistent among all parasite groups (Fig. 1). The difference was most pronounced among trematodes, for which larger numbers of species were available for the comparison. Overall, the type of definitive host used by a parasite accounted for much less of the variance in species roles than the random factors included in the models (Table 3).

The taxonomic affiliations of the parasites and the food web they came from, both included as random effects in the mixed models, accounted for a substantial proportion of the variance in the data (Table 3). Web identity explained between 3 and 31% of the variance in the species roles measured. This effect was particularly pronounced for participation coefficients, which showed clearly different frequency distributions among the six food webs (see Fig. 2). Separate regressions involving the most widely represented families (one nematode family and four trematode families; see Table 4) indicate that among related parasites, species roles sometimes covary with food web size, even if only 5–6 points could be included in each regression. For instance, in the family Rencolidae, values for the four centrality measures were generally higher in larger webs (Table 4). However, food web size is clearly not the only aspect of these systems that influence the variance of parasite species roles.

The proportion of variance in species roles accounted for by the four taxonomic levels combined ranged from 37 to 93%. For all six parasite species roles measured, the amount of variance explained by taxonomy clearly exceeded that explained by web identity (Table 3). This indicates a substantial degree of taxonomic (phylogenetic) dependence in these species roles. In particular, most variance was explained by the family to which parasites belonged, and not by higher taxonomic levels (Table 3). There were very clear differences among parasite families in the values of all properties measured here (see examples in Fig. 3), whether or not these came from the same classes or phyla.

Discussion

The inclusion of parasites in food webs alters the structure and complexity of trophic networks (Lafferty *et al.* 2008; Sukhdeo 2010). Previous analyses of the six food

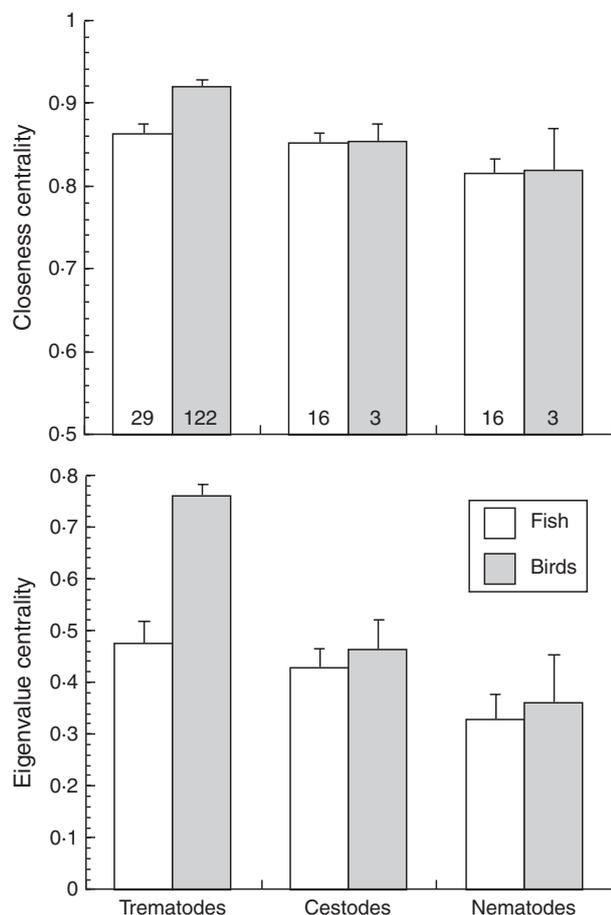


Fig. 1. Mean (\pm SE) values of closeness centrality and eigenvalue centrality for helminth parasites using either fish or birds as definitive hosts in six intertidal food webs. Numbers of species in each group are shown on the bars. The means are derived from rescaled data, that is, centrality measures expressed as proportions of the maximum value observed among helminths in their food web.

webs considered here have confirmed this (Thompson, Mouritsen & Poulin 2005; Lafferty, Dobson & Kuris 2006; Dunne *et al.* 2013). However, these earlier studies investigated only the general effect of parasites on whole-web properties, and not the potential constraints imposed by the identity of parasite species and their phylogenetic affiliations or by other factors on the roles played by each species within the network. Here, we tested different factors that may determine the role and/or network position of parasite species within food webs. First, we found that

the type of definitive host used by adult helminths has a limited impact on the various indices of species roles we investigated. Similarly, we found that the food web from which parasites came also accounted for relatively little of the variance in species roles, despite the six food webs investigated being located in very different geographical areas (Pacific coast of North America, northern Europe, and New Zealand). In contrast, we found that the taxonomic (therefore phylogenetic) affiliation of parasites explained from 37% to a staggering 93% of the variance in their species roles, depending on the particular measure involved. This provides evidence of a strong phylogenetic signal overriding the influence of local factors in determining the centrality of a parasite in a food web and its role in within- and among-module connectedness.

Our modularity analyses with parasites removed from the webs confirmed that parasites themselves are not generating a modular structure in food webs. The same modular patterns were seen whether parasites were included or not; it is with respect to connectivity between modules, and not modularity *per se*, that parasites are important. Parasites, or at least trematodes, were present in all modules identified in all food webs. Almost half of the parasites investigated could be classified as connectors (based on high participation coefficient, P values), compared to only about one-third of free-living species in the same webs. This suggests that parasites are involved in a high number of links between different modules, thus contributing to the cohesion and integrity of food webs. The importance of parasites as connectors is probably mediated to a large extent via concomitant predation links, that is, when a predator consumes the parasites of its prey as well as the latter (Johnson *et al.* 2010). This type of link has been shown to change the motif patterns connecting consumers to their resources in the same intertidal food webs studied here (Dunne *et al.* 2013). When acting as consumers themselves, trophically transmitted parasites feed almost exclusively on host species that form food chains within modules; however, as resources, parasites can be prey for consumers from other modules. A closer look at the Sylt food web, where the trematodes *C. lingua* and *P. atomon* were identified as network hubs, indicates that the low host specificity of these parasites combined with widespread concomitant predation on their various life stages may explain why they play such important roles as within- and among-module connectors. Therefore, we

Table 3. Percentage of variance in different roles of parasite taxa accounted for by either the type of definitive host (fixed effect), the identity of the food web in which they occur (random factor), or four taxonomic levels (nested random factors)

Parasite property	Type of definitive host (%)	Food web identity (%)	Phylum (%)	Class (%)	Order (%)	Family (%)
Node degree	15.5	8.1	25.8	0.9	2.2	35.8
Closeness centrality	12.1	10.5	13.9	1.8	1.9	33.5
Betweenness centrality	2.5	2.7	35.1	0.2	4.3	54.1
Eigenvalue centrality	19.3	3.6	12.1	5.5	3.2	31.1
Within-module degree (z)	2.0	20.3	5.6	4.5	7.8	18.7
Participation coefficient (p)	1.5	31.4	3.9	4.7	2.1	39.5

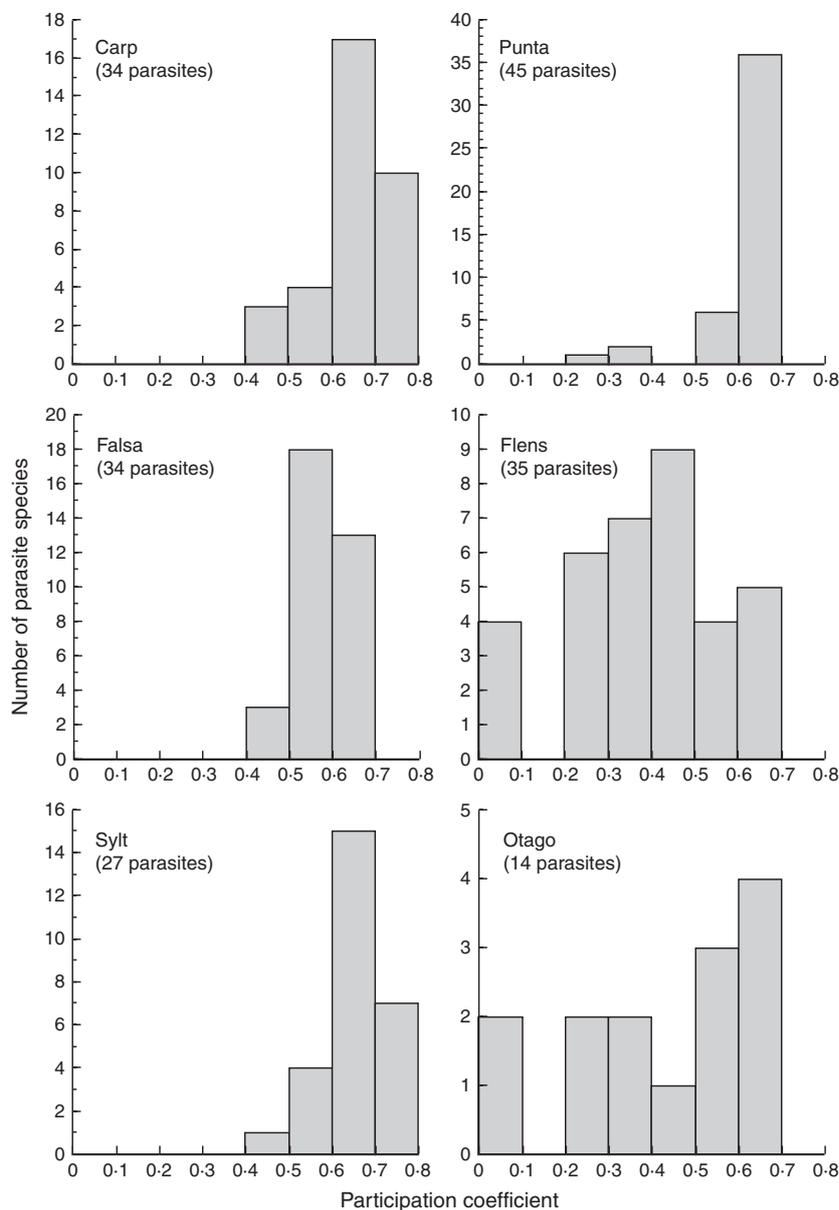


Fig. 2. Frequency distribution of values for the participation coefficient, p , of helminth parasite species in six intertidal food webs.

Table 4. Coefficient of determination (r^2) of regressions between family averages of parasite species roles and food web size, for families represented in either 5 or 6 (shown in parenthesis) of the intertidal food webs

Parasite property	Cystidicolidae (5)	Echinostomatidae (6)	Heterophyidae (6)	Microphallidae (6)	Renicolidae (5)
Node degree	0.339	0.179	0.152	0.508	0.653 [†]
Closeness centrality	0.197	0.120	0.150	0.667*	0.531
Betweenness centrality	0.353	0.397	0.001	0.002	0.889*
Eigenvalue centrality	0.378	0.135	0.177	0.497	0.734 [†]
Within-module degree (z)	0.258	0.009	0.001	0.251	0.018
Participation coefficient (p)	0.038	0.767*	0.517	0.600 [†]	0.416

[†] $P < 0.10$; * $P < 0.05$.

suggest concomitant predation links act to increase connectivity among modules within a food web, providing further evidence of the structural influence of parasites on trophic networks.

At the individual species level, the role of a single parasite species can have impacts on whole-web structure. For

example, in the Otago food web, the microphallid trematode *Maritrema novaezealandensis* ranked in the top two parasite species for node degree, betweenness centrality, closeness centrality and eigenvalue centrality, and third for participation coefficient. In an earlier analysis of a simpler version of the Otago web, Thompson, Mouritsen

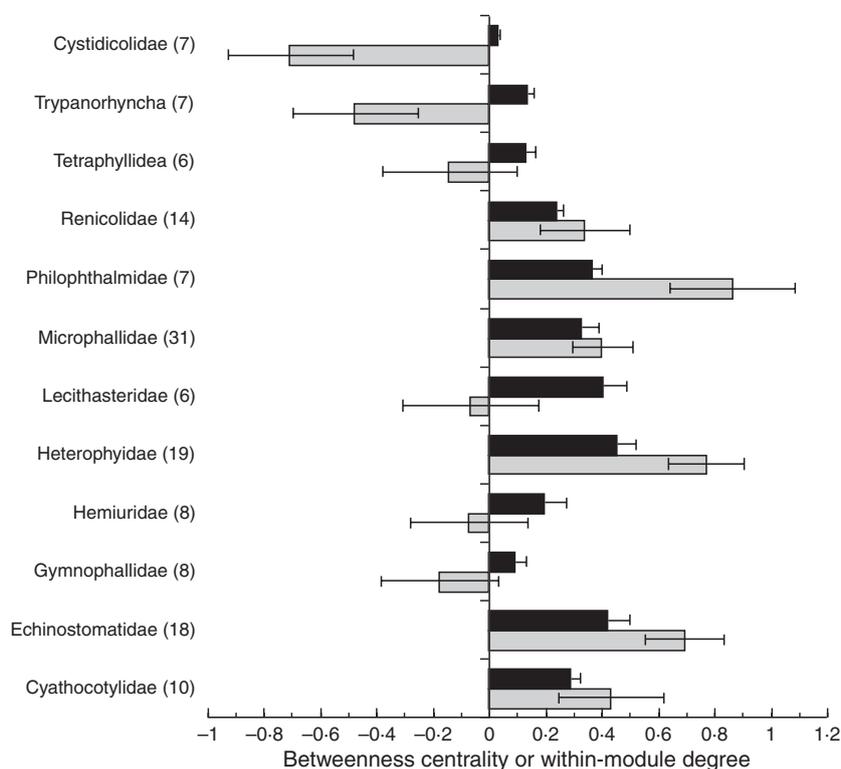


Fig. 3. Mean (\pm SE) betweenness centrality (black bars) and within-module degree, z (grey bars), for helminth parasites averaged across six intertidal food webs. The data are shown for all families (or orders in two cases) represented by at least six species; the numbers of species per family are indicated in parentheses. Cystidicolidae are nematodes, Trypanorhyncha and Tetraphyllidea are cestodes (both orders, not families), and all others are trematodes. Mean betweenness centrality values are derived from rescaled data, that is, they are shown as proportions of the maximum value observed among helminths in their food web.

& Poulin (2005) found that *M. novaezealandensis* had stronger effects on number of links per species and food chain length than any other parasite species in that web. Low host specificity combines with the position of this trematode's hosts within the food web to give it an influential role in web structure and possibly web dynamics, too.

Our analysis focused on the factors that shape and constrain the network roles of parasite species. We found that parasites using bird definitive hosts scored higher for eigenvalue centrality than those using fish, most markedly for trematodes. This suggests that avian trematodes interact with species that are generally better connected within the food web than fish trematodes. The type of definitive host used by a helminth is tied to their phylogenetic position; however, the spread of fish-using taxa and bird-using taxa in the taxonomic hierarchy of the species included in our analysis still allowed the effect of the definitive host type *per se* to be evaluated. Birds generally occupy higher trophic levels than fish, and trophic level can determine the host's biological role within a parasite's life cycle (Poulin & Leung 2011); however, here, we only considered birds and fish as definitive hosts, that is, the end points of trophic transmission. The use of birds instead of fish as definitive hosts can lead to greater parasite gene flow and dispersal among localities (Criscione & Blouin 2004;

Thieltges *et al.* 2009), though not necessarily to broader geographic ranges (Thieltges *et al.* 2011b). Yet, within a local food web, the greater mobility of birds should not impact the network roles of their parasites. Based on the fact that only one of the six different measures of species roles we investigated was influenced by the type of definitive host used, and on the low proportion of variance explained by this factor, we conclude that definitive host type has very little influence on parasite species roles.

Food web identity accounted for a modest amount of the total variance in the six measures of parasite species roles, except for within-module degree, z , and the participation coefficient, p , for which food web identity accounted for 20 and 31% of the variance among parasite species, respectively. For instance, the participation coefficients, which measure the connectedness of a focal species to species from other modules, of parasites in the Otago and Flensburg food webs tend to be lower than those in other food webs (see Fig. 2). We might expect fewer inter-module linkages in the Otago web, which consists of a series of isolated mudflats separated by rocky shores, and in the Flensburg web, which includes a mixture of marine and freshwater taxa, than in the other webs which are either continuous (Sylt) or much smaller in surface area (the three salt marshes from the North American Pacific coast). Other local idiosyncrasies can also affect parasite

species roles. The only local food web property whose effect on species roles could be quantified was food web size, or the total number of species in a web. For some measures of species roles and in some parasite families, web size correlated significantly with family averages. For example, in renicolid trematodes, values for node degree and the three centrality measures were generally higher in larger webs. It is unclear why these trends were only detected in some families and not others, though the number of webs limited the power of these analyses.

By far the most striking result we obtained was the strong phylogenetic signal characterizing all measures of species roles. Depending on the measure considered, from 37 to 93% of the variance among species could be explained by their taxonomic affiliations. Several biological features of parasites are subject to strong phylogenetic constraints. For example, in trematodes the phylum- or order-level identity of the second intermediate and definitive hosts is almost entirely predictable based on their phylogenetic position (Cribb *et al.* 2003). Also, earlier research on trematodes has uncovered distinct phylogenetic niche conservatism of life-history traits (Koehler *et al.* 2012) and host specificity (Mouillot *et al.* 2006). Our results extend this influence of phylogeny to the role played by a species within a food web. Recently, phylogeny has been shown to be the main determinant of several properties of free-living taxa in food webs, namely the number of resources or predators shared by any two species, or their position in smaller subsets of interacting species within the web (Naisbit *et al.* 2012; Stouffer *et al.* 2012). Our results extend the key role of phylogeny to parasites: their roles in food webs are to a large extent predictable based on their phylogenetic position. Thus, among trematodes, heterophyids and echinostomes generally have a high within-module degree of connectedness and high values of centrality measures, whereas hemiurids and gymnophallids generally show the opposite pattern.

The influence of phylogeny on the role played by individual parasite species in food webs probably stems from their phylogenetically conserved life cycle patterns. In trematodes, life cycles typically consist of a first intermediate host, usually a gastropod, from which infective larvae emerge to infect a second intermediate host. The latter may be an invertebrate or vertebrate, depending on the trematode taxon. Finally, predation of the second intermediate host by a vertebrate definitive host allows trophic transmission of the parasite to its last host. Eggs released from the definitive host infect the molluscan host to restart the cycle. The life cycle thus includes three hosts and three transmission events, two via external infective stages (eggs or larvae) and one via predation. Reconstruction of life cycle evolution demonstrates that trematodes originally had two hosts, corresponding to the molluscan first host and the vertebrate definitive host (Cribb *et al.* 2003). The addition of a second intermediate host proceeded separately along several lineages, accounting for the different host taxa now used at this life stage by

different trematodes (Cribb *et al.* 2003). The decoupling of the molluscan and vertebrate hosts resulting from the addition of the second intermediate host creates a range of scenarios in the context of modular food webs, depending on the trematode family involved and the identity of its hosts. For example, in gymnophallids, since both the first and second intermediate hosts are often bivalves and the definitive hosts are generally bivalve-feeding birds like oystercatchers, all hosts frequently belong to the same food chains and the same module. In contrast, in microphallids, the second intermediate host is generally a crustacean, with the definitive host feeding on crustaceans and not on the gastropod first host. Therefore, through phylogenetic conservatism of life cycle patterns, the higher taxon to which a parasite belongs determines its likelihood of being connected to hosts from different food web modules.

Our findings are of course subject to some limitations. For instance, some host-parasite links are missing from the webs investigated here, as the larval stages of some species, particularly cestodes and nematodes, have not yet been found. Also, our analyses are based on matrices characterizing interactions in binary presence-absence format, and not on quantitative webs with data on interaction strengths. Within the same system, the quantitative interaction between a parasite species and its different definitive hosts, measured as prevalence or intensity of infection, can vary widely, even among related host species (Poulin 2005). These inequalities in interaction strength mean that the roles of parasites will need to be re-assessed when complete energy flow networks become available, to uncover the true impact of parasites on web dynamics (Sukhdeo 2010). Nevertheless, our analysis joins a very short list of studies quantifying parasite roles within interaction networks (Bellay *et al.* 2011; Krasnov *et al.* 2012) and is the first to do so within whole food webs. Our findings indicate that parasite species can play greater roles as intermodule connectors, and thus be more important to web cohesion, than free-living species in intertidal food webs. We also demonstrate a strong phylogenetic signal in parasite species roles, which means that the role of any parasite species (in the groups we investigated) is roughly predictable based on its taxonomic affiliations. This may allow inference about the ecological importance of parasites from other intertidal systems, even if based on incomplete food web data, just as it can allow predicting the role of invasive parasite species in food webs. As quantitative webs become available, the next step will be to test the extent to which this phylogenetic determinism also affects energy transfer across species and the strength of links that include parasites.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Species roles for free-living and parasitic taxa in six intertidal food webs.