Host taxonomy constrains the properties of trophic transmission routes for parasites in lake food webs

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Running head: Parasites and trophic links in food webs

Abstract

Some parasites move from one host to another via trophic transmission— the consumption of the parasite (inside its current host) by its future host. Feeding links among free-living species can thus be understood as potential transmission routes for parasites. As these links have different dynamic and structural properties, they may also vary in their effectiveness as trophic transmission routes. That is, some links may be better than others in allowing parasites to complete their complex life cycles. However, not all links are accessible to parasites as most are restricted to a small number of host taxa. This restriction means that differences between links involving host and non-host taxa must be considered when assessing whether transmission routes for parasites have different food web properties than other links. Here we use four New Zealand lake food webs to test whether link properties (contribution of a link to the predator's diet, prey abundance, prey biomass, amount of biomass transferred, centrality, and asymmetry) affect trophic transmission of parasites. Critically, we do this both using models that neglect the taxonomy of free-living species and models that explicitly include information about which free-living species are members of suitable host taxa. Although the best-fit model excluding taxonomic information suggested that transmission routes have different properties than other feeding links, when including taxonomy the best-fit model included only an intercept. This means that the taxonomy of free-living species is a key determinant of parasite transmission routes and that food-web properties of transmission routes are constrained by the properties of host taxa. In particular, many intermediate hosts (prey) attain high biomasses and are involved in highly central links while links connecting intermediate to definitive (predator) hosts tend to be dynamically weak.

Keywords

Introduction

Parasites are increasingly recognized as integral components of ecological communities (Huxham et al., 1996; Lafferty et al., 2006; Dobson et al., 2008; Kuris et al., 2008; Hechinger et al., 2011; Thieltges et al., 2013; Dunne et al., 2013). In some systems, they can reach similar cumulative biomasses to top predators (Kuris et al., 2008), and they often act as prey during their free-living life stages (Thieltges et al., 2013). Parasites can also strongly affect the population dynamics of their hosts (Freedman, 1990; Marcogliese and Cone, 1997) and influence the structure of their communities (Lafferty et al., 2006; Dunne et al., 2013; Cirtwill and Stouffer, 2015). As well as affecting the free-living community, however, many parasites rely on the free-living food web to complete their life cycles. These 'trophically-transmitted' parasites move to a new host when their intermediate host is consumed by an appropriate definitive host. To complete their life cycles, these parasites therefore rely on certain feeding links among free-living species occurring reliably (i.e., frequently enough to allow the parasites to maintain a viable population). Feeding links, however, differ in a number of ways that might affect their suitability as transmission routes. In particular, we might expect that links which are more 'important' to the structure and/or functioning of the food web might occur more reliably than other links. These important links might therefore be 'safer bets' for parasites and more likely to serve as viable transmission routes. There are, however, a variety of ways that the importance of a link can be measured, each of which could be expected to impact parasites for different reasons.

A link might be important because of its *dynamic properties*— its contribution to the

flow of energy and biomass through the food web and, by extension, to the maintenance of free-living populations. Three dynamic properties in particular seem likely to influence the suitability of links as transmission routes. First, we might expect that a link which contributes a particularly large proportion of a predator's diet might be more likely to occur and therefore be more effective as part of a transmission route than links which contribute less to the diet of the predator. This is especially true for definitive hosts, which often experience only minor effects from infections. Because the cost of infection is low and infected prey are often easier to catch and kill, these hosts have little incentive to avoid consuming infected prey (Lafferty, 1992).

Second, parasites might instead tend to be transmitted along links involving highly abundant prey, regardless of the contributions these prey make to the diets of definitive hosts (Canard et al., 2014). Neutral theory suggests that more abundant prey are more likely to encounter and be infected by parasites (Canard et al., 2014) and are more likely to be encountered and consumed by predators (Abrams and Ginzburg, 2000; Wootton, 2005). Abundant prey may also represent a more productive niche that can be exploited by more parasite species (Thompson et al., 2013). Of course, infecting highly abundant prey means that the parasite will often be consumed by predators which are not viable definitive hosts and die. Such losses may be worthwhile, however, if the parasite can still infect its definitive host more frequently than if the parasite had a different life history (Poulin, 2010). Note that while abundant prey can be major contributors to predators' diets as described above, this may not be the case for all predators as some species have strong preferences for particular prey. The contribution of a link to the predator's diet and the abundance of the prey involved therefore provide complementary information about the

impact of a link on the food web.

Third, parasites' transmission routes might not be strongly affected by either the abundance of the prey or the contribution of the link to the predator's diet. Instead, parasites might "go with the flow" and tend to be transmitted along links which transfer a large amount of biomass (Thompson et al., 2013). These energetic "highways" might involve abundant prey, but they could equally involve rare but large prey. Similarly, links which contribute large proportions of the predator's diet may or may not transfer large amounts of biomass in the absolute sense, depending on the size of the predator population and the amount each animal consumes. Whatever the case may be, links which transfer large amounts of biomass represent major flows of energy and nutrients through a community and may therefore occur more reliably than other links (Thompson et al., 2013).

As well as their dynamic properties, links might vary in their suitability as transmission routes because of their *structural properties*— the ways in which links contribute to the structure of the food web. In particular, it has been suggested that parasite life cycles should include transmission along links which are downwardly asymmetric— those in which a generalist predator consumes a prey which has few other predators (Rossiter and Sukhdeo, 2011; McQuaid and Britton, 2013). Such links are likely transmission routes for several reasons. First, a generalist predator is unlikely to have a dramatic impact on any one prey, and is therefore unlikely to cause the extinction of the parasite's intermediate host (Rossiter and Sukhdeo, 2011). Second, the populations of generalist predators may be less variable over time, ensuring a steady supply of hosts for the parasite (Inouye, 1980; Hopkins et al., 2002). Third, by infecting a prey which has few predators, the parasite reduces the loss of propagules consumed by non-host predators (Rossiter and Sukhdeo,

2011; McQuaid and Britton, 2013). Since asymmetric links should provide relatively stable transmission routes with minimal losses, we expect that parasites will be transmitted along such links more often than along less asymmetric links (Rossiter and Sukhdeo, 2011; McQuaid and Britton, 2013).

As well as asymmetry, trophic transmission could be affected by the "centrality" of a link– the number of shortest paths between pairs of species in which it participates (Newman, 2010). A highly central link affects, and is affected by, many species and is therefore more likely to be reached by parasites than a less-central link (Jordán et al., 2007; Lai et al., 2012). Moreover, in order for a link to be highly central, one or both of the *species* involved must be highly central (i.e., be part of many shortest paths). Highly central species tend to host more parasite species than other free-living species (Chen et al., 2008; Thompson et al., 2013), and highly central hosts tend to be particularly important for parasite transmission (Chen et al., 2008). We expect that what is true for central species will also be true for central links, and transmission will be more likely to occur along central links.

Parasites are not always free to follow the best possible transmission route, however, as each parasite is generally limited to hosts from certain taxonomic groups at each life stage (i.e., Combes' compatibility filter [Combes, 2001]). For example, most trematodes use mollusks as hosts for their first parasitic life stage while acanthocephalans always use arthropods as their intermediate host. Previous analyses of parasites' transmission routes have not taken these restrictions into account (e.g., Chen et al., 2008; Rossiter and Sukhdeo, 2011; Thompson et al., 2013), meaning it is possible that parasites tend to infect highly-connected species largely because of the taxonomy of these highly-connected species

6

rather than because these hosts best allow parasites to complete their life cycles. When testing for effects of the properties of feeding links on the potential for these links to transmit parasites, it is therefore essential to control for the potential influence of the taxonomy of free-living species.

Here we test whether links that form part of parasite life cycles (i.e., transmission links) are associated with food-web properties that facilitate transmission, or are more strongly constrained by host taxonomy. Specifically, we expect that links which transmit parasites would (i) contribute larger proportions of predators' diets, (ii) involve more abundant prey, (iii) transfer more biomass, (iv) be more downwardly asymmetric than other links, and (v) be more central than other links, but that any of these associations could be due to the restriction of parasites to particular host taxa. We test each of the above hypotheses using both a set of taxonomically naive models and more conservative, taxonomically informed models that explicitly incorporates the effects of the host specificity of parasites. Comparing these two sets of models allows us to infer the influence of taxonomy on the effect of each property. We note that this is far from an exhaustive list of the link properties which could influence the trophic transmission of parasites. These properties were chosen because they are both likely to affect trophic transmission and could be explicitly tested using our dataset.

Methods

Dataset

We constructed food webs describing the free-living communities of four lakes in the South Island of New Zealand: Lake Hayes (44°58′59.4”S, 168°48′19.8”E), Lake Tuakitoto (46°13′42.5”S, 169°49′29.2”E), Lake Waihola (46°01′14.1”S, 170°05′05.8”E), and Tomahawk Lagoon (45°54′06.0”S, 170°33′02.2”E). To capture the seasonal variation in each community, we constructed three separate food webs describing each community in September 2012, January 2013, and May 2013 (austral seasons: early spring, mid-summer, and late autumn). Our dataset thus consisted of 12 food webs in total (see Table 1 for details). Together, these webs included 2160 links between 110 free-living species, as well as the abundances and densities of all species in each lake. These webs also included quantitative information about the strength of each link (see below).

As well as free-living species, the lake communities also contained 47 parasite life stages, 20 of which were trophically transmitted. Most of these trophically transmitted life stages used fish, amphipods, or mollusks as intermediate (prey) hosts, and all used birds or fish as definitive hosts (see Table *S2, Appendix S1: Section S1* for details). For a detailed description of sampling methods and reconstruction of feeding links, see *Appendix S1: Section S1* and Lagrue and Poulin (2015).

Dynamic and structural properties of links

After assembling the networks, we calculated dynamic and structural properties of each link in order to test whether any of these properties were related to the ability of a parasite

8

to complete its life cycle. We chose properties that would provide indirect measures of a link's importance to free-living species; we did not have access to data such as empirical feeding rates that would provide a more direct measure of importance. To test whether parasites tend to be transmitted along links that contribute large proportions of predators' diets, we defined the contribution of each link based on the proportion of the predator's diet accounted for by that link. For most species, these proportions were determined by gut contents. We note that this approach can underweight the proportion of a predator's diet that is made up by prey which are digested rapidly and does not capture variation in species' diets over time. Nevertheless, gut contents do provide a valuable snapshot of species' diets as it would be extremely difficult to directly observe the majority of feeding interactions among aquatic species in their natural habitat. For birds, which could not be sampled, the contribution of each link to the predator's diet was taken from published information about diets (see *Appendix S1: Section S1* for details). For this and other properties, we took the average across all individuals in a species within the same lake and sampling period. Note that a link which makes a large contribution to the predator's diet might represent either rare but large meals or frequent, small meals. Because the networks in our dataset were based on gut contents rather than direct observation of interactions, we did not have information about interaction frequencies that would allow us to tease these two possibilities apart. We were able to determine the contribution to predator's diet in all 2160 links in our dataset.

We also expected that parasites might tend to be transmitted along links involving highly-abundant prey. These links might make large contributions to the predators' diets as described above, but if predators have strong preferences for certain rare prey then

abundant species might contribute relatively little to their diets. We therefore tested the relationship between prey abundance and transmission as well as that between contribution to diet and transmission. We defined abundance as the number of prey individuals per m^2 of surface in each lake (see *Appendix S1: Section S1* for details). For some resources, such as terrestrial insects which occasionally fall into the lakes, we were unable to reliably estimate the standing local abundance and so we removed these links (see *Appendix S1: Section S1* for details). This left us with 1464 links. Because encounter and consumption rates might depend on the biomass of the prey rather than its abundance, we also calculated the total biomass of the prey in each link. We defined prey biomass as the estimated mass of the prey species per m^2 of surface in each lake. As with abundance, we were unable to reliably estimate the standing local biomass of some species and removed these links from the analysis. This left us with 1627 links.

As another alternative, it is possible that parasites "go with the flow" and tend to be transmitted along links that transfer large amounts of biomass. These links may make large contributions to predators' diets and involve abundant prey, but this depends on the total amount of biomass the predator consumes and the size of each individual prey. We therefore tested the relationship between the amount of biomass transferred along a link and its outcome for parasites in addition to the other relationships described above. We estimated the biomass transfer ω_{ilm} for each link i in lake l during sampling period m as

$$\omega_{ilm} \approx \alpha_{lm} \mu_{lm}^{3/4} \rho_{ilm}, \tag{1}$$

where μ_{lm} is the mean biomass of an individual predator in lake l during sampling period

10

m, α_{lm} is the abundance of the predator in the same sample, and ρ_{ilm} is the proportion of the predator's diet contributed by interaction i in lake l during sampling period m. Following Brose et al. (2008), we used a scaling factor of 3/4 to account for efficiencies of scale in larger species. As biomass transfer, so defined, depends on the predator's diet and local biomass but not on the prey's local biomass or abundance, we were able to estimate the amount of biomass transfer for all 2160 links. We note that calculating biomass transfers using allometric relationships disregards differences in the consumption rates of different species; however, without detailed information about the total biomass consumed by each predator in our dataset, we consider this to be a reasonable approximation.

As well as the above properties, parasite transmission might be affected by the asymmetry of the impact of a link on the predator and prey. To measure this asymmetry, we subtracted the degree of the predator from that of the prey and normalized this difference by the total number of links in the web. That is, we define the asymmetry A_{ij} of a link between species i and j using the equation (Rossiter and Sukhdeo (2011)),

$$A_{ij} = \frac{I_j - I_i}{I_t}, \tag{2}$$

where I_i is the number of prey consumed by the predator, I_j is the number of predators that consume the prey, and I_t the total number of links in the web. A_{ij} is 0 when the link is symmetric (i.e., the predator and prey participate in equal number of links), positive when the prey is involved in more links, and negative when the predator is involved in more links. We expected that links resulting in the transmission of a parasite would have more negative values of A_{ij} (be more downwardly asymmetric). We were able to determine

the asymmetry for all 2160 links.

Finally, because the suitability of a link as a transmission route might depend on its structural importance as well as its role in the food web's dynamics, we tested whether or not the centrality of a link affected its outcome for parasites. To do this, we calculated the "betweenness centrality" of each link. This measure represents the frequency with which a given link lies on the shortest paths between pairs of species (Newman, 2010) and may be calculated using weighted (e.g., by the amount of biomass transferred) or unweighted links. In order to focus on network topology and avoid including information already captured by the dynamic properties described above, we calculated centrality using unweighted links. This approach also echoes the definition of asymmetry in Rossiter and Sukhdeo (2011).

Note that it is possible that some of these dynamic and structural properties capture similar information about a link's importance to the free-living species involved. To account for this possibility, we tested for pairwise correlations among all six properties. We did this using the R (R Core Development Team, 2015) function cor.test from the stats package (R Core Development Team, 2015). In all tests, we considered only the 1463 links for which all properties could be calculated.

Outcomes of links for parasites

Next, we categorized the outcomes of feeding links for each life stage of each parasite species (hereafter "parasite life stage"). As a given link might transmit one life stage while killing another stage of the same species, we performed all of our analyses at the life-stage level. We therefore expanded our dataset by cross-referencing the l links included in each

food web with the p parasite life stages observed in that web, resulting in an $l \times p$ table of feeding links and their outcomes for each lake-season combination (42,022 combinations). Note that the outcome of a given link for a given parasite life stage was assumed to be the same in all lakes and sampling periods in which both the link and the parasite were observed. That is, if a focal parasite life stage was observed in one individual of a free-living species, that species was considered to be a viable host in all of the webs in our dataset. In our analyses, we used only those link-parasite combinations for which all six properties could be calculated (N=28,784).

A link was categorized as a "transmission" link if 1) the focal parasite life stage was known to be trophically transmitted and 2) the predator and prey in the link were observed as hosts for the focal parasite life stage and the next stage in the parasite life cycle (Fig. 1). If the prey was a host for the focal parasite life stage but the parasite life stage could not be trophically transmitted, or if the predator was not a host for the next stage in the parasite life cycle, then the link was categorized as a "loss". This includes cases where the parasite life stage is digested by the predator along with its host (e.g. trematode sporocysts inside a snail host that is eaten by a fish), and other cases where the parasite life stage is killed as an indigestible cyst (e.g. some encysted trematode metacercariae when their second intermediate host is eaten by an unsuitable predator). In rare cases, the parasite may sometimes be able to reproduce by selfing at an earlier life stage (e.g., trematode metacercariae achieving progenesis in their second intermediate host; Poulin and Cribb, 2002). Nevertheless, these parasites should still be under selection to complete their normal life cycles and reproduce sexually. We therefore assumed that completing its full life cycle is the best option for the parasite and, for the two parasites

that may be capable of progenesis in our dataset, categorize links that lead to the normal definitive hosts as "transmission" and links leading to other predators as "loss" even if the parasite can reproduce in an earlier host. The remaining links, where the prey was not a host for the focal parasite life stage, were categorized as "unused". These links should not have any impact on the parasite unless they affect other life stages of the same species.

Throughout our analyses we treated these outcomes as distinct categories. We note that this ignores the possibility that the proportion of parasites in an intermediate host that can infect the predator may vary among the links. For example, some predators may process their prey (e.g., by chewing) more thoroughly and thereby kill more parasites than one which consumes the same prey relatively whole. Alternatively, some predators may simply be more susceptible to infection than other suitable hosts. In either case, parasites may exist at different intensities in different hosts, and changes in intensity of infection between predator and prey could be used to infer continuous values for parasite transmission. However, as neither loss nor unused links *ever* result in the infection of the predator and the completion of the parasite life cycle, treating transmission as a continuous variable would obscure the difference between these two outcomes— a result we chose to avoid.

Outcomes of links as a function of link properties and taxonomy

We first wished to test whether different link properties were associated with the ability of parasites to complete their life cycles (i.e., whether link properties were associated with different outcomes for parasites). To do this, we fit a multinomial logistic regression of outcome against all six properties (contribution to predator's diet, prey abundance, prey

14

biomass, biomass transfer, asymmetry, and centrality) using the R (R Core Development Team, 2015) package MCMCglmm (Hadfield, 2010). To obtain a more normal distribution of the predictors, we log-transformed prey abundance, prey biomass, biomass transfer, and centrality. Contribution to predator's diet and asymmetry were not transformed. As well as the six measures of link importance, we included random intercept effects for lake and season. To account for the fact that different parasite life stages have different numbers of links that result in each outcome, we also included a random intercept effect of parasite life stage. To improve model fitting, we centered and scaled all predictors using the R (R Core Development Team, 2015) base function scale. When fitting the model, we used a burnin period of 5000 iterations and ran the model for 50000 iterations total.

After fitting this "full" model and obtaining its DIC score, we then fit the suite of 62 simplified models that could be obtained by systematically removing all possible combinations of terms from the full model (see *Appendix S1: Section S2* for a list of models). DIC is a Bayesian generalization of AIC and was chosen as the model selection criterion because of its compatibility with the MCMCglmm approach. In all models we considered unused links to be the baseline outcome and all predictors were centered and scaled using the R (R Core Development Team, 2015) base function scale. We also fit a null model containing only an intercept and the random effects.

We expected that any trends we observe could be due to the restriction of parasites to particular host taxa. That is, links transferring large amounts of biomass, for example, could be associated with parasite transmission because these links are important to the free-living species involved, as described above, or because these links involve species which are taxonomically suitable hosts for the parasites. As noted previously, parasites are often

restricted to hosts from a particular taxonomic group (see Table *S2, Appendix S1: Section S1* for restrictions in this dataset). We were therefore able to identify "potential transmission" links, where the prey was a potential intermediate host of the parasite and the predator was a potential definitive host based on the taxonomy of known intermediate hosts. Similarly, we identified "potential loss" links where the prey was a potential intermediate host for the parasite but the predator was not a potential definitive host. Finally, we identified "potential unused" links as those where the prey was not a potential intermediate host regardless of the taxonomy of the predator.

We used relatively broad taxonomic groups (families or classes) when identifying the potential outcomes for links. As the New Zealand lakes in our dataset are relatively species-poor, using a high-resolution taxonomic model (e.g., to the genus level) could restrict the pool of potential hosts to only those in which parasites had already been observed. This would effectively over-specify the models such that the taxonomies of free-living species could completely explain the outcomes of links for parasites, rendering the second set of models useless. For those parasites that relied upon insect hosts, we considered only aquatic insects to be valid potential hosts (although there may be parasites in some systems that infect both terrestrial insects and fish, our dataset did not contain any such parasites and hence transmission could only occur between aquatic insects and their consumers). After determining whether each link was a "potential loss", "potential transmission", or "potential unused" from the perspective of each parasite, we replaced the random effect of parasite life stage with a random intercept effect of the interaction between potential outcome and parasite life stage. This effect thereby accounts both for the different numbers of observed transmission, loss, and unused links for different parasite

16

life stages and for the different numbers of links that could potentially have each outcome based on the host taxa used by the parasite. By comparing the terms retained in these taxonomically-informed models with those in the naive models, we could infer which link traits were tightly correlated with the taxonomies of the free-living species involved and which had effects on trophic transmission above and beyond taxonomic effects.

Results

Correlations between predictors

As we expected, there were significant correlations among many of the properties we investigated (Figs. 2, 3, and *S2, Appendix S1: Section S3*). These correlations, however, tended to be both weak and potentially non-linear. Including several of these properties in the same linear model is therefore likely to represent only limited amounts of redundant information.

Outcomes & link properties

We first tested whether feeding links that form part of parasite life cycles had different properties than those which did not, then tested whether these associations persisted after taking account of the fact that parasites can only infect hosts from a few suitable taxa. Our first set of analyses did not include any information on whether the prey and/or predator in a feeding link were suitable hosts for the focal parasite. The best-fitting of these taxonomically-naive models (DIC=8725) included terms for the log of prey biomass,

the log of prey abundance, the log of biomass transfer, asymmetry, and the log of centrality (Table 2). All other models provided a significantly worse fit to the data (Δ_{DIC}>100).

In the best-fitting taxonomically naive model, transmission and loss links both occurred with much lower probabilities than unused links (β=-10.3, p<0.001 and β=-9.19, p<0.001). Relative to the probability that a link would not affect a parasite, both transmission and loss were more likely along links with higher prey biomasses (β=13.9, p<0.001 and β=16.6, p<0.001, respectively), more central links (β=2.31, p<0.001 and β=2.40, p<0.001, respectively), and links with higher positive asymmetry (β=0.214, p<0.001 and β=0.126, p<0.001, respectively). Loss was also more likely along links involving very abundant prey and links which transferred large amounts of biomass (β=0.619, p=0.052 and β=1.06, p<0.001, respectively), while transmission was less likely as prey abundance and amount of biomass transferred increased (β=-0.766, p=0.038 and β=-2.08, p<0.001). Prey biomass and centrality had much larger effects on the probability of loss and transmission than any other properties (Fig. 4). Prey abundance, on the other hand, is unlikely to substantially affect the probability of transmission or loss as the 95% confidence intervals for this property included zero.

After establishing that feeding links that form part of parasite life cycles do tend to have different food-web properties than other links, we then tested the possibility that these trends might be due to the constraints imposed by the restriction of parasites to specific host taxa. Imposing a correlation structure that took into account whether the predator and/or prey in a feeding link belonged to suitable host taxa dramatically improved the fits of the models we considered (Table *S3, Appendix S1: Section S2*). This suggests that taxonomic constraints on the hosts parasites can infect are stronger

18

determinants of which links are included in parasite life cycles than the food-web properties we investigated here. This possibility is supported by the fact that the best-fitting taxonomically-informed model (DIC=617) was the null model, including only an intercept for each outcome (Table 3). In this model, as in the best-fitting naive model, transmission and loss links occurred with much lower probabilities than unused links (β=-8.86, p<0.001 and β=-8.38, p<0.001, respectively). All other models provided a significantly worse fit to the data (δ_{DIC}>2), meaning that including link properties as well as information on host taxonomies adds very little information to the model. This suggests that most of the association between link properties and outcomes for parasites the we observed can be explained by variation between links involving different free-living taxa.

Only two models including host specificity had Δ_{DIC}<10 relative to the null model. The second-best model (DIC=620) included terms for the contribution of the link to the predator's diet, the log of prey abundance, and asymmetry (Table *S4, Appendix S1: Section S4*). The probability of transmission increased with increasing prey abundance (β=0.176, p=0.842) and decreased with increasing contribution to predator's diet and asymmetry (β=-0.034, p=0.433 and β=-0.058, p=0.562, respectively) while the probability of loss showed the opposite trends (β=-0.437, p=0.600; β=0.046, p=0.550; and β=0.177, p=0.028, respectively). The third-best model (DIC=626) included terms for the contribution of the link to the predator's diet, the log of biomass transfer, and the log of centrality (Table *S5, Appendix S1: Section S4*). In this model, the probability of transmission increased with the log of centrality (β=1.20, p=0.068) and decreased with the contribution of the link to the predator's diet and the log of biomass transfer (β=-0.007, p=0.831 and β=-0.656, p=0.898, respectively) while the probability of loss increased with

19

all three properties (β=1.25, p<0.001; β=0.049, p=0.143; and β=1.04, p=0.126, respectively). These effects were generally quite small and, in almost all cases, the 95% confidence intervals for the effects included 0.

Discussion

Our results demonstrate that the properties of feeding links between free-living species are associated with the ability of parasites to complete their life cycles along these links but that many of the apparent effects of link traits may actually be due to the restriction of parasites to hosts from particular higher taxa. Without taking host specificity into account, we found that a model including all properties except the contribution of a link to the predator's diet gave the most parsimonious description of the outcomes of links for parasites. Despite the fact that these are all indirect measures of the importance of a feeding link, this result clearly shows that the properties of links between free-living species are associated with the consequences of these links for parasites. Prey biomass had the largest effect, with the probabilities of both loss and transmission increasing with increasing biomass. This suggests that free-living species used as intermediate hosts by parasites tend to have high local biomasses, as we expected. These prey may be encountered and infected and/or consumed more frequently than other species (Wootton, 2005; Canard et al., 2014), thereby reliably transmitting parasites to their definitive hosts. Note that this result is based on standing-stock biomasses and not on the total production of each species. Total production may be lower for large, long-lived free-living species and could have different effects on trophic transmission. While testing for such differences is

beyond the scope of the present study, our results when including taxonomic information suggest a more parsimonious explanation. After accounting for the fact that parasites can only infect certain host taxa (e.g., all of the trophically-transmitted acanthocephalans in our dataset used small crustaceans as intermediate hosts, and none of the parasites in our dataset infected plants), prey biomass did not vary between links with different outcomes for parasites. It is therefore very likely that parasites tend to use species with high biomasses as intermediate hosts because of they belong to suitable taxa rather than because of differences in encounter probabilities. This is particularly likely for links involving fish prey, as fish were the most common intermediate host taxon in our dataset (6 life stages) as well as being some of the largest prey items.

Parasitizing host taxa with large standing-stock biomasses entails substantial losses of propagules through consumption by inappropriate predators (Mouritsen and Poulin, 2003; Thieltges et al., 2013). As demonstrated by the many free-living species which employ similar strategies (including such diverse taxa as sea turtles, trees, and frogs [Wilbur et al., 1974]), the loss of most propagules/offspring may be worthwhile as long as a few survive to maturity. These arguments also hold for our finding that both transmission and loss increased with increasing centrality. The transmission of some propagules along central links is enough to maintain parasite populations despite the loss of many other propagules along similarly central links. As with prey biomass, the fact that these relationships were not significant after accounting for taxonomic constraints suggests a taxonomic basis for the trends we observe. Parasites may tend to infect prey that are involved in highly central links because these prey are members of suitable host taxa (generally fish, amphipods, or mollusks in our dataset) rather than because highly central links make good transmission

routes. Similarly, earlier findings that highly central free-living species play host to more parasites (Chen et al., 2008), as do free-living species that have many links to other species (Thompson et al., 2013), could be due to host taxa tending to have similar network properties rather than because of any direct effect of host network properties on parasites.

Our results for asymmetry were less intuitive and are contrary to the predictions of earlier work (Rossiter and Sukhdeo, 2011; McQuaid and Britton, 2013) which argues that transmission should be more likely along links with downward asymmetry (i.e., links involving prey with few predators and predators with many prey) as such links should deliver parasites to their definitive hosts with minimum losses (Rossiter and Sukhdeo, 2011; McQuaid and Britton, 2013). In our dataset, prey hosts tended to be involved in more downwardly asymmetric links (i.e., on average they had few predators which in turn had many other prey). Although infecting such hosts implies that parasites will lose many propagules when prey are consumed by non-host predators, the fact that the predators in such links have few other prey may nevertheless ensure that enough propagules do reach definitive host predators such that parasites can complete their life cycles. As with prey biomass, however, it is also likely that these counterintuitive results are a product of differences between taxa and the restriction of parasites to hosts in certain groups. In our dataset, most trophically transmitted life stages used fish, amphipods, or mollusks as intermediate hosts. While it is not obvious why these taxa tend to have few, generalist predators, this may be an interesting question for future research.

The above results are easily interpreted with regard to the intermediate hosts parasites infect, but provide relatively little information on which interactions involving these intermediate hosts tend to lead to transmission. Our results for biomass transfer and prey

abundance, however, have more direct implications for trophic transmission. Contrary to our expectations, prey hosts involved in transmission links tended to have low abundances and links transmitting parasites between hosts tended to transfer little biomass. Combined with our result for prey biomass, this result suggests a taxonomic basis for earlier work showing that while parasites may "go with the flow" and enter food chains which transmit large amounts of biomass, they are unlikely to be transmitted to their definitive hosts along such chains (Thompson et al., 2013). Instead, prey hosts tended to be relatively rare and the feeding links connecting them to definitive hosts tended to be dynamically weak, transferring little biomass. Intriguingly, such weak links have been touted as critical for maintaining a community's ability to withstand perturbations (McCann et al., 1998; Emmerson and Yearsley, 2004; Banašek-Richter et al., 2009). Where weak links are paired with strong ones, perturbations to the community tend to dissipate. This reduces the likelihood of a permanent change to the system, stabilizing it (McCann et al., 1998; Wootton and Stouffer, 2016). Due to their complex life cycles and dependence on specific hosts, parasites may be unusually vulnerable to perturbations to their communities (Lafferty and Kuris, 2009). Transmission along dynamically weak links could reduce the chances that parasites exacerbate the effects of environmental perturbations on their hosts, reducing the risk of a host population becoming too small to sustain the parasite.

Despite the strong association between link traits and outcomes for parasites that we observed, our analyses demonstrate that, to truly understand trophic transmission of parasites, host specificity *must* be taken into account. Moreover, our results suggest that where parasites' host taxa are known— at least to the class level —this information will be

more useful than link traits when predicting transmission routes. Where traits are more likely to be of use is when investigating *which* species or genera in a suitable class are used as hosts. As most of the parasites in our dataset are relatively generalist and the number of potential hosts is small, however, testing this possibility falls outside the scope of the present study. This may nevertheless be a rewarding line of inquiry for researchers working in more species-rich systems.

Supporting information

Appendix S1: Detailed methods and results

Section S1: Detailed methods for data collection

Section S2: List of models considered

Section S3: Correlation between asymmetry and centrality

Section S4: Supplemental model summaries

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Tables

Table 1: Sizes of the 12 food webs considered in this dataset. For each lake during each season, we give: A) the numbers of free-living species and links between them and B) the numbers of parasite life stages and link-parasite life stage combinations that could result in trophic transmission (Transmission), that would result in the loss of the parasite life stage (Loss), and that did not affect the parasite life stage (Unused).

A) Free-living food web			
Lake	Season	Free-living species	Links
Hayes	Summer	79	159
Hayes	Autumn	76	146
Hayes	Spring	95	184
Tomahawk	Summer	81	183
Tomahawk	Autumn	77	176
Tomahawk	Spring	90	208
Tuakitoto	Summer	91	301
Tuakitoto	Autumn	81	182
Tuakitoto	Spring	87	245
Waihola	Summer	94	238
Waihola	Autumn	83	206
Waihola	Spring	89	226

B) Parasite community					
Lake	Season	Parasites	Transmission	Loss	Unused
Hayes	Summer	12	38 (38)	64 (64)	1602 (918)
Hayes	Autumn	16	34 (34)	74 (74)	1956 (1956)
Hayes	Spring	17	68 (68)	111 (111)	2643 (1470)
Tomahawk	Summer	20	31 (31)	166 (166)	2943 (1923)
Tomahawk	Autumn	17	27 (27)	141 (141)	2484 (1634)
Tomahawk	Spring	24	38 (38)	210 (210)	4048 (2632)
Tuakitoto	Summer	27	52 (52)	128 (128)	5159 (3962)
Tuakitoto	Autumn	27	32 (32)	79 (79)	2213 (1233)
Tuakitoto	Spring	27	47 (47)	116 (116)	4217 (2977)
Waihola	Summer	47	56 (56)	210 (210)	5505 (3910)
Waihola	Autumn	47	64 (64)	140 (140)	3102 (2019)
Waihola	Spring	47	58 (58)	194 (194)	3972 (2740)

Note that, because the same link between free-living species could have different consequences for different parasite life stages, the number of links in B is greater than that in A. In B, values in parentheses are link-parasite life stage combinations left after removing links for which prey abundance or biomass could not be estimated (see Methods). Our sample size (N=28,784) is the sum these parenthetical values.

Table 2: Posterior means and 95% confidence intervals of parameter estimates for fixed effects in the best-fitting model which ignored parasite host-specificity. Note that all predictors were centered and scaled before fitting the model. The values presented here refer to the un-scaled data. This model also contained random effects for lake, season, and parasite life stage.

Parameter	Loss			Transmission		
	Mean	95% CI	pMCMC	Mean	95% CI	pMCMC
Intercept	-9.19	(-11.8, -5.78)	<0.001	-10.3	(-13.5, -6.67)	<0.001
log(Prey biomass)	16.6	(10.8, 21.4)	<0.001	13.9	(7.15, 19.7)	<0.001
log(Prey abundance)	0.619	(-0.014, 1.37)	0.052	-0.766	(-1.32, 0.027)	0.038
log(Biomass transfer)	1.06	(0.541, 1.63)	<0.001	-2.08	(-3.00, -1.32)	<0.001
Asymmetry	0.126	(0.065, 0.188)	<0.001	0.214	(0.130, 0.283)	<0.001
log(Centrality)	2.40	(1.61, 2.95)	<0.001	2.31	(1.32, 3.05)	<0.001

Table 3: Posterior means and 95% confidence intervals of parameter estimates in the best-fitting model which incorporated parasite host-specificity. Note that this model contained only the intercept and random effects for lake, season, parasite life stage, and the set of potential outcomes for each link.

Parameter	Loss			Transmission		
	Mean	95% CI	pMCMC	Mean	95% CI	pMCMC
Intercept	-8.38	(-10.4, -6.41)	<0.001	-8.86	(-10.8, -6.61)	<0.001

Figures

Figure 1: The small subset of species represented here (taken from the dataset used in this study) is used to illustrate the different outcomes of feeding links for parasites. *Maritrema poulini* uses amphipods and *Stegodexamene anguillae* uses small fish as intermediate host prey. These parasites are transmitted to their respective definitive hosts along specific trophic links (predator-prey links). Each trophic link may transmit the parasite to the appropriate definitive host ("transmission" link), the parasite may be consumed by a non-host predator and killed ("loss" link), or the parasite may not be affected by the link ("unused" link). *Maritrema poulini* only uses birds as definitive hosts and is killed (as indicated by the pale, crossed-out symbol) when its amphipod host is consumed by a fish ("loss" link; link 1). For *M. poulini*, "transmission" is only achieved through link 2. *Stegodexamene anguillae* does not infect amphipods and thus trophic links including amphipods as prey are "unused" by this parasite (links 1 and 2). For *S. anguillae*, link 3 is a "loss" link while link 4 is the appropriate "transmission" link to eel definitive hosts; links 3 and 4 are "unused" by *M. poulini*.
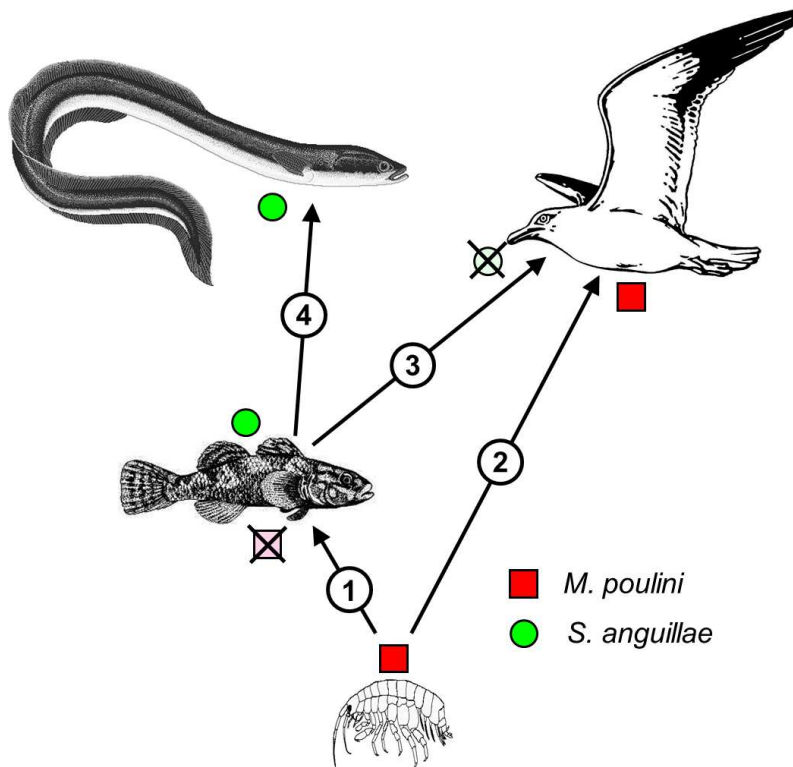
Figure 2: Correlations among dynamic properties of links. The contribution of a feeding link to the predator's diet was not correlated with the log of prey abundance but was positively correlated with the log of prey biomass and the log of biomass transferred. The log of prey abundance, the log of prey biomass, and the log of biomass transfer were all significantly positively correlated with each other. For each pair of properties, we give the R^2 and p-value for the correlation. We also show the means (± 2 SE; points) of one property for 20 bins of the other (sizes of bins vary depending on the regression). Note that, due to the distribution of values for the predictors, not all bins contained observed data points.
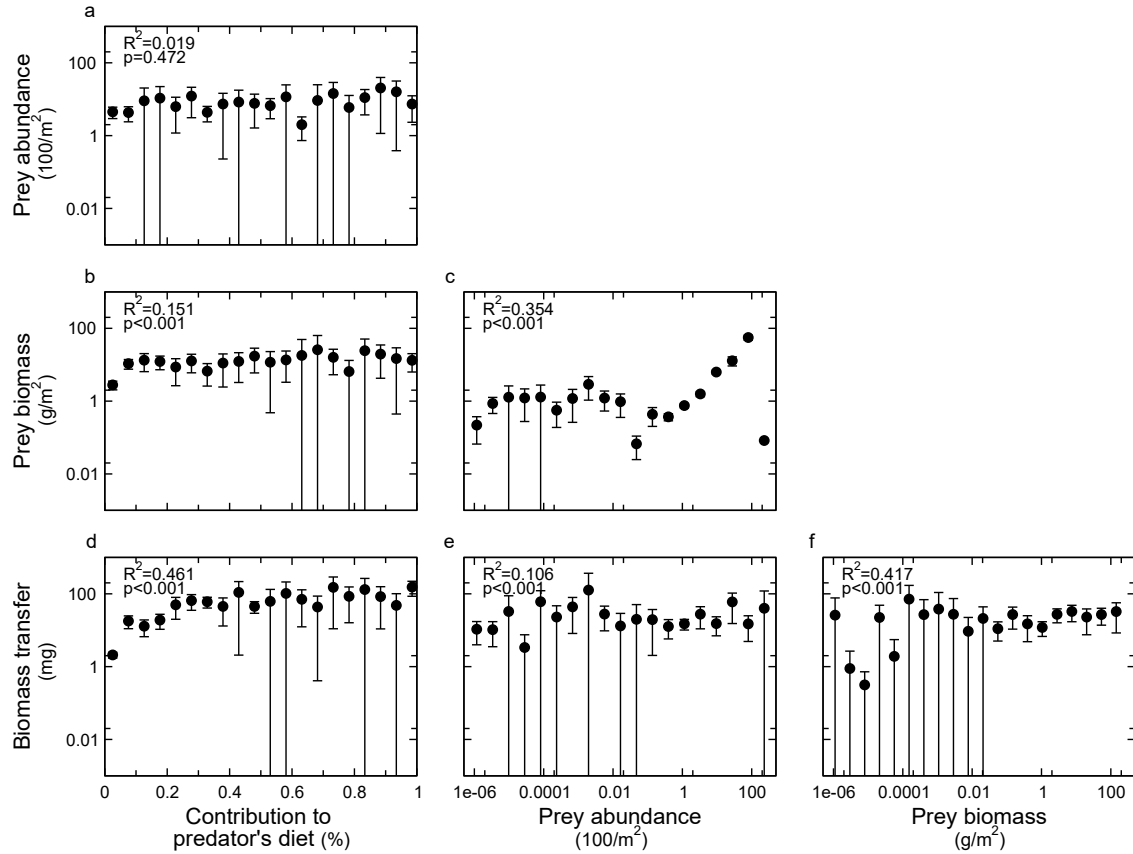
Figure 3: Correlations between structural and dynamic properties of links. Asymmetry was positively correlated with the contribution of a feeding link to the predator's diet, the log of prey abundance, and the log of prey biomass but was not correlated with the log of biomass transfer. The log of centrality was positively correlated with the contribution of a link to the predator's diet but was not correlated with the log of prey abundance, the log of prey biomass, or the log of biomass transfer. For each pair of properties, we give the R^2 and p-value for the correlation. We also show the means (± 2 SE; points) of one property for 20 bins of the other (sizes of bins vary depending on the regression) Note that, due to the distribution of values for the predictors, not all bins contained observed data points. In particular, our data included few links with intermediate asymmetries.
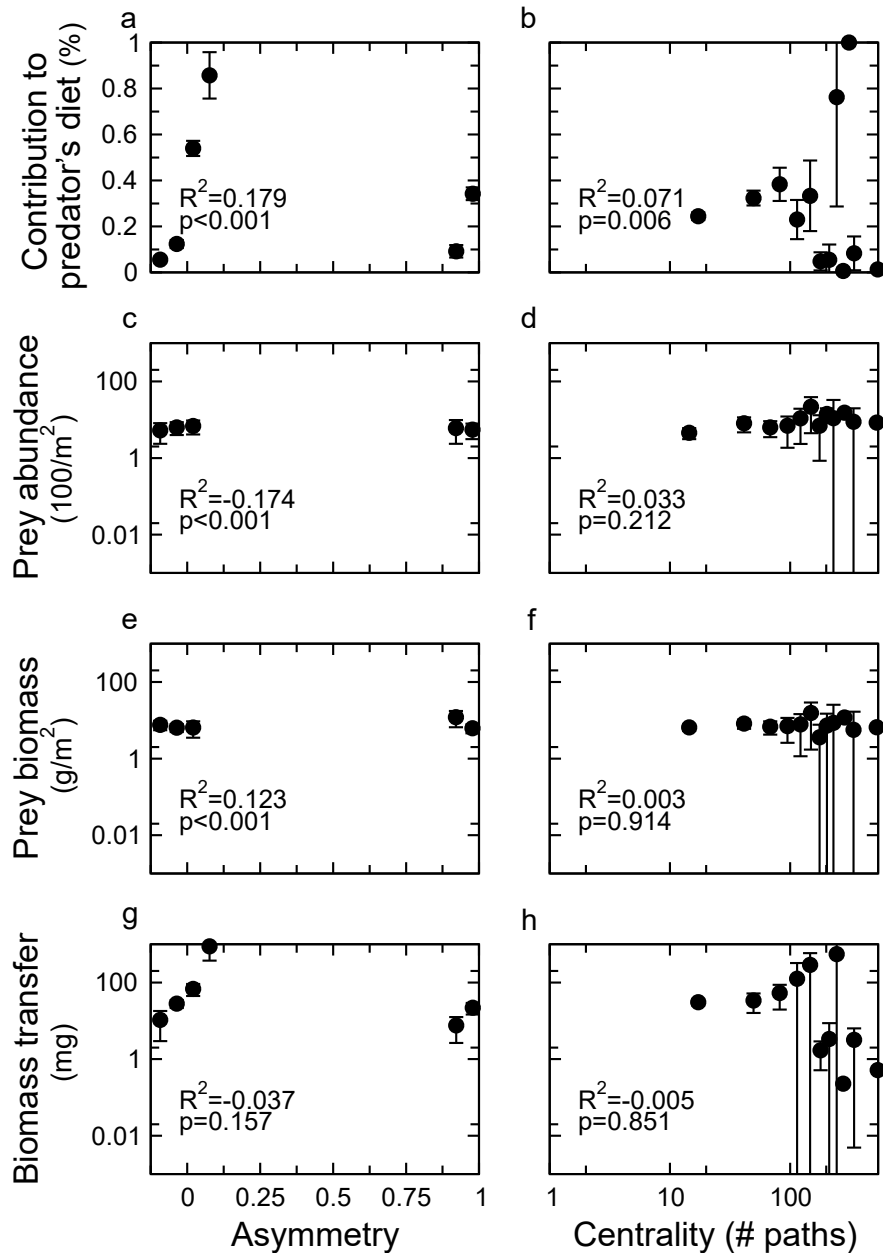
Figure 4: Changes in probability of loss (solid, red lines) and transmission (black, dashed lines) with each property included in the best-fit model ignoring host specificity. Each panel shows the change in probability when varying one predictor with all other predictors held constant at their mean. Note that the probabilities shown exclude random effects for lake, season, and parasite life stage. **A,B)** The probabilities of loss and transmission both increased sharply as the log of prey biomass and the log of centrality increased. **C)** The probability of loss increased with the log of biomass transfer, while the probability of transmission decreased. **D)** The probabilities of loss and transmission both increased slightly with increasingly positive asymmetry. **E)** The probability of loss increased as prey abundance increased, while the probability of transmission decreased.