Species roles and link roles:

a richer perspective on network ecology

Alyssa R. Cirtwill

*Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy
at the University of Canterbury*

School of Biological Sciences
University of Canterbury
New Zealand

2016

Dedicated to my family and to my friends near and far. Your love, support, and encouragement made this possible.

The more accurate the map, the more it resembles the territory. The most accurate map possible would be the territory, and thus would be perfectly accurate and perfectly useless. The tale is the map that is the territory. –Neil Gaiman

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Abstract

Food webs and other ecological networks can be seen as maps of species and their interactions (e.g., predation, pollination, and parasitism). Such mappings frame the complex intricacies of biological communities in a way that is analytically tractable, but also obscure species-level information. This can lead to a gap between studies of networks and the deep literature surrounding species' idiosyncratic ecologies. Species roles— descriptions of the way each species is embedded into its community —offer one way to bridge this gap. As roles provide a species-level perspective on network structure, patterns in species roles can often be related to species traits in a way that the overall structure of a network usually cannot. Thus, role-based approaches give network ecologists a way to use species' natural histories to understand patterns in network structure while also making network analyses more approachable for ecologists with different specialities.

This thesis uses a variety of definitions of species roles to explore a variety of ecological networks, demonstrating the broad range of questions to which species roles may be applied. The first chapter provides an overview of several different role concepts used in network ecology, and the second through fifth chapters each use one or more role concept to investigate specific ecological questions. Chapter two uses species roles to incorporate a predator-prey network into the Theory of Island Biogeography. Chapter three uses species roles to compare the overlap of plants' interaction partners in plant-pollinator and plant-herbivore networks, while chapter four explores the changes to plants' and insects' roles in a single plant-pollinator network over 15 years of climate change. Chapters five and six are focused on aquatic food webs that include parasites. Chapter five compares the roles of parasites and free-living species, as well as different types of interactions between them (i.e., predation among free-living species, parasitism, antagonism among parasites, and

concomitant predation on parasites inside their hosts). Chapter six uses the roles of feeding links between free-living species to better understand the trophic transmission of parasites. Finally, in an appendix we show how individual variation in fishes diets affect their parasite loads.

The key findings of this thesis are i) that using species roles to incorporate information from food webs improves the predictions of the Theory of Island Biogeography, ii) that more closely related plants had more similar sets of interaction partners despite a great deal of variation across networks and between plant families, iii) that the roles of plants and pollinators have shown different changes after 15 years of warming, suggesting that phenological uncoupling may be a risk for this system, iv) that parasites and free-living species have different roles in food webs, but only when concomitant predation was considered, and v) that many properties of feeding links between free-living species affect the outcomes of these links for parasites. As well as providing answers to the driving questions behind each chapter, this thesis demonstrates the breadth of potential applications for species roles. We conclude species roles provide a framework that speaks to the heart of one of the fundamental unsolved questions in ecology— how species' traits relate to the structure of ecological networks.

Preface

This thesis has been written as a series of stand-alone scientific articles which nevertheless form a cohesive unit. The articles all share a common focus on using species roles to combine network theory with ecological questions. As of the date of submission of this thesis, the articles were in different stages of the publication process. The first, *"Species roles in food webs"* was in preparation for submission to *Food Webs* and represents a wider review of the relevant literature than is present in the introductions of the subsequent chapters. The second, *"Knowledge of predator-prey interactions improves predictions of immigration and extinction in island biogeography"*, was published in a special edition of *Global Ecology and Biogeography* June 2015: volume 25, issue 7, pages 900–911. The third, *"Conservation of interaction partners between related plants varies widely across communities and between plant families"* was under revision at *New Phytologist*, manuscript number NPH-MS-2016-21211. The fourth, *"Are high-arctic plant-pollinator networks unravelling in a warming climate?"* was under review at *Ecography*, manuscript number ECOG-02910. The fifth, *"Concomitant predation on parasites is highly variable but constrains the ways in which parasites contribute to food-web structure"* was published in the *Journal of Animal Ecology* May 2015: volume 84, issue 3, pages 734-744. The sixth, *"Taking the scenic route: trophic transmission of parasites and the properties of links along which they travel"* was under review at *Ecology*, manuscript number #ECY16-0885. The appendix, *"Are parasite richness and abundance linked to prey species richness and individual feeding preferences in fish hosts?"* was published in *Parasitology* January 2016: volume 143, issue 1, pages 75-86. In the *"General introduction"* and *"General discussion"* framing these articles, I discuss the relevance of the articles to each other and their application in ecology.

Co-authorship statements

The following pages contain co-authorship statements for each co-authored chapter in this thesis.

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Name: Malyon Bimler Signature: Date: *29-09-2016*

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General introduction

Whether to our delight or our dismay, ecology is not like physics. In physics, a reductionist approach to studying the world (and beyond) has yielded centuries of phenomenal results such as the discovery of the four fundamental forces, Newtonian mechanics, and relativity (Meyer-Ortmanns, 2015). In ecology, meanwhile, many senior researchers despair of uncovering fundamental laws (Lawton, 1999; Simberloff, 2004; Poulin, 2007; but see Turchin, 2001). Exceptions abound to both observed patterns and theoretical predictions (Lawton, 1999; Poulin, 2007). This is likely due to the wide and wonderful variety of species, habitats, and communities that characterises our world. The peculiarities of living things are what draw many ecologists to the field, but they also make any pursuit of general truths very difficult. To put it simply, while a single species can be described relatively well in isolation, knowledge of one species' population dynamics, behaviour, or habitat requirements may not be applicable when studying another species.

One cannot, therefore, understand an ecological community by scaling up from the properties of single species in the same way that one can understand the behaviour of a gas by scaling up from the properties of molecules. Unlike physical systems, natural communities of species display emergent properties that cannot be predicted based on the properties of the species themselves (Emmerson and Yearsley, 2004; Beckerman et al., 2006; Stouffer, 2010). For example, natural communities routinely support higher numbers of species than are stable in naive models (May, 1972). This implies that there is some form of "organisation" in ecological communities that stabilises them and allows them to persist (Dunne et al., 2002, 2004; Fortuna et al., 2010; Stouffer and Bascompte, 2011). To study these structures, it is thus necessary to consider the structure of the community as a whole, and a leading way to do so is within an ecological network framework (Heleno et al., 2014).

Networks are essentially maps of interactions ('links') between species. Networks have been used to map antagonistic (e.g., predation [Paire, 1966] and parasitism [Wells et al., 2013]) and mutualistic (e.g., pollination [Olesen et al., 2007] and seed-dispersal [Schleuning et al., 2011]) interactions in a wide variety of habitats from around the globe. In each case, the network describes the whole community's structure and behaviour with respect to the interaction of interest. By capturing the structure of interactions in a community, networks allow us to address questions about community stability (Dunne et al., 2002, 2004; Fortuna et al., 2010; Stouffer and Bascompte, 2011) and ecosystem functioning (Mello et al., 2011; Burkle et al., 2013; Poisot et al., 2013). Both the density (Dunne et al., 2002, 2004) and arrangement (Fortuna et al., 2010; Stouffer and Bascompte, 2011) of links within a community have been linked to communities' ability to remain stable with high numbers of species. These structural characteristics, moreover, have also been linked to environmental factors such as latitude (Cirtwill et al., 2015), land use (Thompson and Townsend, 2004, 2010), and spatial isolation of the community (Nogales et al., 2015).

In addition to facilitating analysis of the community as a whole, ecological networks can also be used to study species within the broader context of their community. Specifically, we can use ecological networks to describe species' *roles* within their communities– that is, how they interact with other species. By quantifying species' places within ecological networks, roles provide a bridge between species' natural histories and the properties and processes at play at the community level. Integrating the two levels of knowledge allows us to test potential drivers of network structure. For example, species' body sizes have been shown to play a fundamental role in structuring predator-prey interactions (Loeuille and Loreau, 2005; Brose et al., 2006; Curtsdotter et al., 2011; Riede et al., 2011; Brose et al., 2016), and the roles of plants and animals in pollination and frugivory networks have been linked to their phylogenies (Ehrlich and Raven, 1964; Jordano et al., 2003; Rezende et al., 2007; Guimarães et al., 2011; Rohr et al., 2014; Nogales et al., 2015). Moving in the other direction, knowledge of the relationships between species' traits and their roles in food webs are being used to develop probabilistic interaction networks that better account for incomplete sampling of communities (Guimerà and Sales-Pardo, 2009; Dalla Riva and Stouffer, 2015; Poisot et al., 2015). Along with facilitating "pure science" research, species' network roles can also be used to inform conservation plans since species with different roles have different responses to perturbations in their community (Eklöf and Ebenman, 2006; Kaiser-Bunbury et al., 2010; Curtsdotter et al., 2011; Wootton and Stouffer, 2016).

Throughout this thesis, I demonstrate several ways in which species roles can provide a bridge between network ecology and knowledge about species' particular traits. In each case, I endeavour to show that using species roles gives us a unique insight into communities. Like the species they describe, roles come in a variety of shapes and sizes and can be measured in different ways. For a very simple summary, one can count the number of interactions in which a species participates (its degree), or determine how 'high' in the network a species feeds (its trophic level). At the other extreme, concepts like betweenness centrality consider all paths through the network to determine a species' impact (Jordán et al., 2006; Newman, 2010; Lai et al., 2012). In the middle are role concepts that include species' direct interactions as well as indirect interactions that are likely to affect the focal species, but do not include the structure of the entire network in each species' role. One such definition is 'motif roles'; an extension of the use of meso-scale 'motifs'. These are configurations of n species describing unique patterns of interactions that can be used to measure the structure of a network (Milo et al., 2002; Stouffer et al., 2007). Once a network has been described in terms of its component set of motifs, a species' motif role is the list of frequencies with which a species appears in each unique position in each motif (Stouffer et al., 2012; Baker et al., 2015; Cirtwill and Stouffer, 2015). The motif role therefore provides a summary of the species' direct and indirect (up to n-1 steps removed) interactions—a detailed description of the way the species is embedded in the community. These and other role concepts are all valid ways of describing species' places within their ecological contexts, and the choice of role for each study will depend on the precise question being asked.

To clarify the variety of concepts and methodologies used to describe species' roles, I begin this thesis with a review of the literature surrounding species' roles. This review takes the place of the literature review that would normally occur in a thesis introduction. In it, my co-authors and I first summarise several definitions of species roles (including those mentioned above). We then highlight similarities among definitions of role that address similar questions about the ways in which species interact, and finish by noting extensions to role concepts currently being developed. One such extension, the association of species' roles with their phylogenetic history, is also a major focus of Chapter 4.

Having established definitions for a variety of role concepts and their broader context within the literature in Chapter 1, Chapters 2-6 of my thesis each explore one or more role concepts in detail. In the second and third chapters, we define species' roles as their sets of interaction partners. In Chapter 2, these are the predators and prey of arthropod species in a classic island biogeography dataset (Simberloff, 1969). Here, my co-author and I attempt to use knowledge of species' roles in their local community, drawn from a mainland food web (Piechnik et al., 2008), to improve the accuracy of predictions based on the Theory of Island Biogeography (MacArthur and Wilson, 1963). We expect that, as species' roles change, their probabilities of immigrating to or going extinct from a given mangrove island will also change. Specifically, we fit models which include terms for the presence of species' arthropod predators and/or prey and/or their ability to consume basal resources as well as similar "classic" models which include only island size and isolation. We compare models based on both their fit to the empirical data and on the similarity of their predictions. This allows us to determine whether incorporating species' roles into the Theory of Island Biogeography results in a meaningful improvement.

In Chapter 3, we consider plant's roles in terms of their pollinators or herbivores in a wide array of plant-pollinator and plant-herbivore networks. Unlike the food web used to determine arthropods' roles in Chapter 2, these networks are all *bipartite*. That is, they are composed of two groups of species (e.g., plants and pollinators) that interact only with species from the opposite group (i.e., plants are pollinated by animals, not by other plants). We use these networks to investigate the relationship between plants' phylogenies and their roles. We expect that, since related plants tend to have similar traits and since herbivory and pollination interactions are both strongly affected by plants' traits, more closely-related plants will have more similar roles. As herbivory is detrimental to plants while pollination is beneficial, we also expect that the strength of this relationship might differ between network types. Finally, we compare the strength of the relationship between phylogenetic relatedness and similarity of interaction partners across plant families.

In Chapters four and five, my co-authors and I define species' roles more explicitly using the motif roles defined in Stouffer et al. (2012). That is, we decompose networks into their component motifs and track species' participation in each unique position across the set of motifs. In Chapter four, we are interested in the motif roles of plants and their insect pollinators. Our particular focus in this case is the response of each group to climate change and the associated changes to plants' flowering phenologies. As pollinators depend on floral resources for their food, we expect that pollinators' phenologies may also have advanced over time. However, since plants and insects active at different points in the season require different abiotic conditions, we also expect that changes in roles may be linked to the date on which a species becomes active each year. We test all of these hypotheses in a plant-pollinator community in Northern Greenland which has experienced substantial warming over the past 14 years and in which plant phenologies are known to have changed (Høye et al., 2013).

In Chapters five and six, we return to unipartite food webs to explore several aspects of parasites' participation in aquatic food webs. Chapter five compares the roles of parasites and free-living species across seven estuarine food webs to test i) whether parasites' roles are similar to those of free-living species at particular trophic levels and ii) whether parasites' roles change as different types of interactions unique to parasites are included in their roles. We divide free-living species into basal resources (those with predators but no prey), intermediate consumers (those with predators and prey), and top predators (those with prey but no predators), and calculated the median motif roles of each group. We next compare these median roles to those of parasites. To test whether concomitant predation (the consumption of parasites along with their hosts) has a different effect on parasites' roles than interactions in which the parasite is more directly involved (i.e., parasitism, predation on free-living life stages of the parasite, and predation among parasites sharing a host), we calculate parasites roles' both including and excluding concomitant predation and compare each to the roles of free-living species.

Chapter five also extends the motif role concept to links between species. Just as species' motif roles can be described by calculating the frequencies with which a species occupies each unique position in a set of motifs, an interaction's role can be described by calculating the frequency with which it occupies each unique link position in the same set of motifs. This description captures the different ways in which each link contributes to the flow of energy and biomass through a web just as a species' motif role describes the species' participation in the web. To compliment our investigation of the changes to parasites' roles when different types of interactions are included, we also examine the roles of several types of link directly. Because species' motif roles are determined by the interactions in which they participate and vice versa, taking both a species- and link-focused view of network structure provides a unique window into how each type of species and interaction is embedded in the network as a whole.

In Chapter six we build on this concept of links' roles to investigate the consequences of links between free-living species for parasites. Many parasites have complex life cycles which involve multiple hosts. In some cases, parasites move from one host to the next via *trophic transmission* when the parasite's next host consumes its current host. As parasites generally must complete their life cycles in order to sexually reproduce, we expect that they will tend to use transmission routes that are very likely to occur (giving the parasite the best chance of reaching its final host). We used several definitions of links' roles within networks to determine whether links resulting in trophic transmission have different properties from links in which the parasite is killed or links which do not affect the parasites. The latter occurs when the prey in an interaction is not a host for any parasites in the study system. We tested this hypothesis in a spatially and temporally-replicated dataset from four New Zealand lakes (Cirtwill et al., 2016).

In an appendix following the main body of the thesis, I present additional work done during my PhD candidature at the University of Canterbury. As a companion study to the work in Chapter six, my co-authors and I test whether fish with broader diets are more likely to host large numbers of parasites or highly-diverse parasite assemblages. In particular, we are interested in the associations between diet and parasite load across *individuals* of different fish species. Although the six main chapters of this thesis address questions at the level of species and the interactions between them, it is worth remembering that individuals within species do not necessarily all participate in the same interactions or have the same roles. Indeed, several studies have shown that generalist species can be composed of much more specialised individuals (Pires et al., 2011). Investigating spatial, temporal, and intra-specific variation in species' roles is likely to be an important area of study in the future; this appendix provides only one example of the questions that may be asked.

Readers will note that this body of work does not explore a narrow area in great depth but instead applies species roles to a variety of questions in network ecology. This is by design. As species roles, particularly motif roles, are a relatively recent development even within network ecology (itself a young subdiscipline within ecology), it is not yet clear which questions require the most in-depth study. Instead, I have opted to demonstrate the breadth of potential applications for species roles. In addition to contributing to the ecological literature surrounding each chapter, this broad-based approach has also revealed strengths and weaknesses of different role concepts and, more importantly, ways in which I and others can improve them in the future.

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Chapter 1: Species' roles in food webs

Alyssa R. Cirtwill[1], Giulio Valentino Dalla Riva[2], Matthias Dehling[1], Marilia Gaiarsa[3], Malyon Bimler[1], Fernando Cagua[1], & Camille Coux[4,5]

[1]Centre for Integrative Ecology
School of Biological Sciences
University of Canterbury
Private Bag 4800
Christchurch 8140, New Zealand

[2]Beaty Biodiversity Research Centre
Zoology Department
University of British Columbia
2329 West Mall
Vancouver, Canada V6T 1Z4

[3]Departamento de Ecologia
Instituto de Biociências
Universidade de São Paulo
São Paulo, SP, Brazil 05508-900

[4]UMR 1065 Santé et Agroécologie du Vignoble
INRA Bordeaux-Aquitaine
71 Avenue Edouard Bourlaux
33140 Villenave-d'Ornon, France

[5]Centre d'Etudes Biologiques de Chizé
UMR 7372, CNRS & Université de La Rochelle
23 Avenue Albert Einstein
17000 La Rochelle, France

Prepared in the style of *Food Webs*; not yet submitted.

Introduction

Ecologists often wish to understand a species' "place in the biotic environment, its relations to food and enemies" (Elton, 1927 in Johnson and Steiner, 2000) or, in short, its *Eltonian niche*. To do this, one must first map the biotic environment (community) to which the focal species belongs. Food webs provide just such a mapping by connecting species based on their trophic interactions. These interactions include antagonistic interactions such as predation and parasitism, but can also include mutualisms, such as pollination and seed-dispersal, where one species obtains food while aiding the reproduction of the other. Once a food web describing the focal species' community has been assembled, there are several methods that can be used to describe the species' *role* within the web (i.e., how the focal species participates in its community). Because food webs describe energy and biomass flows through a community (Lindeman, 1942; Wootton, 1997), represent ecosystem functions (Memmott et al., 2007; Reiss et al., 2009; Thompson et al., 2012), and even offer insights into the community's overall *stability* (Neutel et al., 2002; Thébault and Fontaine, 2010), describing species' roles in food webs allows us to assess their niches both in terms of species' requirements for survival and their impacts on their communities (Chase and Leibold, 2003).

Roles and Eltonian niches are related, in that both address the ways in which species affect and are affected by each other, but they are not equivalent. This is true even when we completely ignore species' abiotic requirements (Peterson, 2011). Food webs generally only include one type of interaction (e.g., predation or pollination but not both [Fontaine et al., 2011]). A species' role in a food web therefore describes only the portion of its niche that relates to the kind of interaction being described in the food web. For example, the roles of a species of Lepidoptera will be quite different in networks describing pollination, herbivory, and predation. Moreover, the Eltonian niche aims to identify those biotic conditions that are able to support a species on moderate timescales (i.e., from individual lifespans up to thousands of years) (Peterson, 2011), while food webs describe communities at a particular point in time with no guarantee that the species present during sampling will persist. Finally, the portion of a species' niche that is described by its role in a network will be affected by the exact definition of role that is used. Given the variety of definitions used across different fields, it can be difficult to make comparisons across studies. To tackle this problem, here we review several commonly-used concepts of species' roles in food webs. In each case, we summarise the methodology used to obtain the role and highlight its connection to the species' Eltonian niche. We are particularly interested in areas of overlap between role concepts, and take care to point out connections between roles wherever possible. We then outline ways in which researchers identify and group species with similar roles, and conclude with a very brief survey of current limitations to the idea of species' roles, and how researchers are working to overcome these limitations. Terms in italics are defined in Box 1..

Concepts of species' roles in networks

Degree

One of the mathematically simplest definitions of a species' role is it's *degree*: the number of interactions in which the species participates (Fig. 1). Degree depends only on the focal species' *local* neighbourhood within the network. That is, degree only considers other species which directly interact with the focal species. Thus, degree provides a measure of species' participation in a food web without requiring any knowledge of the *global* structure of the web (i.e., the species that indirectly affect the focal species). Degree can also be used to investigate particular subsets of a species' local neighbourhood. If the focal species' role as a predator (or prey) specifically is of greater interest than its overall role, degree can be divided into in-degree— the number of incoming *links* (interactions) — and out-degree— the number of outgoing links (Fig. 1B). Note that this is only applicable in *unipartite* networks as in *bipartite* networks each group of species has only in-links or only out-links and such a division is not meaningful. Whether or not degree is subdivided, in niche terms degree tells us how important the focal species is likely to be, in terms of the interaction described in the food web.

The notion that species with high degrees are particularly important to their communities is based on the fact that if the abundance of such a species changes, this will directly affect many other species (Lai et al., 2012). Perturbations to high-degree species may therefore have larger effects on the food web than perturbations to low-degree species. Moreover, it is more likely that high-degree species will have interaction partners that depend very strongly upon them. As such, the removal of a high-degree species is more likely to cause secondary extinctions than the removal of a low-degree species (Dunne et al., 2002; Memmott et al., 2004; Eklöf and Ebenman, 2006; Kaiser-Bunbury et al., 2010; Curtsdotter et al., 2011). Degree can also have implications for the management of introduced species.

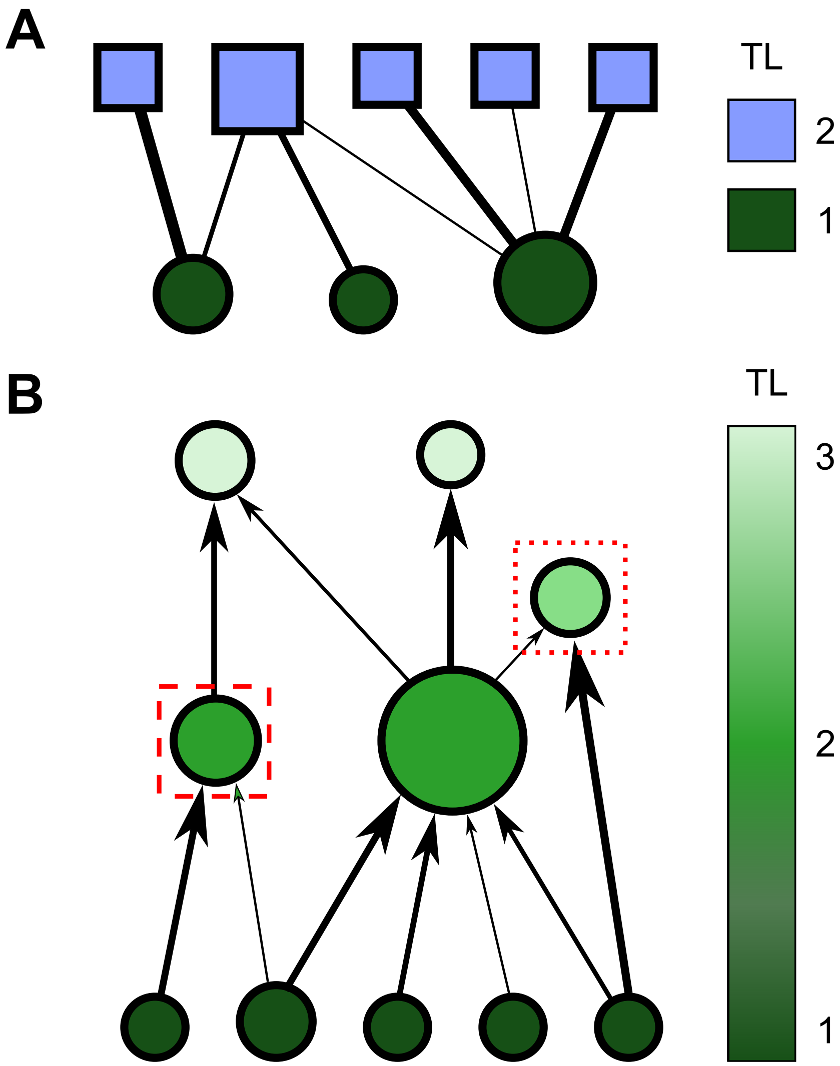

Figure 1: These two food webs each contain species with different degrees and trophic levels. A) In this bipartite food web, pale blue squares represent pollinators and dark green circles represent plants. Note that species do not interact with other species of the same type (i.e., plants do not pollinate other plants). B) In a unipartite food web, any species could potentially interact with any other. Here, degrees can also be subdivided into in- and out-degrees based on a focal species' numbers of prey and predators, respectively. For example, the species highlighted in the red, dashed box has an in- degree of 2 and an out-degree of 1, giving an overall degree of 3. In both networks, the size of a shape increases with its degree while the fill represents trophic level (TL; height in food chains). In A), the two groups of species are at different trophic levels. In B), trophic levels increase from primary producers (TL=1; dark green) to predators (TL=3, very pale green). Most of the species in this food web have integer trophic levels. The species highlighted in the dotted red box, however, is an omnivore with both plant and animal resources. Its trophic level therefore depends on the exact definition of trophic level used. Short-weighted trophic level considers only the most direct path from the focal species to a primary producer; under this definition, the focal species has a trophic level of 2. Prey-averaged trophic level, in contrast, considers the trophic levels of all the focal species' prey. If interaction strengths (indicated by line weights) are not considered, the focal species has a trophic level of 2.5. If interaction strengths are accounted for, however, the focal species' PATL will be closer to 2.

In particular, specialist pollinators are more likely than generalists to interact with exotic plants, suggesting that it may be important to consider the degrees of native species when developing management plans for introduced species (Stouffer et al., 2014).

As well as predicting species' effects on their communities, degrees can also be used to predict which species are most likely to go extinct after the loss of an interaction partner. Specialist predators (those with low in-degrees) are particularly vulnerable to the loss of prey (Allesina, 2012). This difference in vulnerability to secondary extinction in turn has implications for biogeography. As specialists are more likely to go extinct following the loss of a prey species, they are likely to have smaller geographic ranges than generalists (Gaston, 1991). At a landscape level, these trends mean that specialists should appear in fewer patches than generalists (Holt, 2010; Gravel et al., 2011), leading to increased beta diversity (Ødegaard, 2006). This has the potential to create a feedback loop, with geographically-restricted species having access to fewer partners than species with broader ranges and therefore becoming more specialised.

Despite its utility, some have argued that the *qualitative* degree described above, which is calculated based only on the presence or absence of links between species, does not accurately reflect species' specialisation or importance to the community (e.g., Blüthgen et al., 2006). To address this, several *quantitative* extensions of degree have been formulated. These extensions all weight interactions to reflect the importance of the focal species to each of its partners rather than assuming all interactions are equal (Blüthgen et al., 2007; Dormann, 2011; Nilsson and McCann, 2016). Weighted measures may provide a more realistic measure of a species' effect on its interaction partners than qualitative degree (Wootton, 2005; Vázquez et al., 2005). However, calculating weighted degrees requires detailed data that include interaction weights. As these data are more costly and time-intensive to collect, datasets including weights are much rarer than food webs that include only the presence or absence of interactions.

Trophic level

As well as describing the importance of a species' niche, degree can also be used to give an idea of a species' vertical position in a food web— i.e., its *trophic level*. This role concept refers to a species' place in the *food chains* that make up a food web, relative to the primary producers that support the community. Species that do not consume any other species in the web (i.e., those with an in-degree of zero) are primary producers. At the other extreme, species with no predators (i.e., those with an out-degree of zero) are top predators (Fig. 1B). Those with both predators and prey (i.e., non-zero in- and out-degrees) are intermediate consumers. In niche terms, trophic levels tell us whether a focal species relates to its biotic environment as a predator, prey, or both. These categorical descriptions, however, are relatively imprecise. By defining the trophic level of primary producers to be one and those of consumers' to be one greater than that of their prey (Lindeman, 1942), numerical trophic levels can be calculated for each species in a food web.

For species other than primary producers and top predators, degree alone is not enough to calculate trophic levels. Instead, it is necessary to consider the network structure beyond the focal species' local neighbourhood. Specifically, trophic levels can be calculated by following food chains from the focal species to primary producers. Each step up the food chain is a new trophic level, with strict herbivores (that consume only basal resources) assigned a trophic level of two and consumers occupying ever higher values based on their sets of prey species (Lindeman, 1942; Darnell, 1961; Baird and Ulanowicz, 1989; Christian and Luczkovich, 1999). This simple definition was developed under the assumption that species feed on sets of prey with the same trophic level (Lindeman, 1942). As the prevalence and importance of omnivory in food webs has become clear (Holt, 1997; Emmerson and Yearsley, 2004; Thompson et al., 2007), however, non-integer trophic levels have become the norm (Cousins, 1987; Vander Zanden and Rasmussen, 1996; Williams and Martinez, 2004; Thompson et al., 2007). To emphasise this shift, some researchers prefer the term "trophic position" (e.g., Levine, 1980; Cohen et al., 2003). As the two terms refer to the same quantity, we will continue to use trophic level to refer to a species' vertical position in a food web.

A variety of methods have been developed to account for species which feed on prey at different trophic levels (Fig. 1B). Each approach emphasises different interactions. "Shortest trophic level", for example, assumes that because losses occur during the transfer of energy between trophic levels, species obtain most of their energy along the shortest food chain in which they participate (Hairston, Jr. and Hairson, Sr., 1993; Williams and Martinez, 2004). Under this concept, therefore, a species' trophic level is one greater than the lowest trophic level among its prey (Hairston, Jr. and Hairson, Sr., 1993; Williams and Martinez, 2004). Other methods such as prey-averaged trophic level take all food chains in which the focal species participates into account (Williams and Martinez, 2004). Regardless of the precise methodology, however, trophic levels always rank species based on their vertical position in food webs, with primary producers setting the baseline.

Trophic levels can also be calculated independent of food-web topology by using stable isotopes (Peterson and Fry, 1987; Vander Zanden and Rasmussen, 1996; Post, 2002). This approach uses the different rates of bioaccumulation of carbon and nitrogen isotopes to measure species' average trophic levels without requiring knowledge of specific interactions between species. While the stable isotopes approach is therefore useful in cases where the structure of the food web is not known, it is also difficult to use when comparing across food webs. Stable isotope ratios vary between taxa and tissue types depending on their particular biochemistries (Vander Zanden et al., 2015) and between study cites, requiring the use of baseline species in each food web under study (Kling et al., 1992; Cabana and Rasmussen, 1994; O'Reilly et al., 2002; Boecklen et al., 2011). Despite the differences in how trophic levels are calculated from stable isotopes and network topology, they have been shown to be strongly correlated (Williams and Martinez, 2004; Carscallen et al., 2012). This supports the idea that topological definitions of trophic levels are grounded in sound ecological characteristics, and suggests that trophic levels may be comparable across studies even if different methodologies are used.

As well as different carbon and nitrogen isotopes, environmental contaminants such as DDT and mercury tend to accumulate moving up food chains (Rowan and Rasmussen, 1992; Gray, 2002; Wang and Wang, 2005; Tavares et al., 2009; Coelho et al., 2013). Trophic levels can therefore be used to predict the level of contamination in fish species that are targeted for human consumption (Beltran-Pedreros et al., 2011), and assess the risk of contamination for species of conservation concern (Bossart, 2011). The bioaccumulation of DDT in predatory birds is perhaps the most famous example of this process, and identification of this trend and its effects on bird populations led to the banning of DDT in North America (Grier, 1982). Apart from tracking the accumulation of contaminants, a species' trophic level can be used to predict its potential to cause a trophic cascade (Spiller and Schoener, 1994; Dyer and Letourneau, 2003; Borrvall and Ebenman, 2006; Eklöf and Ebenman, 2006; Boersma et al., 2014; Estes et al., 2015; Rodríguez-Lozano et al., 2015), with top predators and primary producers tending to have particularly large effects on the rest of their communities. Like degree, therefore, trophic level offers information about how important a species is to its biotic community.

Motif roles

A major limitation to both trophic level and degree, is that they give little information on a species' indirect interactions— interactions which can have major impacts on the focal species despite not involving the focal species directly (Wootton, 1994; Jordán et al., 2006). This limits the ability of these role concepts to describe species' niches because indirect effects can modulate the relationships between the focal species and their predators or prey. For example, if the focal species' predator has other prey and the focal species becomes rare, the predator might consume more of the alternative prey. The interaction between the predator and its alternate prey might thereby provide the focal species with relief from predation pressure (Hammill et al., 2015). Similarly, the removal of a predator might allow its prey to increase in abundance, having knock-on effects on other predators (Sanders et al., 2013). These patterns of interactions describe network structure at an intermediate scale between the local interactions accounted for in degree and the full, global structure of the network. Some of these *meso-scale* have been shown to affect the focal species' population size and dynamics (Polis et al., 1989; Holt, 1997; Zabalo, 2012), suggesting that meso-scale structures can affect species' Eltonian niches. One way to take these structures into account is by defining species' *motif* roles. These roles extend the concept of network structural motifs— unique patterns of *n* interacting species (Milo et al., 2002) —to the species level and aim to provide a more holistic picture of species' niches by explicitly including direct and indirect interactions (Stouffer et al., 2012; Cirtwill and Stouffer, 2015; Fig. 2).

To determine a species' motif role, the network is first decomposed into a set of motifs (Milo et al., 2002; Stouffer et al., 2007). In unipartite food webs, there are 13 three-species motifs (Stouffer et al., 2007). Some of these motifs, such as "three-species food chains" (Hastings and Powell, 1991; Bascompte and Melián, 2005; Laws and Joern, 2013; Fig. 2), "apparent competition" (two prey sharing a predator [Holt and Kotler, 1987; Bascompte and Melián, 2005; Lefèvre et al., 2009; McKinnon et al., 2013]), and "intraguild predation" (two predators sharing a prey, where one predator also consumes the other [Polis et al., 1989; Holt, 1997; Kondoh, 2008; Zabalo, 2012]) have clear biological meanings and have been studied in isolation. Others, including many of the motifs involving two-way interactions (i.e., A eats B and B eats A), have not yet been interpreted. In bipartite food webs, there are only two three-species motifs. To fully describe species' roles in these networks it is therefore necessary to use larger, less well-studied motifs (Baker et al., 2015). Where possible, however, it is best to use relatively small motifs. This is partly because of computational limitations and the difficulty in interpreting large motifs but also because the impact of indirect effects is expected to decrease moving farther from the focal species (Jordán and Scheuring, 2002; Jordán et al., 2006).

Whatever the size of motifs being used, each motif contains one or more unique positions. In a three-species food chain motif, each species occupies a unique position as the top, bottom, and middle species all have different biological meanings (Stouffer et al., 2012; Cirtwill and Stouffer, 2015). In an apparent competition motif, in

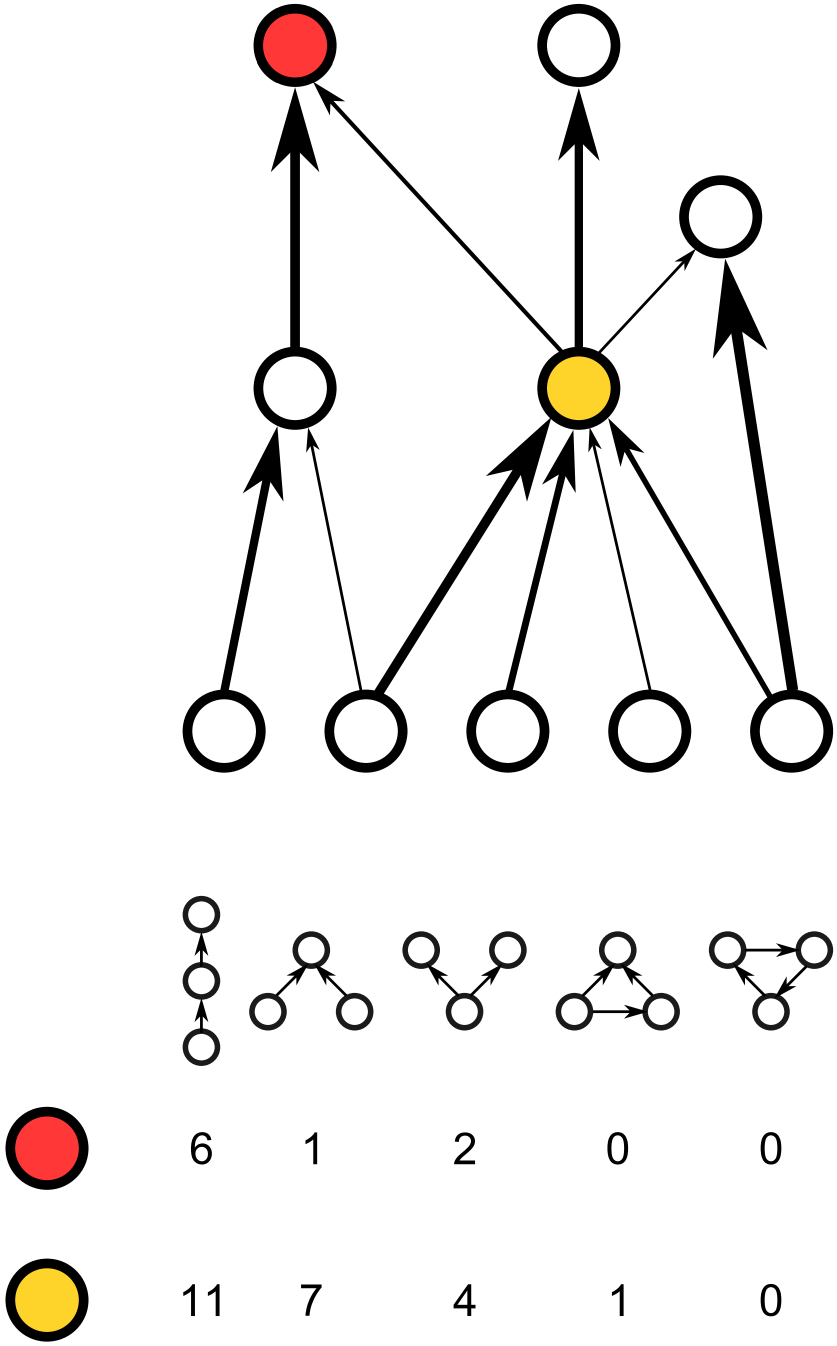

Figure 2: Motif roles describe the way a species is embedded in a food web by decomposing the web into its component motifs (unique configurations of n interacting species) and tracking the participation of the species in each motif. There are 13 different three-species motifs; this simple food web contains only the five motifs that contain only one-way interactions. The motif roles of two species are shown below the food web.

contrast, there are only two unique positions as the two predators are indistinguishable in the context of that motif. Once a network has been broken down into its component motifs, species' motif roles can be calculated by counting the number of times the focal species occurs in each position within each motif (Stouffer et al., 2012; Baker et al., 2015; Cirtwill and Stouffer, 2015). This yields a vector of frequencies which describes the focal species' role in terms of its direct and indirect interactions, providing a detailed picture of the way in which the species is embedded in its community (Stouffer et al., 2012; Baker et al., 2015; Cirtwill and Stouffer, 2015). Because a motif role provides a detailed picture of a focal species' relationships to other species in the community (as predator, prey, competitor, etc.), the motif role can be seen as a description of the species' niche from the perspective of the interaction described in the food web.

Motif roles are a relatively new development, but have already been used to compare the ways in which free-living species and parasites fit into food webs (Cirtwill and Stouffer, 2015), to measure variation in species' roles over space and time (Baker et al., 2015), and to test whether species' roles are phylogenetically conserved (Stouffer et al., 2012). Motifs more broadly have also been linked to community stability, with some motifs appearing much more commonly in stable networks (Stouffer, 2010; Borrelli et al., 2015). This approach has been extended to predict which species contribute most to the stability of their communities (Stouffer et al., 2012). Motifs have also been used to track the extent of regime shifts in the Baltic Sea (Yletyinen et al., 2016), demonstrating the promise of the approach for detailed analysis of particular study systems.

Centrality

Structural roles incorporate meso-scale structures as well a focal species' local neighbourhood. Some measures of *centrality* also take this approach to describe a species' ability to influence the rest of the food web (Estrada, 2007; Lai et al., 2012). These measures extend the thinking behind degree (which considers only the focal species' local neighbourhood) and also consider the focal species' impact through indirect interactions (Jordán et al., 2006; Lai et al., 2012).

Measures of centrality that incorporate meso-scale network structures are usually based on identifying the food chains in which the focal species participates, just as with trophic level. Unlike trophic levels, however, measures of centrality also consider the food chains which do not involve the focal species. Two such measures, "betweenness centrality" (Fig. 3) and "information centrality", both quantify the frequency with which the focal species appears on paths between pairs of other species (White and Borgatti, 1994; Jordán et al., 2006; Estrada, 2007). The main difference between the two is that betweenness centrality includes only the shortest paths between species, while information centrality includes all paths (Jordán et al., 2006; Estrada, 2007).

While betweenness and information centrality are based on food chains (meso-scale structures), other definitions of centrality are based on the global structure of the food web. One such measure, "eigenvector centrality", is based on the defining eigenvector—the eigenvector associated with the largest eigenvalue—of the matrix of interactions for a food web (Bonacich, 1972; Allesina and Pascual, 2009). In this formulation, the centrality of species i is the ith entry in the defining eigenvector (Bonacich, 1972; Allesina and Pascual, 2009; Lai et al., 2012). Eigenvector centrality can be understood as a weighted version of degree, where each neighbour j contributes to the degree of species i in proportion to j's centrality (Lai et al., 2012). At least nine other measures of centrality have been proposed (Jordán

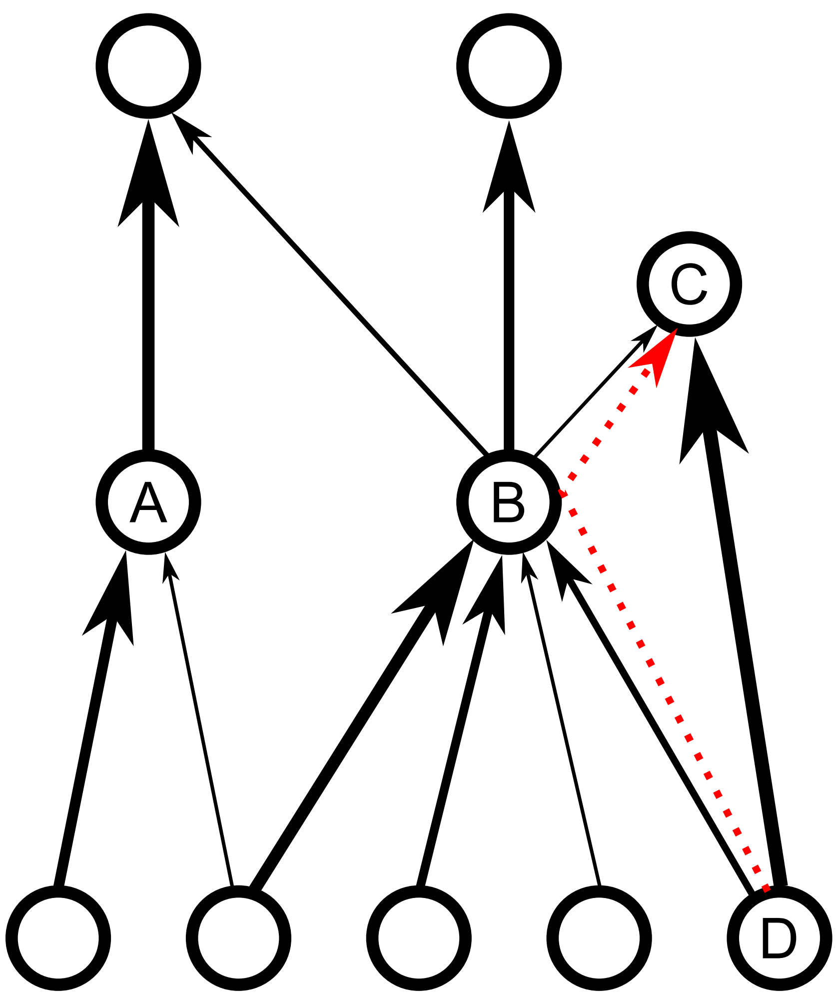

Figure 3: Betweenness centrality defines a species' role as its ability to affect the rest of the food web as determined by the number of times the species appears on the shortest path between pairs of other species. Species A appears on 2 such paths while species B appears on 11. Species B is therefore more likely to have a large effect on its community than is species A. Note that because only the shortest path between a pair of species is considered, the path D-B-C (traced by the dotted arrow) does not contribute to the betweenness centrality of species B.

et al., 2007). Comparative studies have generally found strong correlations between different centrality measures (Jordán et al., 2006; Estrada, 2007). This suggests that the various centrality measures may capture equivalent information about species' niches. We therefore will not describe the other measures in detail here (see Jordán et al. (2006, 2007); Estrada (2007) for detailed descriptions).

The logic behind all of these measures of centrality draws heavily on the keystone species concept— the notion that certain species will have a much larger effect on their community than would be expected based on the species' biomass alone (Paire, 1966; Jordán et al., 2006). Indeed, because highly-central species are expected to affect many other species, centrality has been used to identify potential keystone species in several studies (Jordán et al., 2006; Estrada, 2007; Lai et al., 2012; Mello et al., 2015). Like the keystone species concept, centrality does not tell us so much *what* a species' niche is, but rather suggests which species might have particularly *important* niches.

As well as highlighting species that are potential keystones within free-living food webs, centrality has also been used to understand the transmission of parasites through food webs. Many parasites are trophically transmitted between hosts when the host for one life stage is consumed by the host for the next, and highly-central free-living species tend to host more parasites than other free-living species (Chen et al., 2008; Thompson et al., 2013). This suggests that species which have strong effects on the free-living components of food webs can also be important to the parasite components of the same communities.

Grouping species with similar roles

Structural and regular equivalence

Having completed a brief survey of methods for calculating species' roles within networks, we will now introduce equivalence methods for identifying species with similar roles. These approaches differ from the previous definitions of role by focusing explicitly on the identities of species' interaction partners (Yodzis and Winemiller, 1999). For instance, two species with the same degree may or may not interact with the same partners, but two species are only *structurally equivalent* if they share identical sets of interaction partners (Borgatti, 2002; Fig. 4). In fact, two structurally-equivalent species will have the same roles under any of the definitions above, but not necessarily vice versa. This strict definition can be relaxed slightly to quantify the degree of structural equivalence on a continuous scale by using a distance metric such as Jaccard dissimilarity to compare the overlap in species' interaction partners (Yodzis and Winemiller, 1999). While such quantitative measures provide more information by placing species on a continuous scale from fully equivalent to completely distinct, they are still restricted because species which interact with ecologically similar, but not taxonomically identical, partners will not be considered equivalent. For example, consider two species of herbivorous insects, each of which is specialised on a different plant from the same genus and which is preyed upon by similar spider species. Intuitively, we understand that these two insects have similar roles in their community (and niches) despite having low structural equivalence. To capture this intuitive similarity, another technique is evidently necessary.

As one solution to this problem, some researchers (e.g., Johnson et al., 2001; Luczkovich et al., 2003) have proposed adopting the concept of *regular equivalence* from the study of social networks (White and Reitz, 1983). In this framework, *nodes* within a network are equivalent if they interact with the same "types" of partners (Fig. 4). In a network of several corporations, company presidents are equivalent because they each interact with boards of directors, venture capitalists, etc. (Johnson et al., 2001). Even though the board of directors is made up of different individuals in each company, the boards form a recognisable "type" or "group" of people that interact with company presidents. In ecological networks, researchers often wish to avoid defining such groups *a priori* in order to avoid biasing analyses towards collections of species that are appealing to humans but may not be ecologically relevant. Several algorithms have therefore been developed to do this by iteratively assigning species to groups until the best-fitting arrangement of groups has been reached (Borgatti and Everett, 1993; Johnson et al., 2001; Luczkovich et al., 2003). Happily, the groups determined by such algorithms (e.g., predatory insects, scavengers, and aquatic larvae) usually do tend to be intuitive and biologically meaningful (Johnson et al., 2001; Luczkovich et al., 2003). Thus, by identifying species with similar roles, regular equivalence groups can point to elements of niches that are shared by the species in a group.

Structural and regular equivalence groups are being used increasingly often in food web research, with structural equivalence having the longer pedigree. Structurally equivalent species are often collapsed into *trophospecies* in order to reduce bias in the resolution

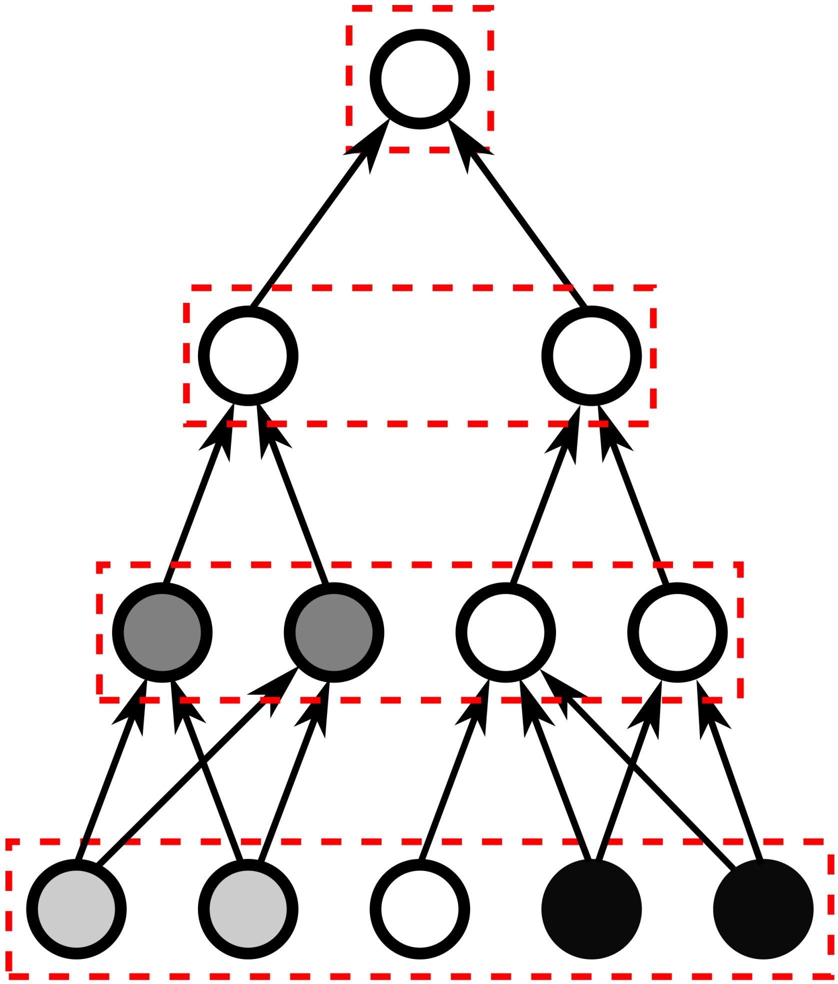

Figure 4: Sets of structurally equivalent species (nodes with the same grey fill) interact with exactly the same sets of partners. Sets of regularly equivalent species (enclosed in red, dashed boxes) interact with partners from the same sets of groups. In this web, regular equivalence groups correspond to trophic levels such that primary producers (bottom group) only interact with herbivores (second group from bottom), herbivores interact with primary producers and consumers (second group from top), and so on. Note that structurally-equivalent species are also regularly-equivalent, but the reverse is not necessarily true.

of unipartite food webs (e.g., Martinez, 1991; Vermaat et al., 2009). Larger, higher-trophic level species are often easier to identify than smaller, lower-trophic level, or cryptic species, leading to better resolution at the top of the food web than among basal species. This greater detail at the top of the food web can then bias estimates of food-web structural properties, hindering efforts to understand the true structure and function of communities. Collapsing structurally-equivalent species into a single node can reduce this bias and facilitate comparisons between communities by ensuring that each node represents a unique niche (Martinez, 1991).

Regular equivalence, on the other hand, has much in common with the concept of functional redundancy, in which species with similar “functions” in a community are grouped together. This redundancy is believed to be important because species with similar niches may be able to compensate if one species becomes rare or goes extinct (Naeem, 1998; Rosenfeld, 2002; Aizen et al., 2012). The loss of a species with a redundant role in a community will therefore have little effect on the rest of the community (Naeem, 1998; Rosenfeld, 2002; Aizen et al., 2012). As well as identifying groups of species with redundant roles, food web models based on regular equivalence groups perform remarkably well (Allesina and Pascual, 2009). This has lead to the suggestion that groups might be the appropriate level of analysis in future studies of food webs, particularly as larger and more detailed data become available (Allesina and Pascual, 2009). This approach holds great promise, especially as more approaches are developed to incorporate more ecological information into regular equivalence groups (Gauzens et al., 2015).

Module-based roles

Another way of grouping species according to their types of interaction partners is through *module* roles, which measure the extent to which species interact with different modules (tightly-knit groups) within a network. Such modules are defined mathematically by interacting more tightly among themselves than with any species that is not a part of the module (Guimerà and Amaral, 2005a,b). They are usually detected algorithmically using techniques such as simulated annealing that aim to find the set of modules that minimises the number of links between different modules (Guimerà and Amaral, 2005a). Once modules have been defined, species can be classified based on A) the focal species' importance to its own module and B) the extent to which the focal species' interactions are distributed across modules (Guimerà and Amaral, 2005a). The focal species' importance within its module is determined by on its "within-module degree", a Z-score of whether the focal species has significantly more interactions with other species in the same module than the average (Guimerà and Amaral, 2005a). Species with a within-module degree of at least 2.5 are designated "hubs" and have significantly more interactions within their module than the average (p«0.005; Guimerà and Amaral, 2005a). Both hub and non-hub species can then be further divided based on the participation coefficient, which measures the evenness of the distribution of the focal species' interactions. Values near 0 indicate species which interact almost entirely within their own modules, whereas values near 1 indicate species who interact with species in all modules equally (Fig. 5).

Using these two parameters, species can be divided into varying numbers of roles. In general, however, hubs with low participation

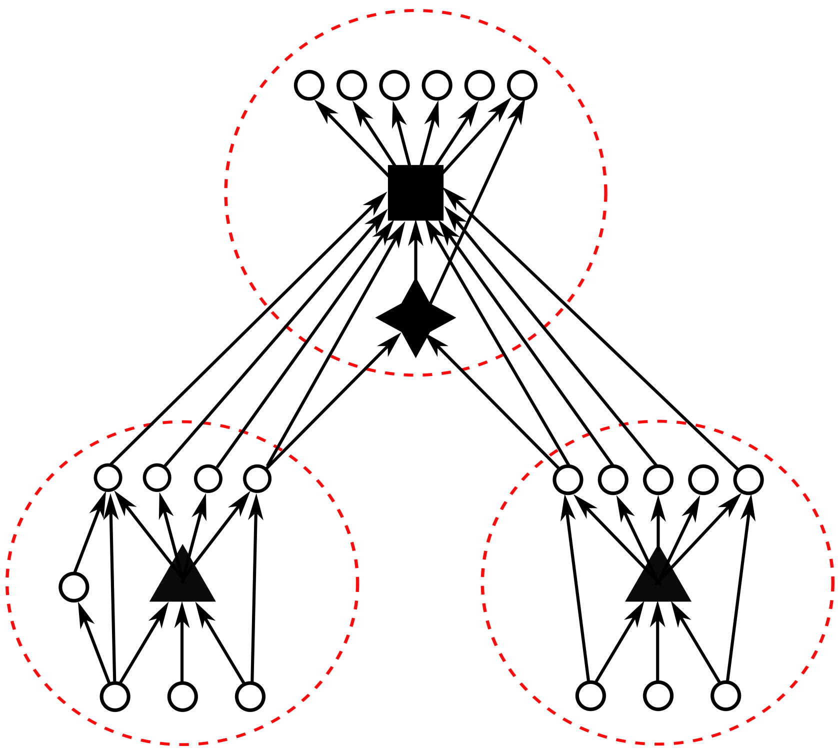

Figure 5: This unipartite food web contains three modules (circled in red, dashed lines). It is possible to group species with similar roles based on how often they interact within their module and with species in other modules. The network hub (black square) interacts with significantly more partners than other species within its module and has many interactions with other modules. Module hubs (black triangles) interact with many partners within their modules, but rarely with species from other modules. The connector (black star) has interactions spread evenly among modules. Finally, peripheral species (white circles) have few interaction partners within their modules and few links to other modules.

coefficients are module hubs, which are important to the cohesion of their modules but have few interactions with other modules, while hubs with high participation coefficients are also important to the coherence of the network as a whole (Guimerà and Amaral, 2005a; Olesen et al., 2007; Poulin et al., 2013). In non-hub species, low participation coefficients indicate peripheral species while high participation coefficients indicate connector species which "glue" different modules together (Guimerà and Amaral, 2005a; Olesen et al., 2007; Poulin et al., 2013).

As with structural roles, module-based roles are relatively new and their potential is only beginning to be explored. So far it has been shown that plants' and pollinators' module-based roles are conserved between the species' native and exotic ranges (Olesen et al., 2007), and that the module-based roles of parasites and free-living species are phylogenetically conserved (Poulin et al., 2013). In seed-dispersal networks, modules tend to include species from different taxa (mammals, birds, fish, etc. [Donatti et al., 2011; Mello et al., 2011]). At a finer scale, however, closely-related species may not belong to the same modules (Donatti et al., 2011) and within-module degree tends not to be phylogenetically conserved (although participation coefficients were [Schleuning et al., 2014]). These results emphasise the importance of both ecological and evolutionary processes in shaping food webs and species' roles within them.

Functional roles

Rather than identifying species with potentially redundant functional roles using regular equivalence, it is also possible to group species according to their *functional roles* directly. This method is based on the premise that species with similar traits (e.g., gape sizes or flower morphologies) should fulfil similar functions in their community (Tilman, 2001; Petchey and Gaston, 2002; Dehling et al., 2016). Extending this notion to interactions, we expect that traits that represent species' functional roles will also influence which interactions they participate in (Thompson and Townsend, 2005; Dehling et al., 2016). One trait that has been found to explain a great deal of variation in predator-prey interactions is body mass, as many taxa feed on smaller prey (e.g., Williams and Martinez, 2000; Stouffer et al., 2006; Petchey et al., 2008; Williams, 2008; Stouffer, 2010; Williams et al., 2010; Gravel et al., 2011; Stouffer et al., 2011; Zook et al., 2011. In most cases, however, more than one trait is necessary to describe all of the interactions in a community (Cattin Blandenier, 2004; Allesina et al., 2008; Allesina, 2011; Eklöf et al., 2013). Moreover, while using empirical traits to create model food webs can reproduce general structural properties, such approaches often fail to predict specific interactions (Petchey et al., 2008; Bartomeus et al., 2016). In an attempt to address both of these shortcomings, some researchers have used artificial traits based on the properties of the observed network (Rohr et al., 2010; Dalla Riva and Stouffer, 2015; Rohr et al., 2016). These abstract traits cannot be directly mapped onto morphological traits, but they can reveal similarities between species that are not evident based on morphology or behaviour. Such hidden similarities, despite the absence of an obvious ecological interpretation, nevertheless identify species that may fulfil redundant functions in the community or strongly compete with each other; i.e., species with similar niches.

An alternative way to identify species with similar functional roles is to analyse the traits of the focal species' interaction partners rather than the traits of the focal species itself (Fig. 6). This approach is common in studies of plant-pollinator communities, where pollination syndromes are often used to predict which species will interact (Waser et al., 1996; Fenster et al., 2004; Ollerton et al., 2009). Pollinators vary in their adherence to classical syndromes (Fenster et al., 2004; Ollerton et al., 2009), but in general species do tend to interact with partners whose traits are relatively similar and match some limiting trait of the focal species (Stiles, 1975; Wolf et al., 1976; Dalsgaard et al., 2009; Stang et al., 2009; Junker et al., 2013; Dehling et al., 2014). By grouping species that interact with partners that have similar traits, we can infer species that have similar functional roles in their community. Grouping species this way is somewhat analogous to grouping regularly-equivalent species based on the types of species with which they interact. The major distinction is that regular-equivalence groups are emergent properties of a network's topology whereas functional roles are linked at least implicitly to a functional mechanism (e.g., fruit size [Dehling et al., 2014, 2016] or flower characteristics [Fenster et al., 2004; Ollerton et al., 2009]). This focus on biologically-explicit groups means that functional roles provide a convenient summary of species' niches in the type of network being studied.

Functional roles have been used to demonstrate co-adaptation between interaction partners, as mutualists are expected to converge on compatible traits (Blüthgen et al., 2007). Species with unique functional roles interact with partners that have extreme or unusual values of the traits that affect the interaction being studied. Because of this, they tend to interact with fewer partners (Junker et al., 2013; Maglianesi et al., 2014; Coux et al., 2016; Dehling et al., 2016) and, as specialists, may then be more vulnerable to extinction (Allesina, 2012).

Limitations to role concepts and future directions

As described above, one of the main limitations of species roles is that while they do offer insight into a species' niche— its "place in the biotic environment, its relations to food and enemies" (Elton, 1927 in Johnson and Steiner, 2000), a role will only capture one aspect of the niche. For some role concepts this might be a specific property such as the niche's position in food chains (trophic level) or the niche's importance (degree and other measures of centrality). Other concepts such as motif roles and functional roles attempt to summarise all of a species' interactions. These roles give a better picture of species' niches from the perspective of food webs, but the fact remains that roles defined in a food web describing only one type of interaction will overlook components of species' niches that do not involve that interaction (Fontaine et al., 2011; Kéfi et al., 2016). Combining different network types has the potential to improve this by integrating different aspects of a species' niche (e.g., as pollinators and as prey [Fontaine et al., 2011]). Kéfi et al. (2016) offer one way forward by identifying species' module roles in a network which

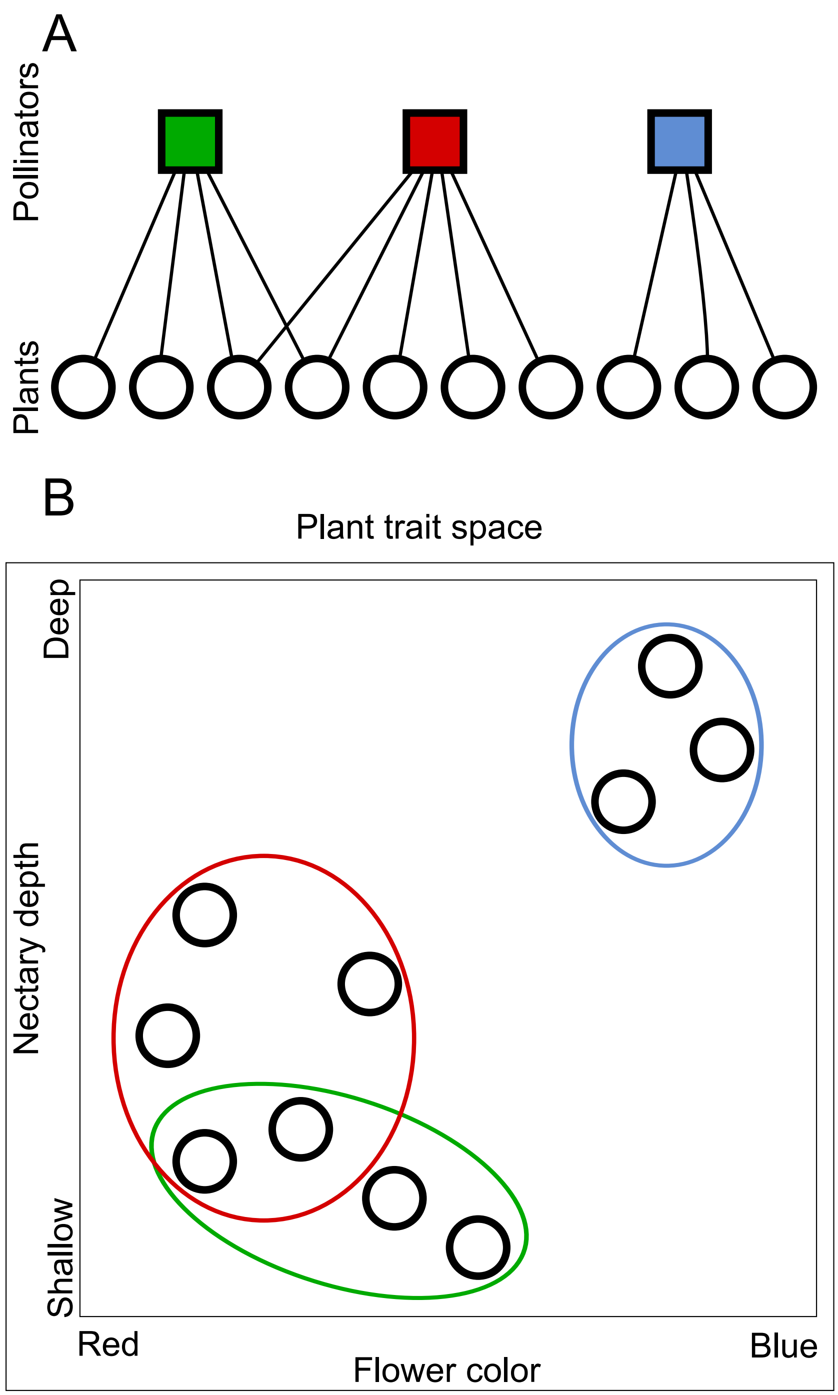

Figure 6: The functional roles framework uses the traits of interaction partners to group species with similar roles. A) In this plant-pollinator network, we are interested in comparing the roles of the three pollinators. B) The functional role of each pollinator is the area of trait space that includes all plants that the pollinator visits. In this community, the red and green pollinators' roles (lower left) overlap while the blue pollinator has a unique role (upper right). Note that the axes used to describe the trait space may be concrete traits, as shown here, or abstract axes describing variation in many traits.

includes trophic interactions and positive and negative non-trophic interactions (including provision of refuges, increased recruitment, competition for space, predator importance, etc.). The roles in this study therefore provide a much more comprehensive picture of species' niches than do roles in webs which describe a single interaction.

Another important limitation in studies of species' roles is the point-sample nature of most ecological networks. Species' niches encompass their relationships to the biotic environment as a whole, but networks provide a spatially and temporally limited snapshot of communities. As more networks are published that include replication over time and/or space (e.g., Olesen et al., 2008, 2011; Leong et al., 2015), we will obtain more thorough descriptions of species' roles. As information about the spatial and temporal variability of species' roles becomes available, we may be able to better understand the differences between species' fundamental Eltonian niches (all of the interactions in which a focal species could reasonably participate) and those that they actually realise in a particular community. This is especially intriguing with respect to species which have moved outside of their historical ranges (i.e., introduced species). It is possible that a species' role in its native community could be used to predict the way in which it will interact with a novel set of potential partners (Aizen et al., 2008; Emer et al., 2016). If this is true, then species' roles will be a powerful tool for conservation biologists.

As well as exploring the spatial and temporal variation of species' roles, researchers are increasingly connecting species' roles to their phylogenies. Related species tend to have similar roles for several of the role concepts we describe above (Stouffer et al., 2012; Poulin et al., 2013; Rohr and Bascompte, 2014). Species' phylogenies are believed to shape their roles because *phylogenetically-conserved* traits affect interactions between species (Gómez et al., 2010; Dalla Riva and Stouffer, 2015). Thus, conserved traits lead to conserved interactions which lead to conserved roles. As well as explaining similarities between the roles of related species, incorporating evolutionary processes into studies of ecological networks can suggest historical drivers of the structure of current communities. Most current studies attempt to explain trends in network structure based on species' traits (Woodward et al., 2005; Brose, 2010) or neutral processes (Siepielski et al., 2010; Canard et al., 2014; Poisot et al., 2015). These approaches have been valuable, but evolutionary explanations may be more parsimonious. Explanations based on species' evolutionary histories may also explain species which seem to lack appropriate interaction partners in modern networks. This is most obvious in the case of "evolutionary anachronisms" such as the large-seeded plants of South America that are believed to have been dispersed by large mammals that are now extinct (Janzen and Martin, 1982). Adaptations to extinct interaction partners can also explain species' interactions with introduced species, as when the plants described above are dispersed by introduced cattle and horses (Barlow, 2000).

Perhaps the most important factor limiting the applicability of species' roles is that role concepts are often abstract and difficult to connect to species' natural histories. This abstraction can be beneficial, as it allows us to identify groups of species when we are not confident that any particular taxonomic level or species trait is the appropriate basis for categories (Luczkovich et al., 2003). However, network researchers must admit that such abstractions can make our work less accessible to non-specialist readers. Mello et al. (2015) suggest that ecological concepts should be used to guide the choice of network measures. We agree, with the proviso that ecological prior knowledge should not be allowed to restrict species' roles so as to ignore unexpected interactions such as frugivory and seed dispersal by crocodilians (Platt et al., 2013) or predation on nestlings by herbivores such as deer and sheep (Furness, 1988; Pietz and Granfors, 2000). Such interactions may be more common than previously suspected. Even if they are indeed rare, rare or weak interactions may still be important for community stability because of their potential for dissipating perturbations (Emmerson and Yearsley, 2004; Allesina and Tang, 2012; Wootton and Stouffer, 2016). After selecting network measures that specifically address the aspects of a species' niche that are of most interest, we also suggest that researchers bear in mind the part of a species' niche that they are analysing (e.g., niche size or vertical position in food chains, or a more holistic summary such as structural roles) and use this to place their results in the context of the focal species' ecology.

Conclusions

Throughout this review, we have sketched some of the questions that have been asked using each role concept. To conclude, we return to the question of why species roles, in general, are useful. Networks allow us to place the focal species in its community context, but the network as a whole is difficult to interpret. By reducing the network to a single value or vector, species' roles compress the network into a tractable form. If we consider food webs as maps of ecological communities, roles provide the topographic lines, borders, and roadways that simplify a map and provide meaning. Just as different types of maps have different themes (e.g., political maps, terrain maps, geological maps, etc.) different role concepts provide different perspectives on a food web. Our task as researchers working with species' roles is to make our choice of role concept, and the aspect of species' niches that it is meant to capture, as clear as cartographers make their maps.

Acknowledgements

We would like to acknowledge the support of the National Science and Engineering Research Council of Canada (to ARC), a Marsden Fast-start Grant (to MD; administered by the Royal Society of New Zealand), the São Paulo Research Foundation (to MPG; FAPESP grants 2013/13319-5 and 2014/20572-1), a University of Canterbury Masters Scholarship and the Miss E. L. Hellaby Indigenous Grasslands Research Trust (to MB), and a University of Canterbury Doctoral Scholarship, Meadow Mushrooms Postgraduate Scholarship, and New Zealand International Doctoral Research Scholarship (to FC). We would like to thank Daniel B. Stouffer for conversation and comments that greatly strengthened this review.

Box 1: Glossary

Eltonian niche	A species' interactions with food sources and natural enemies.
Role	A species' relationship to others in its food web.
Stability	The ability of a food web to withstand perturbations.
Degree	The number of interactions in which a species participates.
Centrality	A species' ability to affect the rest of the network.
Local	The portion of the food web that directly affects the focal species.
Global	The entire food web.
Link	A connection between two nodes, indicating an interaction between them.
Unipartite web	A web containing one group of species that interact amongst themselves.
Bipartite web	A web containing two groups of species where all interactions occur between groups.
Qualitative	A web in which links are present or absent (i.e., not weighted). Also called a *binary* or *topological* web.
Quantitative	A web where links are weighted by frequency, biomass transfer, or some other property. Also called a *weighted* web.
Trophic level	A species' vertical position in a food web or height in a food chain.
Food chain	A path from a primary producer to a top predator, where each step up the chain corresponds to an increase in trophic level.
Meso-scale	The structure of the network including the focal species' local neighbourhood and some indirect interactions, but not the entire network.
Motifs	Unique patterns of n interacting species; building blocks of networks.
Structural equivalence	When a set of species all interact with exactly the same set of partners.
Regular equivalence	When a set of species interacts with partners from the same groups, but not necessarily with the same sets of partners.
Node	A component of a network. In food webs, usually a species.
Trophospecies	A set of structurally equivalent species, collapsed into a single node.
Module	A group of species that interact more often amongst themselves than with other species.
Functional roles	Roles defined by the traits of the focal species' interaction partners.
Phylogenetic conservation	The tendency for related species to have more similar traits because of their shared common ancestry.

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Chapter 2: Knowledge of predator-prey interactions improves predictions of immigration and extinction in island biogeography

Alyssa R. Cirtwill[1] & Daniel B. Stouffer[1]

[1]Centre for Integrative Ecology
School of Biological Sciences
University of Canterbury
Private Bag 4800
Christchurch 8140, New Zealand

Published in *Global Ecology and Biogeography* (2015).
DOI: 10.1111/geb.12332.

This chapter has been modified from its original version. It has been formatted to match this thesis.

Abstract

Aim: MacArthur and Wilson's original formulation of the Theory of Island Biogeography (TIB) included the corollary hypothesis that species richness might affect immigration and extinction rates. Building on this, other researchers have suggested additional top-down and bottom-up effects. We compare these hypotheses to identify the strongest candidates for inclusion in a "trophic TIB".

Location: Six mangrove islands in the Florida Keys, USA

Methods: We studied a classic island-biogeography time series featuring lists of species observed on six mangrove islands during roughly 16 censuses each across 700 days. We first used this time series to determine the number of opportunities for species to immigrate to an island for the first time (N=18,420), to go locally extinct (N=1,943), or to re-immigrate to an island after having previously gone extinct (N=1,813). We then leveraged information on those species' predators and prey to estimate the potential for top-down and bottom-up interactions during each census period. Finally, we constructed statistical models to test for species richness, top-down, and bottom-up effects on per-species immigration and extinction probabilities and validated them by comparing each model with a similar model based on the classic TIB.

Results: We found that models including bottom-up effects gave the greatest improvement over the classic TIB models. Extinction probability in particular decreased sharply for species with both basal resources and animal prey available. Species-richness and top-down effects had far weaker impacts on per-species probabilities of immigration and extinction.

Main conclusions: Our findings suggest that incorporating information on the trophic structure of island communities– particularly the species-specific availability of resources – can substantially alter predictions of extinction probabilities. Immigration probability, on the contrary, appeared largely stochastic. Incorporating trophic information into predictions of extinction rates therefore represents the most promising and best-supported way to extend the TIB.

Keywords

Theory of Island Biogeography, top-down effects, bottom-up effects, community assembly, predator-prey interactions, species richness, food web

Introduction

The Theory of Island Biogeography combines elegant simplicity of formulation (Hubbell, 2009) with the ability to reliably predict properties such as equilibrium species richness across both islands and a range of island-like habitat patches (Simberloff and Abele, 1982; Eadie et al., 1986). As such, it has become one of the cornerstones of ecological theory (MacArthur and Wilson, 1963; Holt, 2010; Hanski, 2010; Harte, 2011). In essence, the TIB supposes that immigration rates should be higher on islands that are closer to a source of immigrants and that extinction rates should be higher as islands get smaller (MacArthur and Wilson, 1963; Schoener, 2010). These two predictions were tested empirically immediately after the publication of the TIB and have generally matched observations well (Diamond, 1969; Case, 1975; Gilpin and Diamond, 1976), although some authors note important differences in immigration and extinction rates across species (Gilpin and Diamond, 1976; Whittaker et al., 2000; Piechnik et al., 2008).

The original TIB partially anticipates these differences by predicting variation in immigration and extinction rates as species richness changes on an island. Specifically, the authors of the TIB predicted that, as species richness on an island increases, immigration rates should decrease while extinction rates increase (MacArthur and Wilson, 1963). The effect of species richness on immigration is expected because species vary in their dispersal abilities (Simberloff, 1969), which could bias island faunas towards the best dispersers. Once these species are already present, the pool of remaining colonists will therefore tend to contain poorer and poorer dispersers, decreasing immigration rates (Schoener, 2010). At the same time, a species-rich island may include more extinction-prone species (e.g., species with low population sizes or specialised diets) and will therefore tend to lose more species than one which is species-poor (Schoener, 2010). Increasing species richness could also directly cause increasing extinction rates if increasing species richness leads to stronger inter-specific competition (Gilpin and Diamond, 1976). However, the effect of competition on island faunas is very difficult to observe experimentally (Simberloff, 1978).

Apart from competition, the presence of other species on an island could affect immigration and extinction rates through top-down and/or bottom-up effects (Knops et al., 1999; Piechnik et al., 2008; Holt, 2010; Gravel et al., 2011). Top-down effects of predators on their prey may increase extinction rates either directly (Savidge, 1987; Hanna and Cardillo, 2014), by causing trophic cascades (Spiller and Schoener, 1994; Ryberg and Chase, 2007; Spiller and Schoener, 2007), or by reducing population sizes such that stochastic extinctions are more common (Ryberg et al., 2012). Alternatively, the presence of predators can mediate competition between species and *decrease* the probability of any of them going extinct (Snyder and Cheson, 2000; Bull and Bonsall, 2010). It is intuitively less likely that there will be top-down effects on immigration rates, as this would seem to require species to adaptively immigrate depending on conditions on islands they have not yet reached. However, given the fact that any new immigrant must persist on an island for some time before being recorded, it becomes easy to envisage effects of predators on *observed* immigration rates following the mechanisms described above. In such a situation, the presence of predators could either reduce observed immigration rates as new arrivals are consumed before being recorded or, alternatively, could reduce competition and thereby increase the survival of new immigrants.

Bottom-up effects of resource availability on the TIB have also been postulated. Species with no resources available should quickly go extinct while species with abundant or varied prey may be more likely to persist (Holt et al., 1999; Holt, 2002; Piechnik et al., 2008; Holt, 2010). It is also possible that the presence of basal resources (e.g., plants, detritus, or bacteria) can affect immigration rates. In order for an island to support resident animal life, it must already have some basal resource present while the converse is not necessarily true (Holt et al., 1999; Holt, 2002, 2010). Basal resources should therefore be present on all islands that support animals as well as some that do not. This might result in a greater inclination of herbivores to immigrate to new islands since doing so entails less risk of starvation. Indeed, while most islands support herbivores, species at higher trophic levels are much rarer (Terborgh, 2009). This suggests that species which cannot consume basal resources may be less likely to immigrate or establish viable populations, perhaps because islands often support fewer prey species (and smaller prey populations) than mainland habitats (Terborgh, 2009).

Finally, top-down and bottom-up effects are known to interact in structuring communities, with the strengths and directions of each type of effect varying over time and across species (Power, 1992; Denno et al., 2002; Gratton and Denno, 2003; Gripenberg and Roslin, 2007). This wide variety of potential effects of interactions between species has prompted the development of "trophic TIB" models that incorporate community structure into island biogeography theory (Holt et al., 1999; Holt, 2002; Ryberg and Chase, 2007; Gravel et al., 2011). Although these models often preserve the TIB's spirit of simplicity and clarity, it is not clear whether they significantly improve on the classic version when confronted with empirical data. Further, most of these models tend to be structured in a way that complicates rigorous comparisons between them.

Rather than investigate a single mathematical model in great depth, here we use empirical data to compare and contrast multiple potential effects of community structure on island biogeography. We are especially interested in measuring the potential effects of predator-prey interactions and examining how they differ when considering immigration and extinction. To this end, we construct a statistical framework with which to test the following non-exclusive hypotheses: 1) immigration probability will decrease with increasing species richness while extinction probability will increase; 2) immigration probability will decrease with the presence of predators while extinction probability will increase; 3) immigration probability will be higher for species that can consume basal resources and extinction probability will decrease; and 4) there will be no effect of the presence of animal prey on immigration probability but extinction probability will decrease for species with prey available. By comparing similarly-structured models built around each hypothesis, our approach allows us to isolate models with little support as well as demonstrating which hypotheses explain similar variation in empirical data. Together, we argue that these two endeavours reveal the strongest candidates for future efforts to extend the TIB.

Methods

Dataset

We studied a classic island-biogeography time series for arthropod immigration and extinction on six mangrove islands (Simberloff, 1969) of known diameter (11-25m) and distance from the mainland (2-533m). In these experiments, each island was artificially defaunated and then censused 16-18 times during the following two years for a total of 96 post-defaunation censuses. Over the course of the experiment, 5 basal resources (mangrove trees, fungus, lichens, detritus, algae) and 231 arthropod species were observed, with most resolved to the species level.

Using this dataset, we were able to directly estimate when the different species immigrated to islands after defaunation. Specifically,

Model	Initial immigration	Repeat immigration	Extinction
Opportunities	18,420	1,813	1,943
Successes	476	127	461
Proportion of successes	0.026	0.070	0.237

Table 1: Number of opportunities for initial immigrations, repeat immigrations, and extinctions (i.e., sample size), and the number of successes and proportion of successes in each case.

for a given island during a given census k, we considered all species that were not observed to be potential immigrants. Note that we did not consider species which were present before defaunation but never returned during the experiment as part of this mainland species pool. All potential immigrants were counted as successful if they were observed during the next census $k+1$ or as failed otherwise. As it is possible that different mechanisms affect species which are frequent immigrants than those that more rarely leave the mainland, we considered initial immigration (i.e., for a given species s and island i, all censuses up to and including the first successful immigration to island i by species s) and repeat immigration (i.e., all immigration opportunities after species s had previously gone extinct from island i) separately. Note that this distinction allowed us to examine factors affecting species which immigrate relatively frequently without defining this set of species *a priori*.

We estimated extinctions on each island in the dataset using a similar procedure. For a given island i during a given census k, any species present could potentially go locally extinct and those not observed during the following census $(k+1)$ were considered to have done so. Species observed again in census $k+1$ were considered to have persisted. See Table 1 for the numbers of potential and observed immigrations and extinctions across the complete time series.

In order to relate these species-occupancy lists to the potential interactions between species on a given island at a given time, we combined them with a published list of potential prey for each species based on interactions observed or inferred on the mainland (see Piechnik et al., 2008 for details on the construction of this list). Potential prey were restricted to other arthropods (hereafter 'animal prey') which had been observed on at least one of the islands during the time series, plus the basal resources which were assumed to be present on all islands throughout the experiment (Piechnik et al., 2008). As basal resources were assumed to be omnipresent throughout the experiment (Piechnik et al., 2008), the ability of a species to consume basal resources (or not) was recorded as one measure of resource availability. The presence of animal prey, on the contrary, varied between censuses. To determine the potential for bottom-up interactions involving animal prey, we compared the list of potential prey for the focal species with the occupancy list for that island and census. If any of the species' mainland prey items were present, that species was assumed to be able to prey on the same species on the island. Similarly, if the focal species featured in the prey lists of any other species on the island at the same time, there was potential for top-down interactions (i.e., predation on the focal species) to occur. Determining the potential for top-down and bottom-up effects on each species on each island at each census allowed us to directly examine the effects of predator-prey interactions on initial immigration, repeat immigration, and extinction probabilities. See Table 2 for further details of the typical values and ranges of these predictors.

Statistical Models

Based on the aforementioned data, we created parallel sets of candidate models for the probability of a given species immigrating to, re-immigrating to, or going extinct from a given island at a given census. For each model, we estimated parameters using the function glmer from the lme4 library (Bates et al., 2014) in R (R Core Team, 2014) with binomial distributions and logit link functions. We then used these models to test our hypotheses relating to the effects of species richness, top-down effects, bottom-up effects, and their interactions using a null model and a model based on the TIB for comparison.

Null Models

The simplest models for initial immigration, repeat immigration, and extinction (henceforth referred to as our initial immigration null model, repeat immigration null model, and extinction null model, respectively) included an intercept and two random effects (*S2.1, Supporting Information S2*). The first random effect was for focal census (that is, the census from which predictor data were drawn, specific to a particular island). It accounted for variation in time between censuses as well as other hidden variables such that the predicted immigration or extinction probability for each census is expected to match that observed empirically.

The second random effect was intended to account for pseudoreplication within the data created by repeated observations

(A) Initial immigration			
Predictor	Min	Max	Mean
Distance	2	533	213
Diameter	11	25	14.9
Time between censuses	10	400	36.5
Species richness	2	47	18.8
Predators	0	1	0.782
Ability to eat plants	0	1	0.578
Animal prey available	0	1	0.440

(B) Repeat immigration			
Predictor	Min	Max	Mean
Distance	2	533	154
Diameter	11	25	15.1
Time between censuses	10	400	68.9
Species richness	11	47	32.3
Predators	0	1	0.933
Ability to eat plants	0	1	0.536
Animal prey available	0	1	0.523

(C) Extinction			
Predictor	Min	Max	Mean
Distance	2	533	164
Diameter	11	25	14.8
Time between censuses	10	400	41.5
Species richness	2	47	30.7
Predators	0	1	0.956
Ability to eat plants	0	1	0.600
Animal prey available	0	1	0.514

Table 2: Number of opportunities for initial immigrations, repeat immigrations, and extinctions (i.e., sample size), number of successes and proportion of successes in each case, and minima, maxima, and means for model predictors. As each set of models was based on slightly different data, we present the means and ranges for each separately.

of population-level behaviour of the same species across the experiments. For initial immigration, this was a species-by-island random effect as all potential immigrations of a given species to a given island were drawn from the same mainland population. On average, there were 8.2 pseudoreplicates per level of this random effect.

For repeat immigration and extinction, we further distinguished between different "event windows" to produce a species-by-island-by-window random effect. That is, we considered repeat immigration opportunities for species s to island i after the species' first extinction on island i up to and including the first successful repeat immigration—the first event window—to be independent from opportunities for species *s* to re-immigrate to island *i* after it had gone extinct a second time up to and including the second successful repeat immigration—second event window. For extinction, we distinguished between opportunities for extinction associated with different event windows for species *s* on island *i* (e.g., potential extinctions after an initial immigration, potential extinctions after the first repeat immigration, and so on). These two models included fewer pseudoreplicates per random effect (mean 4.7 and mean 3.6, respectively) than did the initial immigration model.

Theory of Island Biogeography Models

We next tested initial immigration, repeat immigration, and extinction TIB models based on the original formulation of island biogeography. The two immigration TIB models each included terms for distance, diameter, and their interaction. The extinction TIB model included only the diameter term as isolation was not hypothesised to affect the extinction of established populations (MacArthur and Wilson, 1963). In addition, each model included a term for the time between the focal census and the next census (i.e., the amount of time a species would have to immigrate or become extinct) since this interval varied across censuses (Table 2). To account for potential differences in the strength of the time effect on different islands, we also included all interaction terms between diameter, distance (immigration models only), and time between censuses (Table *S2.1*, *Supporting Information S2*). As in the null models, random effects of census and source population were also included.

Species-richness Models

We then extended the TIB models to test the hypotheses that initial and repeat immigration probability will decline and that extinction probability will increase with increasing species richness. To do this, we studied statistical models including all terms in the corresponding TIB models, species richness during the focal census, and interactions between species richness and all other terms in the TIB models (Table *S2.1*, *Supporting Information S2*).

Top-down Models

Next, we tested the hypotheses that top-down effects decrease the probability that a new immigrant survives long enough to be observed and increase extinction probabilities for species that have already been observed. This was done by adding a term quantifying the presence of any of the focal species' predators during the focal census to the corresponding TIB models. We also included interaction terms between the presence of predators and all terms in the TIB models. In order to ensure that any observed effect of top-down interactions was distinct from the effect of species richness, we further compared each top-down model to a similar top-down & species-richness model which included all terms in the top-down model, as well as terms for species richness and interactions between species richness and all other terms in the top-down model (Table *S2.1*, *Supporting Information S2*).

Bottom-up Models

To test the bottom-up hypothesis that the ability to eat basal resources, having access to animal prey, or both, will increase a species' initial or repeat immigration probability, we created a statistical model that combined all of the terms in the corresponding TIB model with new terms that quantify whether or not the focal species consumes basal resources, whether or not any of the focal species' animal prey were available during the focal census, and their interaction. The bottom-up model also included interactions between terms in the TIB model and the terms describing bottom-up effects. As with the top-down model, we ensured that species-richness and bottom-up effects were distinct by comparing each bottom-up model to a bottom-up & species-richness model including all terms in the bottom-up model, terms for species richness, and interactions between species richness and all other terms in the bottom-up model (Table *S2.1*, *Supporting Information S2*).

Top-down & Bottom-up Models

Finally, we tested the possibility that top-down and bottom-up effects act synergistically. To do this, we examined a top-down & bottom-up model including all of the terms in the bottom-up model as well as terms for the presence of predators and interactions between the presence of predators and all terms in the bottom-up model. In keeping with the spirit of elegant simplicity of the original TIB, we did not include terms for species richness in this model (Table *S2.1*, *Supporting Information S2*). This decision was supported by our finding that the trophic & species-richness models described in *S2.1*, *Supporting Information S2* were all very similar to the trophic-only models (see *S2.4 & S2.5, Supporting Information S2*).

Model simplification

For each of the aforementioned statistical models, we started by fitting the most complex models including all interactions. Where a full model was non-convergent (i.e., parameter estimates could not be robustly determined, indicative of over-fitting), we removed all interactions of the highest order (e.g., 6-way interactions) and attempted to re-fit the model; we repeated this procedure (i.e., removing 5-way interactions, etc.) until we obtained a convergent model from which we could proceed with simplification. We then measured the AIC of these "full" models as well as each of the suite of potential simplified models. Simplified models were obtained by systematically removing all possible combinations of terms from the full model. When an interaction term was included in a simplified model, all main effects involved in that interaction term were also retained.

Once the AIC of each model was calculated, we selected the model with the lowest AIC as the best-fitting model. We performed this simplification automatically using the R (R Core Team, 2014) function dredge from package MuMIn (Bartón, 2014). We then used the R (R Core Team, 2014) function glmer from the package lme4 (Bates et al., 2014) to estimate the standardised effects (βs) for each fixed effect in the best-fitting models as well as their corresponding p-values. Note that all standardised effects presented in the results reflect the per-unit (e.g., per 1m increase in diameter) impact of each predictor on logit-transformed initial immigration, repeat immigration, or extinction probability.

Hypothesis Comparison

We also wished to quantify the degree to which different hypotheses give similar predictions across the dataset. If the specific predictions of the species-richness and top-down models for extinction agree, for example, this would indicate that the effect of species richness on extinction rates is capturing the same variability in the data as does the effect of predators. To compare the models and hypotheses in this way, we first generated 10,000 simulated datasets for each model using the R (R Core Team, 2014) function rbinom and the models' predicted probabilities of immigration or extinction. If, for example, a given model predicted that species *s* on island *i* at census *k* had an immigration probability of 0.005, approximately 50 of the simulated immigration events would be successful. Next, we used the best-fit parameters of the various models (when fit to the empirical data) to calculate the likelihood of observing each simulated dataset. We repeated this procedure for each pair of initial immigration, repeat immigration, and extinction models, including comparisons of every model to itself, producing 10,000 likelihoods for each pairwise comparison.

To quantify the degree of similarity between the set of likelihoods obtained when data generated using model A were fit by model A to those obtained when the same data were fit by a different model B, we calculated the area under the receiver operating characteristic (ROC) curve. The area under the curve (AUC) represents the probability that a randomly chosen likelihood for model A is greater than a randomly-chosen likelihood from model B. When models A and B explain exactly the same variation in the data, and therefore fit data generated by A or B equally well, AUC=0.5; as model B's ability to fit data generated by model A decreases, the AUC increases towards 1. An AUC close to 0.5 therefore indicates that the two models explain very similar variation while an AUC close to 1 indicates that the models account for very different variation.

Results

Initial Immigration

The best-fit versions of all alternate models for initial immigration had significantly lower AIC's than the null model and explained greater variance (Table 3A). The best-fit species-richness, top-down, bottom-up, and top-down & bottom-up models all provided significantly better fits to the data than the TIB model (χ^2=8.97, df=2, p=0.011; χ^2=8.68, df=3, p=0.034; χ^2=11.7, df=4, p=0.020; and χ^2=16.425, df=5, p=0.006, respectively). The top-down & bottom-up model provided the best fit to the data, and significantly improved upon both the top-down and bottom-up models (χ^2=7.74, df=2, p=0.021 and χ^2=4.74, df=1, p=0.029).

In the top-down & bottom-up model, and similar to the other models, a species' probability of immigration decreased with increasing distance from the mainland ($\beta_{Distance}$=-56.3) and increased with increasing intervals between censuses (β_{Time}=18.1, Fig. 7; Table

Model							
(A) Initial immigration							
Effect	TIB	SR	TD	BU	TD & BU	TD & SR	BU & SR
Dist.	-	-	-	-	-	-	-
Diam.	+	+	-	-	-	+	+
Time	+	+	+	+	+	+	+
Species richness		+				+	+
Predators			+		+	0	
Animal prey				+	+		+
Dist.:Diam.	+	+	+	+	+	+	+
Dist.:Animal				+	0		0
Diam.:Species		+				+	+
Diam.:Predators			+		+	0	
Diam.:Animal				+	+		+
Time:Predators			-		-	0	
Dist.:Diam.:Animals				+	0	0	0
AIC	4271	4266	4268	4267	4264	4266	4264
Marginal R^2	0.061	0.068	0.070	0.070	0.075	0.068	0.072
Conditional R^2	0.213	0.214	0.214	0.228	0.223	0.214	0.222

NB: The best-fit TD & SR model was identical to the SR model. The marginal R^2 of the Null model was 0 and the conditional R^2 of the Null model was 0.169.

(B) Repeat immigration							
Effect	TIB	SR	TD	BU	TD & BU	TD & SR	BU & SR
Diameter	-	-	-	+	+	-	+
Time	-	-	-	-	-	-	-
Basal resources				-	-		-
Diameter:Time	-	-	-	-	-	-	-
Diameter:Basal				-	-		-
Time:Basal				+	+		+
AIC	922	922	922	912	912	922	912
Marginal R^2	0.026	0.026	0.026	0.060	0.060	0.026	0.060
Conditional R^2	0.141	0.141	0.141	0.222	0.222	0.141	0.222

NB: The best-fit SR, TD, and TD & SR models were identical to the TIB model, while the best-fit TD & BU and BU & SR models were identical to the best-fit BU model. The marginal R^2 of the Null model was 0 and the conditional R^2 of the Null model was 0.148.

(C) Extinction							
Effect	TIB	SR	TD	BU	TD & BU	TD & SR	BU & SR
Diameter	+	-	+	0	0	-	+
Time	+	+	+	+	+	+	+
Species richness		+				+	+
Basal resources				-	-		-
Animal prey				+	+		-
Diameter:Time	+	-	+	0	0	-	0
Diameter:Species		-				-	-
Time:Species		+				+	+
Time:Basal				-	-		-
Time:Animal				0	0		-
Species:Basal							-
Basal:Animal				-	-		0
Diameter:Time:Species		+				+	0
AIC	1912	1904	1912	1874	1874	1912	1864
Marginal R^2	0.114	0.153	0.114	0.231	0.231	0.114	0.251
Conditional R^2	0.296	0.373	0.296	0.497	0.497	0.296	0.524

NB: The best-fit TD and TD & SR models were identical to the best-fit TIB model, while the best-fit TD & BU model was identical to the best-fit BU model. The marginal R^2 of the Null model was 0 and the conditional R^2 of the Null model was 0.325.

Table 3: Terms included in the best-fit models for A) initial immigration, B) repeat immigration, and C) extinction when comparing a null model (not shown), a model based on the Theory of Island Biogeography (TIB), and models based on the TIB that also include effects of species-richness (SR), top-down interactions (TD), bottom-up interactions (BU), top-down & bottom-up interactions (TD & BU), top-down interactions & species-richness (TD & SR), or bottom-up interactions & species-richness (BU & SR). In all cases, 'Dist.' is short for distance and 'Diam.' is short for diameter. Each '+' indicates a positive effect, '-' indicates a negative effect, and a '0' indicates that the effect was not included in the best-fit model. An empty cell indicates that the term was not part of the model and hence could not appear in the best-fit version. For the full list of terms included in each model, see *S2.1, Supporting Information S2*. Below the individual effects, we give the Akaike Information Criterion (AIC) and marginal and conditional R^2 values for each model, where marginal R^2 is the amount of variance explained by a model's fixed effects and conditional R^2 is the amount of variance explained by both fixed and random effects (Nakagawa and Schielzeth, 2013). Sample size for all initial immigration models was 18,420 opportunities for species to immigrate, for all repeat immigration models was 1,813 opportunities for species to re-immigrate following an extinction, and for all extinction models was 1,943 opportunities for species to go extinct.

S2.7). Unlike in the TIB model, a species' probability of immigration decreased with increasing island size

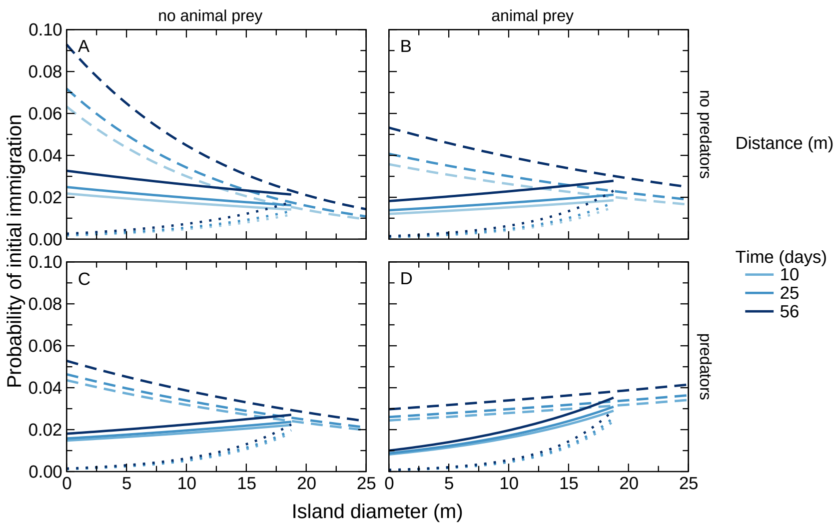

Figure 7: Per-species probabilities of initial immigration in the top-down & bottom-up model were affected by the presence of animal prey, the presence of predators, island diameter, distance from the island, and time between censuses (based on N=18,420 potential initial immigrations). In each panel, we show the model predictions for different scenarios with line colour indicating island distance and line type indicating interval between census. Light lines are for islands close to the mainland (2m), medium lines for moderately isolated islands (163m), and dark lines for very isolated islands (533m). Similarly, dashed lines are for the lowest observed interval between censuses (10 days), solid lines for the mean interval between censuses (25 days), and dotted lines for the mean interval between censuses plus one standard deviation (56 days). **(A)** When neither predators nor animal prey were present, predicted immigration probability decreased with increasing island diameter except for islands that were farthest from the mainland. **(B) & (C)** The presence of either animal prey or predators weakened this trend such that immigration probability increased with island diameter for all islands except those closest to the mainland. **(D)** When both animal prey and predators were present, immigration probability increased with increasing island diameter for all islands. In all cases, increasing the time between censuses increased the probability of immigration. As no large islands were observed at moderate to high degrees of isolation, the corresponding predictions are truncated to reflect the observed range only.

($\beta_{Diameter}$=-0.711), but this effect was overwhelmed by a positive interaction between distance and diameter ($\beta_{Distance:Diameter}$=333). Probability of immigration also increased for species with either predators or animal prey present. Both of these trends were stronger on larger islands ($\beta_{Diameter:Predators}$=1.29, $\beta_{Diameter:Animal}$=1.32).

Despite the statistical improvement of the other alternate models over the TIB, each model described data generated by any of the others well (Fig. 8; Fig. *S2.2*). In addition, each alternative model provided a good fit to data generated by the null model, and vice versa. This means that all models captured similar variation in the empirical data; the extra terms in the alternative models therefore may represent over-fitting.

Repeat Immigration

The best-fit versions of all alternate models for repeat immigration had lower AIC's and explained greater variance than the null model (Table 3B), although the TIB model did not significantly improve on the null model (χ^2=6.09, df=3, p=0.107). The best-fit species-richness and top-down models were identical to the best-fit TIB model, while the best-fit top-down & bottom-up model was identical to the best-fit bottom-up model (*S2.2*, *Supporting Information S2*). Contrary to our expectations, none of the best-fit alternate models included any effects of distance from the mainland on repeat immigration. The bottom-up model provided the best fit to the data, significantly improving upon the fits of the null and TIB models (χ^2=22.4, df=6, p=0.001, and χ^2=16.0, df=3, p=0.001, respectively).

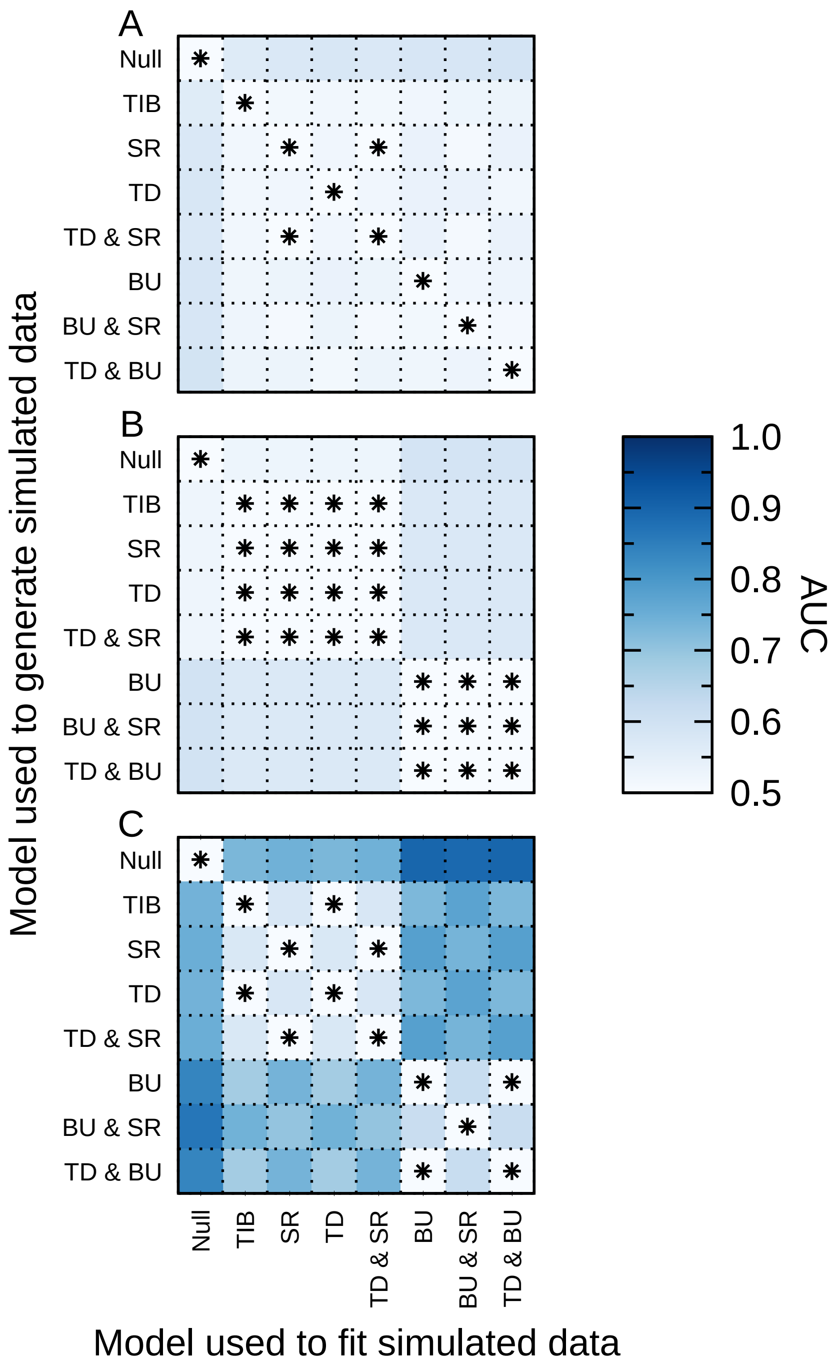

Figure 8: Hypothesis comparison of best-fit statistical models based on the AUC statistic. **(A & B)** All best-fit models for initial immigration generated very similar predictions, as did all models for repeat immigration. **(C)** Among best-fit models for extinction probability, there were two clusters of models which generated predictions that were similar to each other but distinct from those in the other cluster. In all panels, comparisons are made between a Null model, a model based on the Theory of Island Biogeography (TIB), and models based on the TIB that also include effects of species richness (SR), top-down interactions (TD), top-down interactions and species richness (TD & SR) , bottom-up interactions (BU), bottom-up interactions and species richness (BU & SR), or top-down & bottom-up interactions (TD & BU). Each cell containing an asterisk indicates that two best-fit models were identical.

Again contrary to our expectations, a species' probability of repeat immigration in the bottom-up model decreased as the interval between censuses increased (β_{Time}=-76.8, Fig. 9, Table *S2.8*). This effect was stronger on larger islands, but weaker for species able to consume basal resources ($\beta_{Diameter:Time}$=-431; $\beta_{Time:Basal}$=-2.52).

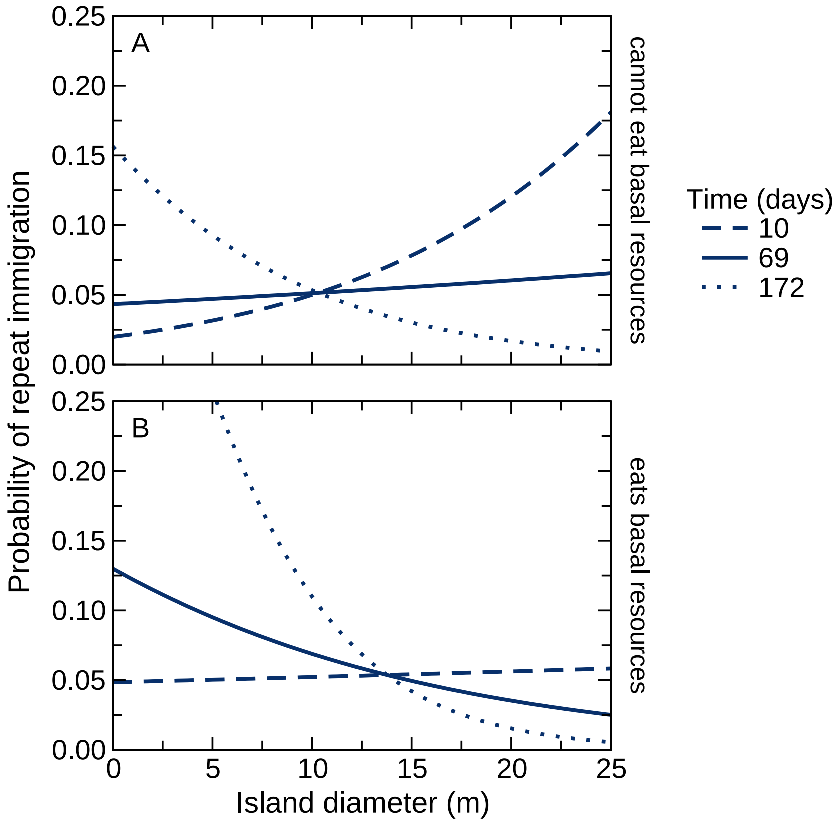

Figure 9: Per-species probabilities of repeat immigration in the bottom-up model were affected by the ability to consume basal resources, island diameter, and interval between censuses (based on N=1,813 opportunities for species to re-immigrate). In both panels, we show model predictions for different scenarios with line type indicating interval between census; dashed lines are for the lowest observed interval between censuses (10 days), solid lines for the mean interval between censuses (69 days), and dotted lines for the mean interval between censuses plus one standard deviation (172 days). **(A)** For species unable to consume basal resources, repeat immigration probability increased with increasing island diameter except when the interval between censuses was very large. **(B)** For species able to consume basal resources, repeat immigration probability increased with increasing diameter when the interval between censuses was short and decreased with increasing island diameter when the interval between censuses was moderate to large.

Species able to consume basal resources were, however, less likely to immigrate to larger islands ($\beta_{Diameter:Basal}$=-2.52).

Despite the statistical improvement of the bottom-up model over the null and TIB models, all models captured very similar variation in the empirical data (Fig. 8). Similarly, while the bottom-up model explained significantly greater variance than the null model (Table 3B), this increase was relatively small. This suggests that the additional terms in the bottom-up model may indicate over-fitting, and that its counterintuitive predictions may be spurious.

Extinction

Compared to the initial and repeat immigration models, the best-fit alternate models for extinction showed much greater improvements over the extinction null model (Table 3C). The best-fit top-down model was identical to the best-fit TIB model and the best-fit top-down & bottom-up model was identical to the best-fit bottom-up model (*S2.2, Supporting Information S2*). In addition, the best-fit

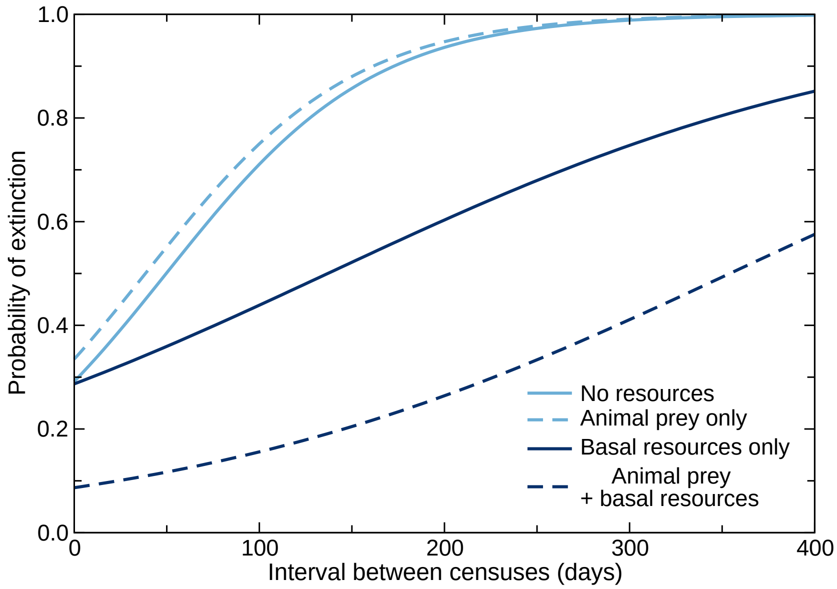

Figure 10: Per-species probabilities of extinction in the bottom-up model were affected by the presence of animal prey, the ability to eat basal resources, and time between censuses (based on N=1,943 opportunities for species to go extinct). **(A)** For species unable to eat basal resources, extinction probability increased rapidly with interval between censuses. Extinction probability saturated near 1 after roughly 300 days. Species with animal prey available were slightly more likely to go extinct. **(B)** Species able to eat basal resources had lower probabilities of extinction overall, and probability of extinction increased more slowly with interval between censuses. Species with both basal resources and animal prey available were least likely to go extinct.

species-richness and bottom-up models both improved significantly on the best-fit TIB model (χ^2=16.6, df=4, p=0.002 and χ^2=41.9, df=2, p<0.001, respectively).

The effects included in the alternate extinction models varied a great deal. Notably, the bottom-up model did not include any effects of island diameter. The TIB and species-richness models both did, although the TIB model predicted that species where more likely to go extinct on larger islands while the species-richness model predicted the opposite trend (Table *S2.8*). The bottom-up model predicted that probability of extinction would be lower for species able to eat basal resources, especially those which also had access to animal prey, but that species with access to animal prey only would be more likely to go extinct (β_{Basal}=-0.470, β_{Animal}=-1.64, $\beta_{Basal:Animal}$=0.201; Fig. 10).

As a consequence of the significant trophic effects included in the bottom-up model, it described data generated by the null, TIB, and species-richness models poorly, and vice versa (Fig. 8). This suggests that adding bottom-up effects and removing the effect of diameter allowed this model to capture different variation in the data than that accounted for by the other models. While the model containing both bottom-up and species-richness effects provided a significantly better fit to the data than the bottom-up model (χ^2=19.5, df=5, p=0.002), it nevertheless captured very similar variation in the

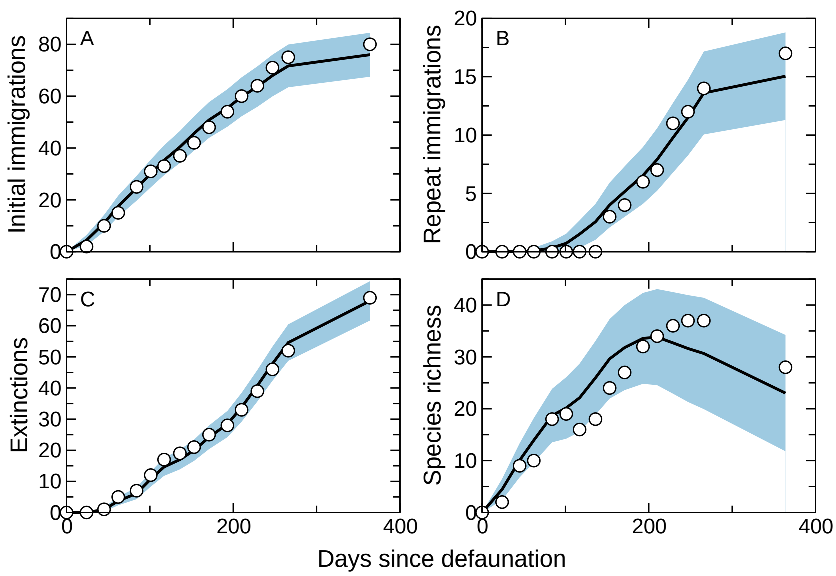

Figure 11: Initial immigrations, repeat immigrations, extinctions, and species richness over time for a representative island (island E9, 18m in diameter, 379m from the mainland). **(A)-(D)** We show the cumulative values for the observed experiment (white circles) along with the equivalent values as predicted by the the best-fitting models for initial immigration, repeat immigration, and extinction (i.e., species-richness, bottom-up, and bottom-up models, respectively). We obtained the model predictions for total species richness at each census by adding predicted immigrants and subtracting predicted extinctions. In all panels, the solid line indicates the mean prediction while the shaded area corresponds to one standard deviation. Comparable figures for all other islands can be found in *S2.6, Supporting Information S2*.

data (average pairwise AUC=0.618; Fig. *S2.5*) As such, we expect that the extra terms in the bottom-up & species-richness model may constitute over-fitting.

Discussion

We compared statistical models based on several factors predicted to affect per-species probabilities of initial immigration, repeat immigration, or extinction in the context of island biogeography theory. In our dataset, species richness generally had little impact on immigration or extinction. Top-down and/or bottom-up effects, however, were included in each best-fit model. When directly compared to the empirical data, it is apparent that each of our best-fit models provides an excellent fit to the observed sequence of initial immigrations, repeat immigrations, and extinctions on all islands (Fig. 11 and *S2.6, Supporting Information S2*). This success of our trophic TIB models therefore stands in contrast to previous examinations of these same data where, when focusing on changes in species richness over time, it has been suggested that stochastic models of immigration and extinction may accurately describe the system (Simberloff, 1969; Simberloff and Wilson, 1969) and that colonisation as a whole does not depend on trophic interactions (Simberloff and Simberloff, 1976). These differences also suggest that considering immigration and extinction separately provides an extra level of detail which allows us to better disentangle the underlying ecology of island biogeography.

Although the best-fitting initial and repeat immigration models showed varying structures (for example, there was evidence that initial immigration varied with the availability of animal prey and repeat immigration with the ability to consume basal resources), they generated very similar predictions for patterns of immigration. This indicates that our expectations that island characteristics and interactions between species would affect immigration probabilities were incorrect. In particular, the prediction– based on the TIB (MacArthur and Wilson, 1963) –that immigration probability would decline with increasing distance from the mainland was ultimately not supported in this system. One possible explanation is that many of the arthropods in this system are highly mobile and can easily reach all of the mangrove islands in this study (Simberloff, 1969). This scenario would appear even more likely because potential colonists were restricted to arthropods that were observed on the islands prior to defaunation (Wilson and Simberloff, 1969), meaning that they were all previously successful immigrants.

Alternatively, it is possible that immigrants in this system are not arriving from the mainland but rather from other mangrove islands. There are many small mangrove islands in the area of the study islands that could serve as sources of colonists in addition to the mainland (see maps in Wilson and Simberloff, 1969). As the source of arthropod immigrants was not determined, the distance from each island to the mainland may not always be the best reflection of the distance immigrants actually travelled. In this regard, the mangrove islands in this study are quite different from isolated oceanic islands but similar to habitat patches which interact both amongst each other and with a larger source habitat. Limitations of the TIB when dealing with complex geographies are well known (Hanski, 2010), and the inability of the TIB to account for multiple sources of colonists (Hanski, 2010), the existence of predator-free refuges (Ryberg et al., 2012), or varying island-mainland geographies (Taylor, 1987) may all contribute to the relatively poor fit of TIB-based immigration models to this dataset. They may also help to explain the apparently stochastic immigration patterns observed here.

Just as the expected distance effects were not observed in the immigration models, the best-fitting extinction model did not include the expected effect of island diameter. It is possible that the islands in this study were similar enough in size that arthropod population sizes did not vary greatly between islands, or that other factors had stronger effects. For example, populations on small islands might be maintained by occasional arrivals from the mainland (i.e., the 'rescue effect'), preventing extinctions. While the bottom-up model for extinction did not include any effect of island diameter, it did include effects for the ability to consume basal resources and the presence of animal prey which suggest that, all else being equal, having access to both plant and animal prey makes extinction less likely than having access to only one type of resource.

The synergistic effects of basal and animal resources are surprising in light of the fact that many arthropod species form part of the aerial plankton in the region (Simberloff, 1969), and others such as *Diptera* that were seen on the islands were not recorded during the experiment (Simberloff, 1969). As such, recorded animal prey may have been only a small part of the diet of even obligate insectivores. The strength of the observed effects therefore strongly suggests that bottom-up effects provide a promising avenue for extending the TIB, in agreement with previous work (Gravel et al., 2011). The reduction in extinction probability where both types of resources were available also suggests that prey switching between basal resources and animal prey may be particularly important in determining extinction probabilities (Murdoch, 1969; Coll and Guershon, 2002) as well as potentially influencing immigration order (Piechnik et al., 2008). It is also possible that the availability of many prey species might encourage further migration from the mainland and provide stronger rescue effects for these species.

Overall, our results suggest that incorporating bottom-up interactions provides the greatest improvement over the classic TIB. However, we note that our relatively weak results for top-down effects contrast with the strong effects of predators observed in other island systems (Spiller and Schoener, 1994; Kotiaho and Sulkava, 2007; Spiller and Schoener, 2007). The apparent weakness of top-down effects in this system could be due to the presence of transient predators which were observed visiting the islands during the experiment but not recorded in the censuses because they do not breed on mangroves (Simberloff, 1969). The effects of these predators cannot be measured from the available data, but could potentially be large. Further complicating matters, the effects of *resident* arthropod predators are difficult to detect in this system because they were almost always present (Table 2), making the effects of predators a "black box" in this system. Given these caveats, and because a rich record exists of top-down and bottom-up effects acting simultaneously to structure mainland communities (Power, 1992; Amarasekare, 2008), we advocate that the potential for top-down effects still be considered along with bottom-up effects in any further attempts to combine food-web ecology and island biogeography: “two of the most important conceptual frameworks in community ecology” (Holt, 2010).

Acknowledgements

We thank Kate Wootton, Camille Coux, Jason Tylianakis, and Nick Baker for comments on the manuscript. This research was supported by an NSERC-PGS-M graduate scholarship and a University of Canterbury Doctoral Scholarship (to ARC) and a Marsden Fund Fast-Start grant (UOC-1101), administered by the Royal Society of New Zealand (to DBS). We are thankful to the BlueFern University of Canterbury Super Computer for computing facilities.

Supporting information

S2.1: Full models
S2.2: Best-fit models
S2.3: Summary tables for best-fit models
S2.4: Details of models not described in the main text
S2.5: Cumulative species richness plots for islands not shown in the main text

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Chapter 3: Conservation of interaction partners between related plants varies widely across communities and between plant families.

Alyssa R. Cirtwill[1], Giulio V. Dalla Riva[2], Nick J. Baker[1], Joshua A. Thia[1,3], Christie J. Webber[1], Daniel B. Stouffer[1]

[1]Centre for Integrative Ecology
School of Biological Sciences
University of Canterbury
Private Bag 4800
Christchurch 8140, New Zealand

[2]Biomathematics Research Centre
School of Mathematics and Statistics
University of Canterbury
Private Bag 4800
Christchurch 8140, New Zealand

[3]Present Address: School of Biological Sciences
University of Queensland
St Lucia
Brisbane, QLD 4072, Australia

Manuscript prepared in the style of *New Phytologist*.

This chapter has been modified from its original version. It has been formatted to match this thesis.

Summary

- Related plants are often hypothesised to interact with similar sets of pollinators and herbivores, but empirical support for this idea is mixed. Here we argue that this may be because some plant families vary in their tendency to share interaction partners.
- We introduce a novel approach with which to quantify overlap of interaction partners for each pair of plants in 59 pollination and 11 herbivory networks. We then tested for relationships between phylogenetic distance and overlap within each network, and whether these relationships varied with the composition of the plant community. Finally, we tested for different relationships within well-represented plant families.
- Across all networks, more closely-related plants tended to have greater overlap, and this tendency was stronger in herbivory networks than pollination networks. These relationships were also significantly related to the composition of the network's plant component. Within plant families, relationships varied greatly in both network types.
- The variety of relationships between phylogenetic distance and interaction partners in different plant families likely reflects a variety of ecological and evolutionary processes. To understand the distribution of interactions within a community, it is therefore important to consider factors affecting particular plant families.

Keywords

defensive syndrome, ecological networks, herbivory, niche overlap, phylogenetic signal, pollination, pollination syndrome, specialisation

Introduction

Interactions with animals affect plants' life cycles in several critical ways (Mayr, 2001; Sauve et al., 2015). On one hand, pollination and other mutualistic interactions contribute to the reproductive success of many angiosperms (Ollerton et al., 2011). On the other, herbivores consume plant tissues (McCall and Irwin, 2006) which costs plants energy and likely lowers their fitness. In both cases, these interactions do not occur randomly but are strongly influenced by plants' phenotypes. For example, plants that produce abundant or high-quality nectar may receive more visits from pollinators (Robertson et al., 1999) whereas plants that produce noxious secondary metabolites may suffer fewer herbivores (Johnson et al., 2014). A plant's traits are also likely to determine which specific pollinators and herbivores interact with that plant. Plants with different defences (e.g., thorns vs. chemical defences) may deter different groups of herbivores (Ehrlich and Raven, 1964; Johnson et al., 2014), and the concept of pollination syndromes has often been used to group plants into phenotypic classes believed to attract certain groups of pollinators (Waser et al., 1996; Fenster et al., 2004; Ollerton et al., 2009).

If attractive and/or defensive traits are heritable, then we can reasonably expect that related plants will have similar patterns of interactions with animals (Schemske and Bradshaw, 1999). Recent studies that have investigated this question at the level of whole communities, however, have yielded mixed results (Rezende et al., 2007b; Gómez et al., 2010; Rohr and Bascompte, 2014; Fontaine and Thébault, 2015; Lind et al., 2015). In particular, significant phylogenetic signal in plants' interaction partners —the tendency for more closely-related plants to have more similar interactions— tends to be rare in empirical networks (Rezende et al., 2007b; Lind et al., 2015; but see Elias et al., 2013; Fontaine and Thébault, 2015). Further, plants' roles within networks tend to be less phylogenetically constrained than those of animals (Rezende et al., 2007b; Chamberlain et al., 2014c; Rohr et al., 2014; Vamosi et al., 2014; Lind et al., 2015).

Several mechanisms that might weaken the conservation of interactions have been identified in the literature. Pollination and herbivory may be affected by a wide variety of traits, and not all of these are likely to be phylogenetically conserved (Rezende et al., 2007a; Kursar et al., 2009). If, for example, floral displays are strongly affected by environmental conditions (Canto et al., 2004), then pollinators may not be predicted by plants' phylogenies. Even if the traits affecting pollination and herbivory are heritable, plants may experience conflicting selection pressures that weaken the overall association between plant phylogeny and interaction partners (Armbuster, 1997; Lankau, 2007; Siepielski et al., 2010; Wise and Rausher, 2013). For instance, floral traits that are attractive to pollinators can also increase herbivory (Strauss et al., 2002; Adler and Bronstein, 2004; Theis, 2006). Conversely, herbivory can reduce pollination by inducing chemical defences (Adler et al., 2006) or altering floral display or nectar availability (Strauss, 1997). Observed patterns of similarity in plants' interaction partners therefore represent a mixture of environmental effects and various selection pressures as well as plants' shared phylogenetic history.

A further complication is the possibility that the relationship between plants' relatedness and the similarity of their interaction partners is not constant across plant clades. Closely-related plants in one clade might be under strong selection to favour dissimilar sets of pollinators to avoid exchanging pollen with other species (Levin and Anderson, 1970; Bell et al., 2005; Mitchell et al., 2009). Similar pressures could also affect related plants' defences against herbivores if congeners tend to grow in the same places such that herbivores could easily move between them. Unrelated plants might also converge upon similar phenotypes, attracting a particularly efficient or abundant pollinator (Ollerton, 1996; Ollerton et al., 2009). Likewise, herbivores may be able to depredate sets of unrelated plants if they have evolved similar defences (Pichersky and Gang, 2000). In either case, dissimilarity of interactions among related species or similarity of interactions among unrelated species could result in low apparent phylogenetic signal across an entire plant community. Moreover, all of the aforementioned hypotheses are non-exclusive; different processes likely affect different clades, and these processes might be associated with different pressures imposed by pollination and herbivory.

Here we use a novel approach to investigate how the patterns of overlap in interaction partners between pairs of plants (henceforth "niche overlap") vary over phylogenetic distance. Whereas previous studies have focused on the presence or absence of phylogenetic signal across entire networks, we are able to investigate the relationship between niche overlap and phylogenetic distance in within networks as well as different plant families. Specifically, we test whether niche overlap decreases over increasing phylogenetic distance in a large dataset of pollination and herbivory networks, whether the plant family composition of a community affects the relationship between niche overlap and phylogenetic distance in that community, and whether the relationship between niche overlap and phylogenetic distance differs across plant families.

Materials and methods

Network data

We studied phylogenetic conservation of interactions within a set of 59 pollination and 11 herbivory networks. These networks span a range of biomes (desert to scrub forest to grassland) and countries (Sweden to Australia), and range in size between 18 and 996 total species (mean 160.93, median 96) with seven to 131 plant species (mean 38.06, median 28). To ensure that we were analysing interactions influenced by similar sets of traits across networks, we restricted our herbivory networks to insects consuming leaves. This excluded sap-sucking, leaf-mining, and galling insects as well as seed predators and xylophagous insects; all of these interactions involve different plant tissues and means of feeding than leaf consumption and so may be influenced by different plant and insect traits. We also excluded networks which focused on plants from a single genus as these did not contain sufficient variation in phylogenetic distance between plants. See *Table S3.1, Supporting Information S3* for details on the original sources of all networks.

Phylogenetic data

In order to fit the plant species in all networks to a common phylogeny, we first compared all species and genus names with the National Center for Biotechnology Information and Taxonomic Name Resolution Service databases to ensure correctness. This was done using the function 'get_tsn' in the R (R Core Team, 2014) package taxize (Chamberlain and Szocs, 2013; Chamberlain et al., 2014a). Species which could not be assigned to an accepted taxonomic name (e.g., 'Unknown Forb') were discarded, as were those with non-unique common names and no binomial name given (e.g., 'Ragwort) or binomial names that could not be definitively linked to higher taxa (e.g., '*Salpiglossus sp.*'). We were left with 2341 unique species in 1027 genera and 195 families. On average, 11.43% of plants were removed from each network (median 4.60%, range 0-55.10%).

We then estimated phylogenetic distances between species. To accomplish this, we constructed a phylogenetic tree for our dataset based on the phylomatic 'mega-tree' of higher plants (version

20120829; Reveal and Chase, 2011). Where possible, we dated nodes on the mega-tree according to Wikström et al. (2001). These dates included divergence times in millions of years (My) between families and within some families, but did not give dates for divergences within genera. For those nodes that were not included in the mega-tree, we used the branch length adjustment algorithm bladj (Webb et al., 2008) to estimate the ages of all undated nodes. This means that the ages in our phylogenies are approximations, but the presence of even a subset of properly dated nodes within a phylogeny improves upon undated, purely taxonomic approaches (Webb, 2007). To obtain trees for each network, we pruned the dated mega-tree to include only species in that network.

Calculating niche overlap within communities

To fully describe the extent to which two plants' niches overlap, we defined the overlap between two plants' sets of interaction partners by recording the frequencies with which pairs of animals (where each animal interacted with at least one plant) fall into three unique patterns (Fig. 12). In the first pattern, both plants interact with both animals, indicating total overlap for that quartet. In the second pattern, one plant interacts with both animal partners while the other interacts with only one animal, indicating partial overlap. In the third pattern, each animal interacts with only one plant, indicating no overlap. Taken together, the frequencies of these three patterns of overlap can be used to describe the degree to which two plants have similar interaction partners.

Using the three patterns defined above provides more detail than other measures of overlap, such as the proportion of one species' partners that are shared with another as given by Jaccard similarity. In particular, comparing the probability of observing each pattern rather than one of the other two provides a measure of indirect interactions between plants by considering pairs of animal partners rather than each animal separately. For example, a pair of plants which share two interaction partners are more likely to influence each other via these partners than two plants which do not share interaction partners. Moreover, our measure of overlap has greater statistical power than Jaccard dissimilarity because it includes information on the *number* of shared interaction partners as well as the proportion. For instance, a pair of plants which together interact with 100 animals provides more information about shared overlap than a pair of plants which together interact with only one animal whereas the Jaccard similarity of both would simply be one.

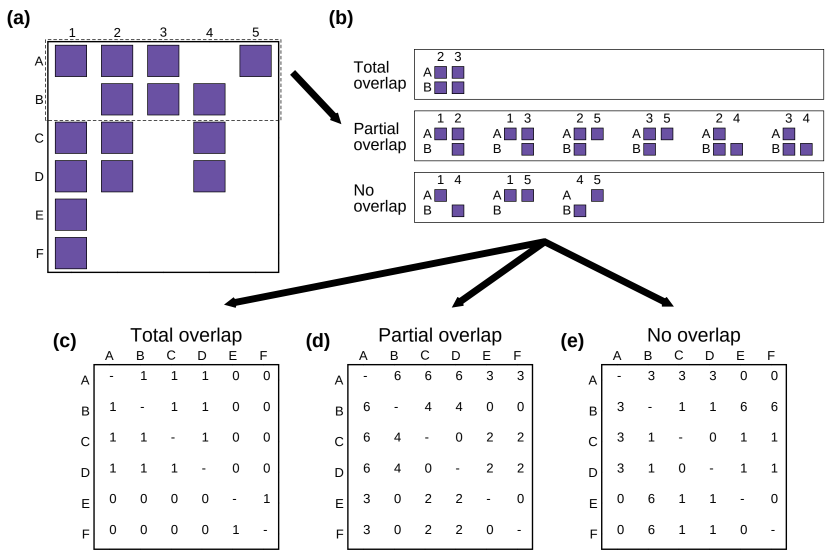

Figure 12: Visual depiction of our decomposition of pairwise niche overlap of plants' interaction partners. **(a)** First, consider the representation of any pollination or herbivory network as an adjacency matrix. Here, filled cells indicate an interaction between a particular plant (letters on rows) and an animal (numbers on columns). **(b)** For a given pair of plants (e.g., plants A and B), we then considered the set of animals that interact with at least one of the focal plants. Taking each pair of animals in this set in turn, we assigned the resulting quartet (the two focal plants plus two animals) to one of three patterns of overlap. In the **total overlap** pattern, both plants interact with both animals. In the **partial overlap** pattern, one plant interacts with both animals and the other plant interacts with only one. Finally, in the **no overlap** pattern each animal interacts with only one plant; note that this includes cases where both animals interact with the same plant (e.g., animals 1 and 5 and plant A) as well as cases where each animal interacts with a different plant (e.g., animals 1 with plant A and animal 4 with plant B). **(c-e)** The number of times each pattern occurred was used to summarise the pairwise niche overlap between the two plants and then related to their phylogenetic distance.

Statistical analysis

To determine how overlap of interaction partners breaks down over phylogenetic distance, we modelled the probabilities of observing each pattern of overlap relative to the other two patterns. We expected that the frequency of the high- and moderate-overlap patterns would decrease with increasing phylogenetic distance between two plants while the frequency of the low-overlap pattern would increase. As we expect pollination and herbivory networks could show different patterns of overlap, we included effects of network type and the interaction between network type and distance. Lastly, to account for the possibility that different communities show different characteristic relationships, we also included random effects of network ID on the slope and intercept, giving a mixed-effects logistic regression of the form

$$logit(\omega_{pnij}) \propto \delta_{ij} + \rho_n + \delta_{ij}\rho_n + N_n + \delta_{ij}N_n, \quad (1)$$

where ω_{pnij} is the probability of overlap pattern p occurring between species i and j in network n, δ_{ij} is the phylogenetic distance between plants i and j, ρ_n is the network type (one in pollination networks, zero in herbivory networks), and N_n and $\delta_{ij}N_n$ are random slope and intercepts for network n. All models were fit using R function glmer from package lme4 (Bates et al., 2014). Sample size for these models was the sum (over all pairs of plants) of the number of pairs of animals where each plant and each animal has at least one interaction partner. Over all networks, there were 43,288,090 such sets of plants and animals, with a median of 72 (mean 671 +/- 2247) pairs of animals per pair of plants and median 58,528 (mean 636,590) plant-animal sets per network.

Linking network-level trends and community composition

Next, we examined the connection between our network-level observations and the plant families present in each community. Specifically, we tested the hypothesis that varying relationships between phylogenetic distance and pairwise niche overlap are due to the different distributions of families across networks. To do this, we performed a non-parametric permutational multi-variate analysis of variance (PERMANOVA; Anderson, 2001) using the change in log odds of observing each pattern of overlap to predict the Bray-Curtis dissimilarity of networks based on the composition of their plant component (defined as the proportions of the plant community made up by each plant family present in the entire dataset). We used Bray-Curtis dissimilarity because, for a given pair of networks, only those plant families that appear in at least one network are considered (Anderson, 2001; Cirtwill and Stouffer, 2015; Chapter 5); that is, the absence of rare plant families will not make two networks appear more similar than they actually are.

Note that a PERMANOVA does not assume that the data are normally distributed, but rather compares the pseudo-F statistic calculated from the observed data to a null distribution obtained by permuting the raw data. As pollination and herbivory networks might have different community composition and the change in log odds of observing different patterns of overlap, we stratified these permutations by network type. That is, the change in log odds for a pollination network could only be exchanged for that of another pollination network. Stratifying the permutations in this way ensures that the null distribution used to calculate the P-value is not biased by including combinations of changes in log odds and community composition that would not occur because of inherent differences in the two network types (e.g., *Pinaceae* only appeared in herbivory networks and should not be assigned to pollination networks). We used 9999 such stratified permutations to obtain the null distribution and obtain a P-value.

The PERMANOVA tests whether there is an association between community composition and network-level patterns but does not give any information on *which* plant families have the greatest effects. To address this, we supplemented the PERMANOVA with three constrained correspondence analyses (CCAs) which placed plant families along an axis representing the change in log odds of observing each pattern of overlap. A correspondence analysis (CA) is similar to other multivariate analyses such as principal components analysis in that it reduces multivariate data to a set of orthogonal axes. A subset of axes that explain the majority of variation in the data can then be interpreted to elucidate trends that were difficult to interpret in the full multivariate space. A constrained correspondence analysis (CCA) creates an extra axis based on some constraint - in this case, the change in log odds of observing each pattern of overlap.

Calculating niche overlap within families

Finally, we wished to compare the breakdown of overlap of interactions in different plant families. To do this, we used the same definitions of overlap and phylogenetic distance as in the within-network analysis but restricted our regressions to pairs of plants from the same family and the same network. In order to fit our regression models, we had to eliminate any family-network combination where there was no variation in the probabilities of any pattern of overlap or in phylogenetic distance. This occurred, for example, when all plant pairs in a given family in a given network were taken from the same genus (as divergence times in our dataset were not resolved within genera; Wikström et al., 2001). Unlike in our previous analysis, we analysed data from pollination and herbivory networks separately as most well-represented plant families appeared in only one network type. For those families which appeared in both network types, we ran separate analyses on each subset of data.

For each plant family, within each network type, we then fit one of two similar sets of models. First, when family f was found in several networks of network type t, we fit mixed-effects logistic regressions for each pattern of overlap ω_{pntfij} of the form

$$logit(\omega_{pntfij}) \propto \delta_{ij} + N_n, \qquad (2)$$

where ω_{ptnfij} is the probability of overlap pattern p in network n of network type t for plants i and j in plant family f, δ_{ij} is the phylogenetic distance between plants i and j, and N_n is a random effect of network n. Second, if a plant family was represented in only one network and therefore necessarily in only one network type, we omitted the network-level random effect giving mixed-effects logistic regressions of the form

$$logit(\omega_{pntfij}) \propto \delta_{ij}. \qquad (3)$$

We fit Eq. 2 using the function 'glmer' from the R package lme4 (Bates et al., 2014) and fit Eq. 3 in R using the function 'glm' from the same package.

Results

Within-network conservation of niche overlap

Overlap of interaction partners decreased significantly with increasing phylogenetic distance in pollination networks ($\beta_{\delta+\delta\rho}$ = -17.14 My^{-1}, P<0.001 for total overlap; $\beta_{\delta+\delta\rho}$ = -9.47 My^{-1}, P<0.001 for partial overlap). In herbivory networks, these negative relationships were even stronger (β_{δ}=-40.81 My^{-1}, P<0.001; β_{δ}=-16.47 My^{-1}, P<0.001 for total and partial overlap, respectively). In both cases, the trends for the no-overlap pattern were opposite to those described above, as expected (see *S3.2*, *Supporting Information S3* for details). That is, a pair of plants in the same genus was more likely to share interaction partners than a pair of plants in the same family in both types of networks, but a pair of congeners would be less likely to share pollinators than to share herbivores.

Further, plants were slightly less likely to share pollinators than herbivores regardless of their phylogenetic distance (β_{ρ}=-0.94, P=0.014 and β_{ρ}=-0.40, P=0.110 for total and partial overlap, respectively). This may be due to the greater proportion of specialist pollinators than specialist herbivores. In our dataset, an average of 48% (+/- 14) of pollinators in a given web were extreme specialists (i.e., visited only one plant species) compared to 29% (+/- 29) of herbivores (z=5.62, df=68, P<0.001 for a binomial regression of specialists and generalists over network type).

Despite these general trends, there was substantial variation between pollination networks, with overlap of interaction partners decreasing with increasing phylogenetic distance in some networks and increasing in others (Fig. 13). For example, the probability of observing the no-overlap pattern ranged from approximately 0.3 to over 0.95 over the entire range of divergence times observed in our dataset. Herbivory networks were less variable in the directions of relationships between overlap and phylogenetic distance, but there was nevertheless a great deal of variation in probabilities across networks. In one network, for instance, the probability of observing the no-overlap pattern increased from 0.1 to 0.7 over 800 My of divergence time while in other networks the probability was much more constant. Overall, overlap of interaction partners decreased with increasing phylogenetic distance in 77% of pollination networks ($\beta_{\delta+\delta\rho+\delta N}$<0 in 46 of 59 networks for total overlap and in 45 of 59 networks for partial overlap). All herbivory networks, on the other hand, showed decreasing overlap with increasing phylogenetic distance ($\beta_{\delta+\delta N}$<0 in 11 of 11 networks for total and partial overlap).

Linking network-level trends and community composition

In each PERMANOVA, the change in log odds of observing a given pattern of overlap in a given network was significantly associated with the composition of the plant community in that network ($F_{1,68}$=1.79, P=0.019; $F_{1,68}$=1.92, P=0.010; and $F_{1,68}$=1.81, p=0.015 for total overlap, partial overlap, and no overlap, respectively). In the CCAs of community composition constrained by the change in log

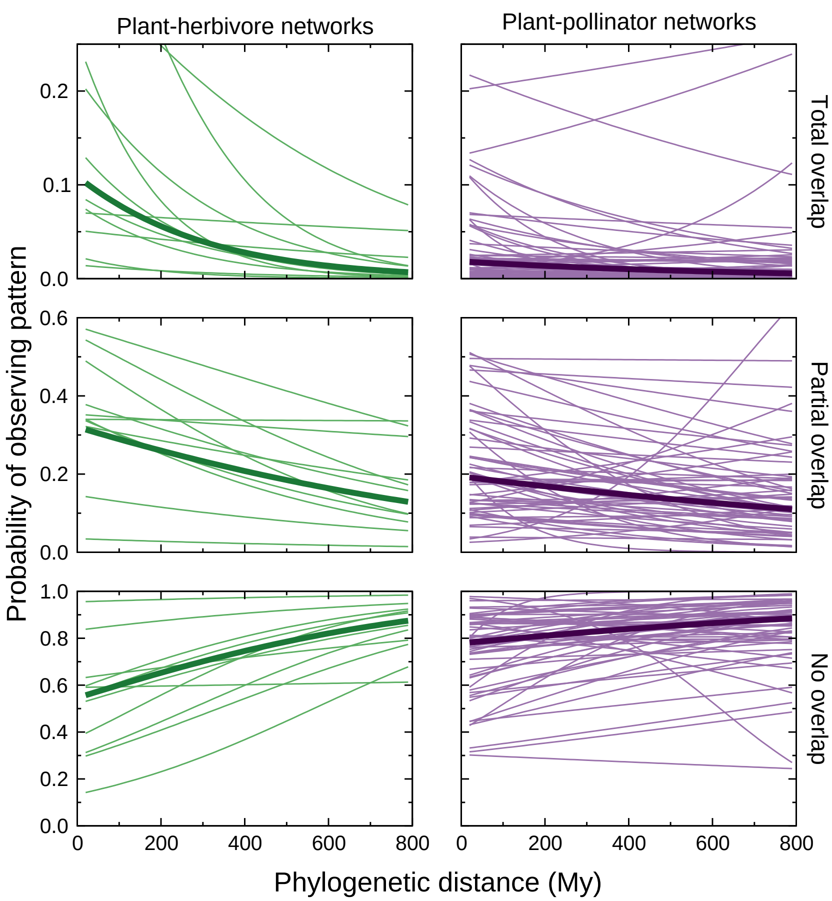

Figure 13: Results of a mixed-effects logistic regression of pairwise niche overlap against phylogenetic distance for plants in 11 herbivory networks (left; green) and 59 pollination networks (right; purple). In both network types, the probability of observing the total and partial overlap patterns tended to decrease as phylogenetic distance increased while the probability of the no-overlap pattern tended to increase (thick, dark lines). There was also substantial variation between individual networks (thin, pale lines) of both types. The model is described in Eq. 1.

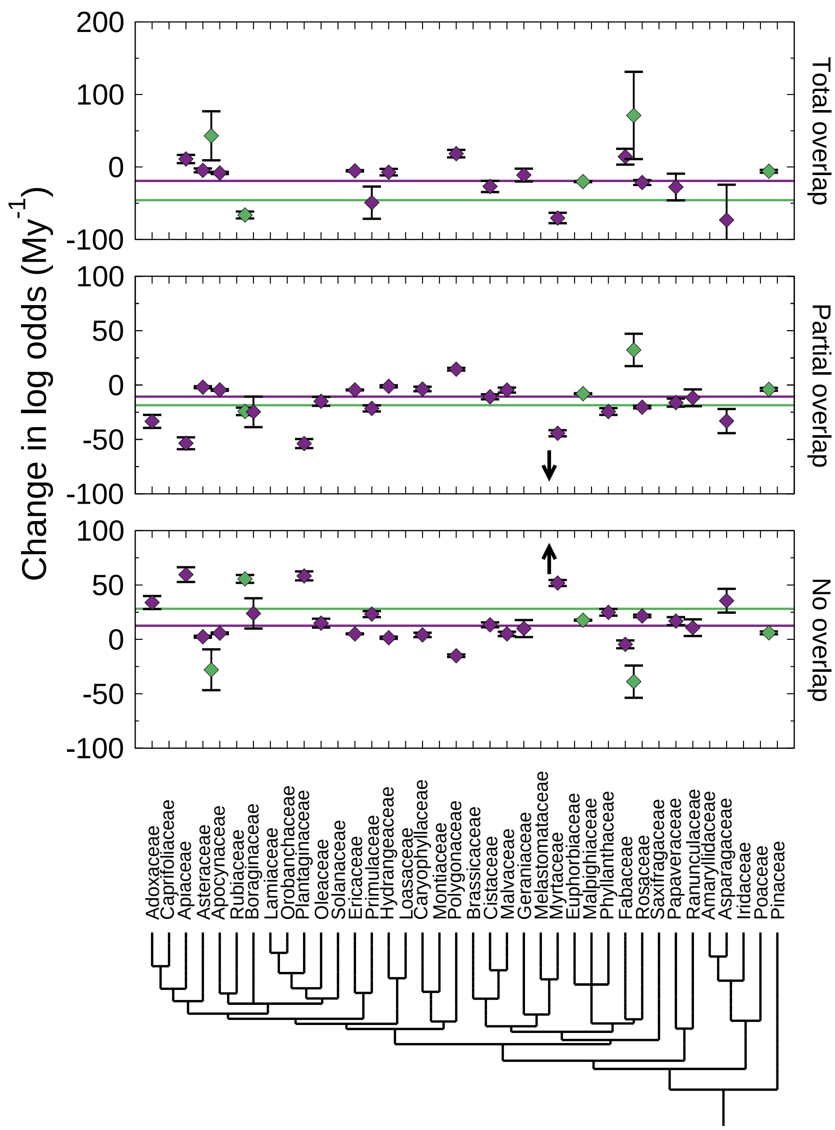

Figure 14: Change in log odds of observing different patterns of pairwise niche overlap per million years of divergence time between a pair of plants in 38 separate plant families. Families in pollination networks are indicated by dark purple diamonds while families in herbivory networks are indicated by pale green circles. Note that changes in log odds are analogous to the slopes of the regression lines from Eq. 2-3 in logit-transformed space and represent the change in the probability of observing a pattern of overlap per million years of divergence time. We also show the slope of the relationship between the log-odds of observing each overlap pattern and phylogenetic distance across all plant families in herbivory (pale, green horizontal line) and pollination (dark, purple horizontal line) networks. Arrows indicate significant values for *Melastomataceae* in herbivory networks which fell outside the plot margins. See Figure *S3.1; Supporting Information S3* for more details. The phylogenetic tree below the plots indicates the relatedness between plant families. Error bars represent 95% confidence intervals.

odds of observing each pattern of overlap, the largest decreases in partial overlap with increasing phylogenetic distance were associated with *Begoniaceae*, *Gleicheniaceae*, *Myricaceae*, *Siparunaceae*, and *Apocynaceae* (the ordering of plant families was qualitatively similar for total overlap and no overlap– see *S3.3, Supporting Information S3*). The largest increases in partial overlap with increasing phylogenetic distance were associated with *Surianaceae*, *Malpighiaceae*, *Goodeniaceae*, *Plumbaginaceae*, and *Resedaceae*.

Table 4: Change in log odds (per million years of phylogenetic distance) of observing total, partial, or no overlap in herbivores between a pair of plants in the same family.

Family	Total overlap		Partial overlap		No overlap	
	Odds ratio	*P*-value	Odds ratio	*P*-value	Odds ratio	*P*-value
Asteraceae	42.96	**0.013**	2.04	0.820	-27.95	**0.004**
Rubiaceae	-66.12	**<0.001**	-24.24	**<0.001**	55.60	**<0.001**
Melastomataceae	-528.51	0.190	-923.08	**<0.001**	905.82	**<0.001**
Euphorbiaceae	-20.29	**<0.001**	-7.92	**<0.001**	17.60	**<0.001**
Fabaceae	71.05	**0.021**	32.33	**<0.001**	-38.87	**<0.001**
Poaceae	-5.91	**<0.001**	-3.97	**<0.001**	6.01	**<0.001**
Pinaceae	-47.11	0.351	-13.22	0.340	16.95	0.215

Only seven plant families were sufficiently diverse in our dataset to permit this analysis (see *Materials and Methods* for details). For each pattern of overlap, we show the change in log odds per million years and the associated *P*-value. Statistically significant values are indicated in bold.

Within-family conservation of niche overlap

The relationship between within-family niche overlap and phylogenetic distance varied widely in both pollination and herbivory networks. For the families that were well represented in pollination networks, overlap decreased with increasing phylogenetic distance in 18 (Table 5). There was no significant relationship between overlap and phylogenetic distance in a further 15 plant families. Finally, the overlap between pairs of *Polygonaceae* increased with increasing phylogenetic distance. Of the seven plant families that were sufficiently well represented in herbivory networks, overlap decreased with increasing phylogenetic distance in four (Table 4; Fig. 14). Two families did not show significant relationships between phylogenetic distance and overlap, and in one family, *Fabaceae*, overlap of interaction partners increased with increasing phylogenetic distance.

Discussion

We found broad support for the hypothesis that more closely-related pairs of plants have a higher degree of niche overlap. Using a novel method which considers all pairs of plants together, the probability of two plants sharing the same animal interaction partners decreased with increasing phylogenetic distance. Considering networks separately, ≈78% of the pollination and all of the herbivory networks exhibited the expected trend of decreasing overlap with increasing distance.

Within families, however, there was much greater variability. More than half of the plant families in each network type behaved as we hypothesised, with more closely-related plants having greater niche overlap than distantly related plants. This relationship between overlap and phylogenetic distance is consistent with the idea that traits affecting interactions are heritable (Schemske and Bradshaw, 1999) and changing gradually such that closely related plants resemble their common ancestor— and each other —more than

Table 5: Change in log odds (per million years of phylogenetic distance) of observing total, partial, or no overlap in pollinators between a pair of plants in the same family.

Family	Total overlap		Partial overlap		No overlap	
	Odds ratio	*P*-value	Odds ratio	*P*-value	Odds ratio	*P*-value
Adoxaceae	-	-	-33.37	**<0.001**	33.87	**<0.001**
Caprifoliaceae	-	-	1.04	0.588	-1.23	0.522
Apiaceae	11.02	**<0.001**	-53.50	**<0.001**	59.57	**<0.001**
*Asteraceae**	-4.74	**<0.001**	-2.00	**<0.001**	2.38	**<0.001**
Apocynaceae	-8.26	**<0.001**	-4.48	**<0.001**	5.67	**<0.001**
Rubiaceae	-	-	-	-	-	-
Boraginaceae	26.40	0.470	-24.67	**<0.001**	23.85	**<0.001**
Lamiaceae	5.81	0.528	1.90	0.255	-2.10	0.205
Orobanchaceae	241.20	0.998	261.90	0.995	-262.55	0.995
Plantaginaceae	-529.93	0.940	-53.81	**<0.001**	58.36	**<0.001**
Oleaceae	-11.01	0.367	-14.95	**<0.001**	14.90	**<0.001**
Solanaceae	-	-	12.33	0.743	-25.57	0.484
Ericaceae	-5.32	**<0.001**	-4.48	**<0.001**	5.02	**<0.001**
Primulaceae	-49.15	**<0.001**	-21.46	**<0.001**	23.22	**<0.001**
Hydrangeaceae	-7.14	**0.002**	-1.16	**0.027**	1.47	**0.004**
Loasaceae	482.42	0.998	478.88	0.995	-485.71	0.995
Caryophyllaceae	-3.42	0.167	-3.63	**<0.001**	4.09	**<0.001**
Montiaceae	346.61	0.999	406.10	0.998	-406.90	0.998
Polygonaceae	18.37	**<0.001**	14.63	**<0.001**	-14.99	**<0.001**
Brassicaceae	-6.04	0.260	-1.34	0.302	1.57	0.218
Cistaceae	-26.90	**<0.001**	-10.81	**<0.001**	13.33	**<0.001**
Malvaceae	-1.29	0.558	-4.59	**<0.001**	5.02	**<0.001**
Geraniaceae	-11.17	**0.014**	-1.25	0.730	9.96	**0.013**
*Melastomataceae**	47.20	0.998	52.97	0.993	-53.08	0.993
Myrtaceae	-70.37	**<0.001**	-44.38	**<0.001**	51.83	**<0.001**
Malpighiaceae	-0.83	0.610	-0.26	0.850	0.99	0.513
Phyllanthaceae	-389.36	0.995	-24.36	**<0.001**	24.88	**<0.001**
*Fabaceae**	14.19	**0.011**	3.18	0.091	-4.60	**0.012**
Rosaceae	-21.45	**<0.001**	-20.31	**<0.001**	21.50	**<0.001**
Saxifragaceae	-4.00	0.053	0.40	0.722	0.79	0.474
Papaveraceae	-27.67	**0.003**	-16.16	**<0.001**	16.80	**<0.001**
Ranunculaceae	69.01	0.996	-11.70	**0.003**	10.73	**0.006**
Amaryllidaceae	0.65	0.933	-1.01	0.465	0.97	0.480
Asparagaceae	-73.15	**0.003**	-33.10	**<0.001**	35.56	**<0.001**
Iridaceae	253.09	0.998	1.68	0.773	-2.30	0.691
*Poaceae**	343.63	0.996	343.55	0.990	-344.97	0.990

We were able to fit these models to 35 plant families (see *Materials and Methods* for details). Families marked with an asterisk were also highly diverse in herbivory networks. Statistically significant values are indicated in bold. Dashes indicate plant families where the corresponding overlap pattern was either extremely rare or omnipresent and the relevant model was uninformative.

they do distantly related plants. In some families, such as *Asteraceae* in pollination networks, the positive slope of this relationship was very shallow while in others, such as *Melastomataceae* in herbivory networks, the positive slope was extremely steep. This could indicate different rates of phenotypic drift or evolution in different families.

In contrast, *Polygonaceae* in pollination networks and *Fabaceae* in herbivory networks showed the opposite pattern. In these families, closely-related plants had *lower* overlap than more distantly-related pairs of plants. There are several possible reasons a plant family might display this pattern. First, part of the family may have recently undergone a period of rapid diversification with closely-related species developing novel phenotypes that attract different animal interaction partners (Linder, 2008; Breitkopf et al., 2015). It is also possible that the animals have undergone an adaptive radiation to specialise on their most profitable partner (Janz et al., 2006). Second, this pattern could be the result of ecological or environmental filtering (Mayfield et al., 2009; Ackerly, 2003). Closely-related species which have high degrees of overlap in their interaction partners might compete too severely to coexist. This is especially likely for plants sharing pollinators, where the loss of pollen to related species might severely limit reproductive success (Levin and Anderson, 1970; Bell et al., 2005; Mitchell et al., 2009). Indeed, animal pollination and seed dispersal have been shown to act as filters for several plant clades (Mayfield et al., 2009). Distantly-related plants with similar interaction partners, on the other hand, may differ in some other aspect of their niches and so be able to coexist. Plants sharing herbivores are unlikely to compete for these interaction partners, but the presence of both plants in a community could support higher herbivore populations than could one species alone (Russell et al., 2007). If the plants compete for some other resource, the combined impact of herbivory and competition could eliminate the rarer plant species. Distantly-related plants sharing herbivores, conversely, would be less likely to compete for vital resources and more likely to persist.

The remaining families did not show significant relationships in either direction. That is, the niche overlap between two plants did not vary linearly over phylogenetic distance. Once again, there are several possible drivers for this trend (or lack thereof). These plants might be highly specialised on different interaction partners and therefore have low overlap at all levels of relatedness. In other plant families with more moderate levels of specialisation, it is possible that pollination and/or herbivory do not exert large selection pressures on the plants. If traits affecting pollination or herbivory are not heritable in these groups (Kursar et al., 2009) and that their phenotypes are constrained by other factors (e.g., drought tolerance), then we should not expect a relationship between phylogenetic distance and overlap of interaction partners. Alternatively, pollination and/or herbivory might exert large pressures that maintain the clade within a pollination or defensive syndrome. These syndromes are commonly believed to predict the pollinators or herbivores with which a plant will interact (Waser et al., 1996; Fenster et al., 2004; Ollerton et al., 2009; Johnson et al., 2014). As some recent studies have suggested that pollination syndromes do not accurately predict plants' visitors in all plant families (Ollerton et al., 2009), it may be of interest for future researchers to test whether syndromes are better predictors in families with weak relationships between overlap and phylogenetic distance. Lastly, it is possible that the absence of a linear relationship between niche overlap and phylogenetic distance is because the data actually exhibit a strongly non-linear one. This could result, for example, from an early burst of diversification followed by a period of stasis (Davis et al., 2014).

For those families which were well-represented in both pollination and herbivory networks, we can also contrast the trends in the two network types. In all five such cases, there was a significant relationship between overlap and phylogenetic distance in only one network type (counting the singular relationships in *Rubiaceae* in pollination networks as non-significant). This may indicate that one type of interaction places greater constraints upon these families than the other. Plants may not be able to respond to selection on both types of interaction simultaneously because traits affecting pollination can also affect herbivory, and vice versa (Strauss, 1997; Strauss et al., 2002; Adler and Bronstein, 2004; Adler et al., 2006; Theis, 2006). Associations with pollinators and herbivores may also be constrained by the larger structure of the community. In one recent study, plants which are visited by many pollinators are also consumed by many herbivores (Sauve et al., 2015). This may be because pairing antagonistic and mutualistic interactions balances the indirect effects of these interactions, leading to a more stable community (Sauve et al., 2014). As more networks describing pollination and herbivory in the same community become available, it will be interesting to test this hypothesis more thoroughly.

Altogether, our study has revealed a wide variety of relationships between overlap of interaction partners and phylogenetic distance between plants in the same family. Regardless of the precise mechanisms behind these relationships, it is clear that the differences between families can affect the relationship between overlap and phylogenetic distance at the network level. Interestingly, in our analyses the plant families associated with the steepest relationships between niche overlap and phylogenetic distance at the network level did not show particularly steep relationships within themselves. This result suggests that it is not just which plant families are present but the additional relationships between the families that affects conservation of interactions at the network level and is consistent with previous work showing that the shape of phylogenetic tress, as well as the phylogenetic distances between species, can affect the strength of phylogenetic signal (Chamberlain et al., 2014b). Our results emphasise the importance of considering conservation of interactions at multiple scales. We hope that these results will help to guide future work investigating the genetic and environmental drivers underpinning these relationships.

Acknowledgements

We would like to thank the authors of the published networks used in this study. We are grateful for the use of the Edward Percival field station in Kaikoura, New Zealand in May, 2014. This research was supported by an NSERC PGS-D graduate scholarship (to ARC), a Marsden Fund Fast-Start grant (UOC-1101) and a Rutherford Discovery Fellowship, both administered by the Royal Society of New Zealand (to DBS), a BlueFern HPC PhD scholarship (to NJB), and by the Allan Wilson Centre (to GVDR).

Author contribution

ARC, DBS, GVDR, and NJB designed the research. ARC, JAT, and CJW collected published data. ARC and GVDR performed the analyses. All authors contributed to the manuscript.

Supporting information

S3.1: Original sources for networks
S3.2: Supplemental within-network results
S3.3: Supplemental within-family results

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Chapter 4: Are high-arctic plant-pollinator networks unravelling in a warming climate?

Alyssa R. Cirtwill[1], Tomas Roslin[2,3], Claus Rasmussen[4], Jens Mogens Olesen[5], Daniel B. Stouffer[1]

[1]Centre for Integrative Ecology
School of Biological Sciences
University of Canterbury
Private Bag 4800
Christchurch 8140, New Zealand
[2] Department of Ecology
Swedish University of Agricultural Sciences
P.O. Box 7044
SE-750 07 Uppsala, Sweden
[3] Spatial Foodweb Ecology Group
Department of Agricultural Sciences
Uiversity of Helsinki
PO Box 27 (Latokartanonkaari 5)
FI-00014 Helsinki, Finland
[4] Department of Bioscience
Aarhus University
Ole Worms Allé 1
DK-8000 Aarhus C, Denmark
[5] Department of Bioscience
Aarhus University
Ny Munkegade 114
DK-8000 Aarhus C, Denmark

Manuscript prepared in the style of *Global Change Biology*.
This chapter has been modified from its original version. It has been formatted to match this thesis.

Abstract

The Arctic is currently experiencing severe climate change, resulting in substantial changes to plants' flowering periods and insects' emergence dates. This has raised concerns that the two groups of species may be becoming phenologically uncoupled. If this is the case, networks of plant-pollinator interactions could be disrupted, with adverse consequences for both plants and insects. We investigated this possibility using a temporally-replicated network from a well-studied High Arctic site at Zackenberg, Greenland. Specifically, we tested for turnover in community composition and change in the dates at which species became active in the plant-pollinator network before and after 15 years of warming. We then looked for effects of these changes on species' roles within the network. Our results suggest that the plant-pollinator network is beginning to unravel, with changes to the roles of plants active early in the year and insects late in the year being most pronounced. This is consistent with phenological uncoupling and suggests that, if the trends we observed continue, the pollination network at this site may be disrupted. As the Arctic is the "canary in the coal mine" for the effects of climate change, we expect that similar changes may also occur at more temperate sites in the future.

Keywords

pollination, phenological uncoupling, network structure

Introduction

Plant-pollinator interactions are currently being strongly influenced by climate change (Hegland et al., 2009; Miller-Struttmann et al., 2015). In particular, the differential effects of climate change on species' phenologies are likely to disrupt entire networks of plant-pollinator interactions (Parmesan, 2006; Tylianakis et al., 2008; Settele et al., 2014). If plants and their pollinators respond to climate change in different ways, changes to the active periods of plants and their pollinators can disrupt pollination— even if the species themselves remain present in the community (Tylianakis et al., 2008; Hegland et al., 2009; Petanidou et al., 2014; Forrest, 2015). Advancing phenology in response to global warming has been reported across biomes (Menzel et al., 2006; Høye et al., 2007; Hua et al., 2016), raising concerns about just such an uncoupling of trophic interactions (Both et al., 2006; Thackeray et al., 2010; Rasmussen et al., 2013; Gezon et al., 2016; Hua et al., 2016; Schmidt et al., 2016). For example, if early-emerging pollinators respond to higher temperatures and emerge before plants blossom, they may have difficulty finding food. Late-emerging pollinators, on the other hand, may encounter different plant species and may or may not be able to obtain nectar from or pollinate them. In this context, a species' sensitivity to climate change is likely to vary with its range of alternative resources. Equally, the vulnerability of an interaction is likely to o depend on the phenologies of the species involved.

Severe effects of climate change on plant-pollinator interactions are particularly likely in the Arctic— where substantial warming has already taken place (Høye et al., 2013) —challenging the ability of organisms, processes, and ecosystems to adapt (Post et al., 2009). Moreover, the climate of the Arctic is predicted to change faster than that of most other regions (IPCC, 2013; Settele et al., 2014), making understanding the effects of climate change on arctic communities a priority for current research (Settele et al., 2014). Previous work suggests that rapid climactic shifts in the Arctic have already led to equally rapid phenological shifts (Høye et al., 2007; Høye and Forchhammer, 2008b; Høye et al., 2013; Schmidt et al., 2016). Even more importantly, recent studies suggest that arctic plants and their pollinators respond differently to climate warming (Høye et al., 2007; Høye and Forchhammer, 2008b; Høye et al., 2013; Schmidt et al., 2016).

In a well-studied plant-pollinator community at Zackenberg, Northeast Greenland, flowering dates in the plant community have been shown to advance along with earlier snowmelt (Høye et al., 2007). Moreover, although variation in plant phenology across the landscape is pronounced in the Arctic, this variation tends to decrease under both natural and experimental warming (Post et al., 2008; Høye et al., 2013). This means that differences in flowering time between patches are unlikely to "rescue" pollinators which require floral resources later in the summer. To complete this picture of changing flower availability, late-flowering plants at Zackenberg have shown greater changes to their phenologies than have early-flowering plants (Høye et al., 2013; Schmidt et al., 2016). In contrast to alpine communities, in which early-flowering plants have shown greater advances in flowering time (Aldridge et al., 2011; Iler et al., 2013), this suggests that the window of floral resources in the Arctic is both changing and shrinking as the climate changes (Høye et al., 2013; Schmidt et al., 2016). We note, however, that data on individual pollinators' responses to changes in the mosaic of flowering plants are still lacking, meaning that conclusions about the consequences of change in plants' phenologies must be considered tentative. Nevertheless, these changes have the potential disrupt the network of plant-pollinator interactions at Zackenberg and similar sites, posing a significant challenge to plant-pollinator interactions in the future (Memmott et al., 2007; Hegland et al., 2009; Post et al., 2009).

As most of the pollinator community at Zackenberg has also emerged earlier, tracking changes in snowmelt (Høye et al., 2007; Høye and Forchhammer, 2008b), it is possible that pollination interactions, and the integrity of the plant-pollinator network, might be maintained despite climate change. However, phenological changes vary greatly between taxa (Høye and Forchhammer, 2008b) and more recent studies have found declines in pollinator populations in the Arctic (Potts et al., 2010; Høye et al., 2013). This suggests that high-arctic plants and their pollinators may indeed be vulnerable to phenological uncoupling (Høye et al., 2007; Høye and Forchhammer, 2008a; Olesen et al., 2011; Rasmussen et al., 2013) and that pollination networks may be disrupted (Schmidt et al., 2016). At Zackenberg specifically, many species also have very short active periods (4-8 days) (Rasmussen et al., 2013). For these species in particular, a shift in the phenology of an important interaction partner could have large effects.

To test whether changes in plants and pollinators' phenologies are leading to changes in the interactions between them, we draw on a set of temporally-replicated plant-pollinator networks from Zackenberg, Greenland which spans 15 years of warming. Given the substantial phenological change in both plants and pollinators at this site, we expected (1) that there would be substantial turnover in plant and/or pollinator communities and (2) that dates at which pollinators begin visiting plants, and plants begin receiving visitors, will have changed between decades. If there is substantial turnover or phenological change, then interactions between plants and their pollinators may be disrupted. We therefore expect (3) that the structure of interaction networks will change over time. If network structure changes over time, we then expect (4) that species' structural roles within these networks (i.e., the patterns of their interactions with other species) will also change over time. Moreover, we expect (5) that species which become active at different times of the year will have different roles in the plant-pollinator network and (6) that the roles of species which become active at different times of the year will change in different ways between decades. Finally, we expect (7) that the change in a species' role will depend both on the magnitude of the change in its phenology and on the direction of that change. For clarity and later reference, these seven hypotheses are summarised in Table 6.

Materials and Methods

Study site

At the Zackenberg research station in High Arctic NE Greenland (74° 28′ N, 20° 35′ W), the local climate has changed dramatically

<table>
<tr><th colspan="2" rowspan="2">Hypothesis</th><th colspan="2">Support</th></tr>
<tr><th>Plants</th><th>Pollinators</th></tr>
<tr><td>1.</td><td>Community composition changed between decades.</td><td>Weak</td><td>Strong</td></tr>
<tr><td>2.</td><td>Phenology changed between decades.</td><td>Mixed</td><td>None</td></tr>
<tr><td>3.</td><td>Network structure changed between decades.</td><td colspan="2">Strong</td></tr>
<tr><td>4.</td><td>Species' roles changed between decades.</td><td>Strong</td><td>Strong</td></tr>
<tr><td>5.</td><td>Species' roles were correlated with their phenology.</td><td>Strong</td><td>Strong</td></tr>
<tr><td>6.</td><td>Change in roles between decades was correlated with phenology.</td><td>None</td><td>Mixed</td></tr>
<tr><td>7.</td><td>Amount of change in roles was correlated with amount of change in phenology.</td><td>None</td><td>Mixed</td></tr>
</table>

Table 6: Summary of the hypotheses we tested in this study and the strength of evidence for them. Note that the aspect of phenology we are most interested in throughout this study is the date at which species become active in the plant-pollinator network.

during the study period (1996-2011). The average near-surface air temperature across June, July and August has increased at a rate of 1.3-1.8°C per decade since 1996, whereas the timing of snowmelt has advanced at a rate of between 9.8 and 12.8 days per decade (Høye et al., 2013; Mortensen et al., 2014). Over the same period, the flowering season of focal plants has shortened at the landscape scale at a rate of 3.7 days per decade (Høye et al., 2013).

Data collection

We use plant-pollinator data compiled over four summers, in 1996 and 1997 (Olesen et al., 2008) and 2010 and 2011 (Rasmussen et al., 2013). Each study period lasted from the last snowmelt in spring to the first frost and snowfall in autumn. In 1996 and 1997, this covered 43 and 69 days, respectively, of which 25 in each year had sufficiently fine weather to permit observation (Olesen et al., 2008). In 2010 and 2011, the study period covered 70 and 69 days, respectively, of which 54 and 52 days were spent observing in the field (Rasmussen et al., 2013). All observation days had weather suitable for foraging insects. During each field day (lasting from 09:00 to 17:00), two individuals of each species of flowering plant were observed for 20 minutes each (i.e., 40 minutes of observation for each plant species), and all insect visitors to flowers were recorded as potential pollinators (Olesen et al., 2008; Rasmussen et al., 2013).

Quantifying species turnover and changing phenologies

We first assessed the amount of turnover in plants and pollinators across years (Hypothesis 1). Using the 1996 community as a baseline, we calculated the number of plants and pollinators in 1997 that had also been detected in 1996, the number of new plants and pollinators observed in 1997, and the number of plants and pollinators observed in 1996 that were not found in 1997. We then repeated this procedure between 1997 and 2010 and between 2010 and 2011. As well as comparing numbers of persistent species, newly-observed species, and species disappearing from sight from one year to the next, we also quantified turnover between all pairs of years using Whittaker's beta diversity index (β_W; Whittaker, 1972). This index,

$$\beta_W = (\gamma - \alpha)/\alpha, \tag{4}$$

compares the total number of species detected across both years (γ) with the mean number of species detected in one year (α) and varies between 0 (identical species in both years) and 1 (complete turnover of the community). We calculated turnover separately for plants and pollinators.

As well as changes in which species were detected, we were interested in changes to these species' phenologies (Hypothesis 2). For both plants and pollinators, we calculated the change in each species' dates of first interaction between each pair of networks from different decades (i.e., 1996-2010, 1996-2011, 1997-2010, and 1997-2011) in which the pollinator was detected. To capture the phenologies of species which were observed in only one year in a given decade, we included all between-decade pairs of networks, thus mimicking our analysis of species turnover above. To explicitly test whether the phenology of the community has changed over time, we compared mean dates of first interaction between decades (1996 and 1997 vs. 2010 and 2011) using a two-tailed two-sample t-test and compared variances in emergence times between decades using an F-test. In addition to comparing the distributions of the entire communities, we also compared the distributions of newly-arrived and persistent species.

We note that empirically-observed dates of first interaction are highly dependent on sampling effort, species' abundances, and their interaction frequencies; to ensure that our results are not biased by missing interactions, we repeated all subsequent analyses using simulated dates of first interaction. These simulated dates were based on the full set of observed interactions for each species and allowed us to determine how robust our results may be to noise in the observed first dates of interaction. In general, analyses involving plants' first dates of interaction were more robust than those involving insects, but in both cases the majority of simulated datasets led to qualitatively the same conclusions as the observed data (see *Supplemental Information: S4.1* for further details).

Quantifying network structure

To test our remaining hypotheses, we compiled plant-pollinator networks for each year (1996, 1997, 2010, and 2011) and for each month of sampling within each year (June, July, August), giving 16 networks in total. Of the observations collected in 1996, 94 were not precisely dated and were instead associated with a range of tentative dates. As all of these dates were from late in the summer, they were not likely to affect our estimates of species' dates of first interaction. Our results were qualitatively identical whether these tentatively-dated observations were included only on the best-guess date of observation, included only in the yearly networks (i.e., excluded from the monthly networks), or included for each network covering any part of the range of tentative dates (see *Supplemental Information: S4.2-S4.3* for details). Thus, we present results based on networks which included the tentatively-dated observations only in the networks describing the best guess for the date of observation as this approach preserves the number of interactions that were actually observed.

We then quantified the structure of each network based on the organisation of interactions into two- to six-species "motifs". These motifs can be thought of as the building blocks of networks (Milo et al., 2002, 2004; Stouffer et al., 2007; Baker et al., 2015). Each motif represents a unique way in which sets of species interact, and hence a unique contribution to the transfer of energy and other ecosystem processes (i.e., pollination) within a community. As the number of individual motifs in a network tends to increase with the number of species in the network, we converted the counts of each motif to relative frequencies by dividing by the total number of motifs in the network. This ensures networks from different years do not appear to have different structures simply because they have different numbers of species and/or interactions.

Comparing network structure over time

Having determined the structure of each network, we then aimed to test whether this structure changed over time (Hypothesis 3). To do this, we first quantified differences between networks' motif profiles (i.e., structures) using Bray-Curtis dissimilarity (Anderson, 2001; Baker et al., 2015). This dissimilarity measures differences between networks based only on motifs which appear in at least one of the networks. Thus, two networks with different structures will not appear more similar to each other just because they have a large number of shared "double zeros" (motifs which do not appear in either network). We then used a non-parametric permutational multivariate analysis of variance (PERMANOVA Anderson, 2001) to test whether network structure varied over time. We were particularly interested in the change in network structure after several years of warming and so we compared network structure between decades (i.e., 1996-1997 to 2010-2011). With PERMANOVA, we achieved this by comparing the spatial medians of network structures associated with each decade– these median structures can be considered the “typical" structures for each decade.

Similar to a traditional ANOVA, a PERMANOVA uses a pseudo-*F* statistic to compare differences among and within groups. Unlike an ANOVA, however, the PERMANOVA does not assume that the data follow any particular distribution. Instead, the raw data are permuted to obtain the null distribution of the test statistic and a *p*-value is computed using this distribution. Where possible, we used 9999 permutations to calculate the null distribution. In PERMANOVAs where there were fewer than 9999 possible unique permutations of the data, we used the maximum number of permutations possible (as noted below). All PERMANOVAs were performed using the adonis function in the R (R Core Team, 2014) package vegan (Oksanen et al., 2014).

We first compared the structure of yearly networks across decades. In this case, there were only 24 possible permutations of motif profiles between decades. We next compared the structure of monthly networks across decades. As there were many more monthly than yearly networks, we were able to use 9999 permutations to obtain the null distribution. For these networks, it was additionally possible that changes in the plants and pollinators active in each month might drive large amounts of variation in network structure between decades. To control for the possibility that such month-to-month variation in network structure might mask differences in network structure between decades, we stratified permutations by month. This stratification ensures that motif profiles are only shuffled between networks describing the same month (e.g., the motif profiles of June 1996 and June 2010 could be swapped but the motif profiles of June 1996 and July 1996 could not).

To visualise the change in network structure over time, we also performed a nonmetric multidimensional scaling (NMDS) analysis to align the motif profiles of all networks along two major axes explaining the most variation in structure. The NMDS also aligns the motifs themselves along the same axes, allowing us to interpret changes in structure based on the motifs which exert the greatest influence on these axes. We analysed the structures of yearly and monthly networks together using the metaMDS function in the R (R Core Team, 2014) package vegan (Oksanen et al., 2014).

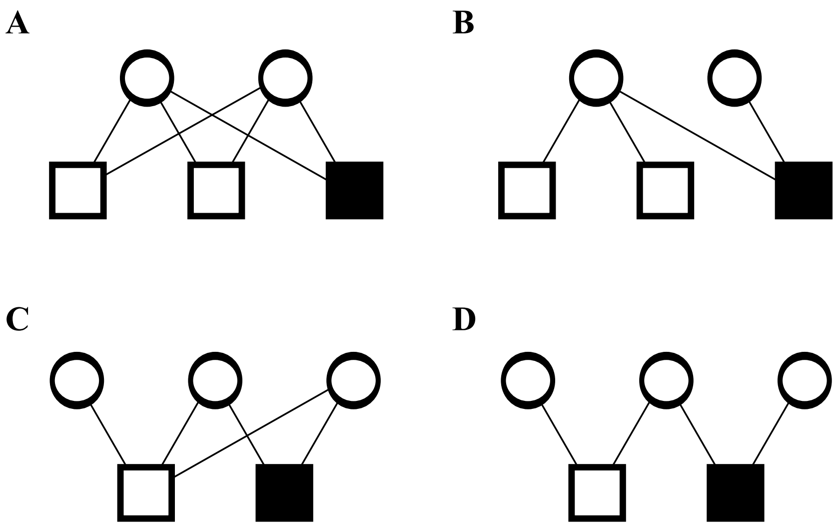

Figure 15: In this study, we use motifs (unique patterns of 2-6 interacting species) to describe both the structure of networks and species' roles within them. We show four small networks with different structures. All networks all contain 5 species but some have different numbers of links. However, even those with the same number of links (i.e., B and D) have different arrangements of those links. By describing network structures using motifs, we can capture these differences in a way that is not possible with simpler measures of network structure. Along the same line, all of the plants (squares) highlighted in black interact with two pollinators (circles), but their roles within their networks are different. For example, the focal plants in networks A and C interact with two generalist partners, while the focal plants in networks B and D interact with one specialist and one generalist pollinator. Moreover, by incorporating indirect interactions, structural roles based on motifs also allow us to distinguish between a plant in a network where every species is a generalist (i.e., network A) and one which also includes specialists (e.g., network C). As direct and indirect interactions both affect the pollination service the focal plant receives, and therefore the plant's population dynamics, structural roles provide a more comprehensive picture of changes to species' roles than simpler measures such as number of interaction partners.

Comparing species' roles over time

As with network structure, we used the decomposition of each network into its component motifs to calculate the role of every species within its network (Stouffer et al., 2012; Baker et al., 2015; Cirtwill and Stouffer, 2015; Chapter 5), and then to compare these roles over time (Hypothesis 4). To do so, we determined the number of times the species appears in the two-species motif, each of the two possible three-species motifs, four possible four-species motifs, etc. (Baker et al., 2015). As each motif includes one or more unique positions that a species might occupy, we next identified which position the species took within each motif. There are 74 unique positions that an species can occupy in two- to six-species motifs, resulting in vectors of length 74 describing the role of each species in these plant-pollinator networks (Baker et al., 2015). These multidimensional roles capture the ways in which species are embedded into their networks in more detail than simpler measures like degree (Fig. 15), allowing us to better understand how pollination is changing over time at Zackenberg.

We were primarily interested in whether species' roles change *shape* over time– that is, whether a species tends to take different positions within the network in different years (Hypothesis 4) rather than participating in different numbers of motifs. However, roles as defined above also vary in magnitude, with species involved in more interactions also tending to occupy more positions within the network. This is analogous to networks containing different numbers of species and interactions having different numbers of motifs. Roles with different magnitudes may therefore appear different even if the species involved occur with the same frequencies across all motif positions. To prevent apparent changes in shape driven solely by a species having different numbers of interaction partners in different years, we therefore normalised the role vectors of all species by dividing each role vector by the total number of positions in which that species occurred. This converts counts of occurrences in different positions to relative frequencies, and we used these normalised roles in all subsequent analyses.

We then tested whether species' roles within networks changed between decades. As when comparing network structure, we first quantified differences between roles using Bray-Curtis dissimilarity (Anderson, 2001; Baker et al., 2015; Cirtwill and Stouffer, 2015; Chapter 5). Since this ensures that two species' roles will not be considered more similar if the species share many "double zeros"– positions in which neither species occurs. We then used PERMANOVAs to compare roles between decades. We were able to use 9999 permutations to obtain the null distribution for all PERMANOVAs of species' roles and hence did so.

Testing the effect of emergence date on species' roles

Next, we tested whether changes to species' phenologies, particularly their dates of first interaction at the start of each sampling season, could explain patterns in their roles (Hypothesis 5) or the ways in which these roles changed over time (Hypothesis 6). To address Hypothesis 5, we added the effect of date of first interaction to the PERMANOVAs used to compare species' roles over time. To address Hypothesis 6, we also included an interaction term between date of first interaction and year or month. As above, we used 9999 permutations to obtain the null distribution of roles in each case.

To test the possibility that changes in roles are driven by changes in network structure over time, we performed a constrained analysis of principal coordinates (CAP) that accounted for network structure. This analysis, similar to a redundancy analysis, measures the variance in the response (roles) explained by a set of predictors. We used date of first interaction as a predictor and included network structure as a "conditioning" variable. When testing the ability of decade to explain variation in pollinators' roles, the CAP compares a model including only the conditioning variable (in this case, a distance matrix based on network structure) with a model including the conditioning variable and any other predictors. As with the PERMANOVAs above, we used Bray-Curtis dissimilarities to describe both differences in network structure and differences in species' roles. We performed the CAP using the capscale function in the R (R Core Team, 2014) package vegan (Oksanen et al., 2014). These CAP analyses also allowed us to visualise species' median roles over time, as with the NMDS used to visualise network structure.

As well as being interested in the effects of dates of first interaction *per se*, we were interested in whether the *change* in these dates was related to the amount of change in species' roles (Hypothesis 7). That is, did pollinators that became active much earlier in the 2010's than in the 1990's have more dissimilar roles in those years than pollinators that became active at very similar times in each decade? To test this, we combined the Bray- Curtis dissimilarities between species' yearly roles in different decades (i.e., between 1996 and 2010, 1996 and 2011, 1997 and 2010, and 1997 and 2011) with the differences in species' dates of first interaction between these years. Negative values for change in date of first interaction indicate that a species became active earlier in the later network while positive values indicated a shift to becoming active later in the year.

We then measured the correlation between within-species differences in emergence date and within-species role dissimilarities. As we expected that species' roles might respond differently to advancing or retreating phenologies, we analysed species which became active on an earlier date in the 2010's than in the 1990's separately from species which became active on a later date in the 2010's than in the 1990's. In each case, and as in our previous analyses, we did not assume that this statistic would follow a normal distribution but rather obtained the null distribution through permutations. Moreover, as some species' dates of first interaction were more variable than others, we stratified permutations to within species (i.e., the difference in emergence dates for *Aedes impiger* from 1996 to 2010 could only be swapped with the difference in dates of first interaction for *Aedes impiger* from 1996 to 2011, 1997 to 2010, or 1997 to 2011). We used 9999 permutations to obtain the null distribution.

We followed a similar approach to test the effect of the magnitude of change in date of first interaction on the change in species' roles in monthly networks. In this case, we were more interested in changes across years than within years (i.e., from June 1996 to June 2010 rather than from June 1996 to July 1996). We therefore only calculated dissimilarities between networks describing

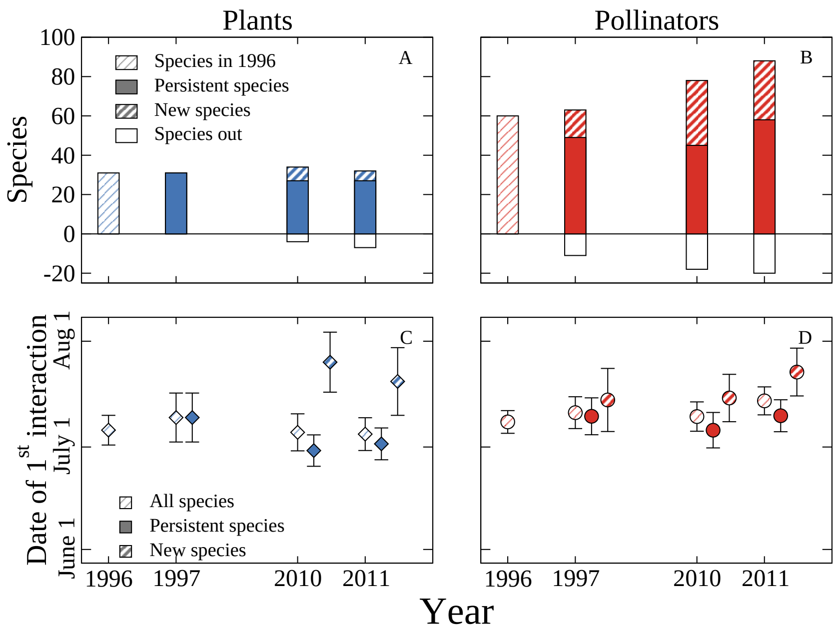

Figure 16: From 1996 to 2011, the composition of the Zackenberg plant-pollinator community changed dramatically. A-B) We show the number of species in each group that were recorded in the previous year (solid), the number of species detected in the previous year that were not observed in the focal year (no fill), and the number of species that were detected in the focal year but not in the previous year (striped fill). The height of the bar indicates the total number of plants or pollinators observed each year. The majority of plant species were recorded in all four years. The pollinator community, however, both increased in species richness and showed substantial turnover (Table 7). C-D) We show the mean date of first interaction (±2SE) for plants or their pollinators for each year. In both communities, mean dates of first interaction were not significantly different between decades or between any two years.

the same month in different years. Using these dissimilarities and the changes in dates of first interaction described above, we once again tested for correlation between the magnitude of change in dates and the magnitude of change in roles. As with the yearly roles, we performed separate tests for species emerging or flowering earlier in the 2010's than in the 1990's and those emerging or flowering later in the 2010's. In both cases, we used 9999 permutations to obtain the null distribution and permutations were stratified within species.

Results

Did community composition change between decades?

Both the richness and composition of the network varied between years, partially supporting Hypothesis 1 (Table 6). While numbers of plant species were relatively constant over time, more pollinator species were observed in each year from 1996 to 2011 (Fig. 16 A-B). Similarly, few plant species either appeared or disappeared between years while there was a great deal of turnover of pollinator species (Fig. 16C-D, Table 7).

Did species' phenologies change between decades?

Perhaps more importantly, the dates of first interaction did not vary between decades for either plants or pollinators ($F_{1,126}$=0.995, p=0.321 and $F_{1,287}$=1.52, p=0.219, respectively). This suggests that, despite the species turnover at Zackenberg, interactions between species were more constant over time and gives no support for Hypothesis 2 (Table 6). However, for those plants which persisted between years, dates of first interaction were significantly earlier in 2010-2011 ($F_{1,83}$=6.34, p=0.018). No new plants were detected in the community in 1997, but those that appeared in 2010 and 2011 had their first visitors substantially later than the other plants in the community. Among the pollinators, dates of first interaction did not differ between decades for either persistent or newly-arrived species ($F_{1,150}$=0.299, p=0.591 and $F_{1,75}$=0.538, p=0.466).

Did network structure change between decades?

The motif structure of yearly networks changed significantly between the mid 1990's and the early 2010's ($F_{1,2}$=6.27, p=0.042 for a PERMANOVA of structures of yearly networks across decades). The motif structure of the monthly networks also changed between the mid 1990's and the early 2010's, but only when permutations were stratified by month ($F_{1,10}$=2.32, p=0.064 for an unstratified PERMANOVA of structures of monthly networks across decades; p=0.030 for a similar PERMANOVA stratified by month). That is, while network structure did change across the decades, this change could be masked by the substantial variation in network structure between months within the same year if the network is not resolved to finer timescales. Overall, however, these results support Hypothesis 3 (Table 6).

These trends in network structure for both the yearly and monthly networks were also visually apparent in the NMDS of network structure across years. Negative values of the first NMDS axis were associated with several motifs representing tightly knit groups composed of generalists interacting with other generalists, while positive values were associated with motifs representing more loosely connected sets of species involving specialists interacting with generalists. Moving from negative to positive values of the second NMDS corresponds to an increase in the relative frequency of

Years		Plant turnover	Pollinator turnover
1996	1997	0.000	0.203
1996	2010	**0.169**	**0.391**
1996	2011	**0.111**	**0.432**
1997	2010	**0.169**	**0.362**
1997	2011	**0.111**	**0.417**
2010	2011	0.182	0.301

Table 7: Turnover at Zackenberg (measured using Whittaker's beta diversity index) was higher among insect pollinators than plants. Turnover among pollinators was higher between years in different decades (bolded) than between years in the same decade, while turnover in the plant community was similar across all years.

five- and six-species motifs and a decrease in two- and three-species motifs. From the 1990's to the 2010's, the yearly networks increased along the first NMDS axis and decreased along the second NMDS axis (Fig. *S4.5A*, *S4.5, Supporting Information S4*). This suggests that the yearly networks developed a more 'open' structure over time, with fewer plants sharing all (or almost all) of their pollinators with other plants and fewer 'connector' species connecting small motifs into larger ones. This trend towards more specialised pollinators is supported by the lower mean degrees (number of interaction partners) of pollinators in 2010 and 2011 than in 1996 and 1997 (4.43 and 3.23 partners for the 1990's and 2010's, respectively; p=0.007 for an anova of degree by decade). Plants, meanwhile, had similar numbers of interaction partners in both decades (8.79 and 8.14, respectively; p=0.573).

In the monthly networks, the amount of change in network structure varied greatly between months (Fig. *S4.5B-D*). The June networks in 1996, 2010, and 2011 had similar structures, but the 1997 network was lower along the first NMDS axis and higher along the second NMDS axis, while the July networks had very similar structures in each year. In both months, pollinators' mean numbers of interaction partners were similar between decades (1.958 and 1.907 partners in June, p=0.862 for an anova of degree by decade; and 3.857 and 3.270 for July; p=0.216). Plants' degrees were also similar between decades in the June and July networks (3.76 and 4.12, p=0.699 for June; 6.87 and 6.26, p=0.524 for July). The August networks, in contrast, showed greater variation in structure. As with the yearly networks, they increased along the first NMDS axis and decreased along the second axis. Once again, this corresponds to the August networks developing a more 'open' structure with fewer species sharing interaction partners, and was associated with a significant decrease in pollinators' mean degrees (2.786 and 1.856 partners, p=0.006). As with the other months and the yearly networks, plants' degrees were not significantly different between decades (4.33 and 5.06 partners, p=0.505).

Did species' roles change between decades?

The yearly roles of both plants and pollinators varied over time ($F_{1,126}$=5.35, p<0.001 and $F_{1,287}$=12.7, p<0.001, respectively, for PERMANOVAs of yearly roles against decade; Fig. 17 A). Likewise, plants' and pollinators' monthly roles both varied over time ($F_{1,230}$=3.20, p=0.003 and $F_{1,455}$=8.82, p<0.001, respectively, for PERMANOVAs of monthly roles against decade). Moreover, the

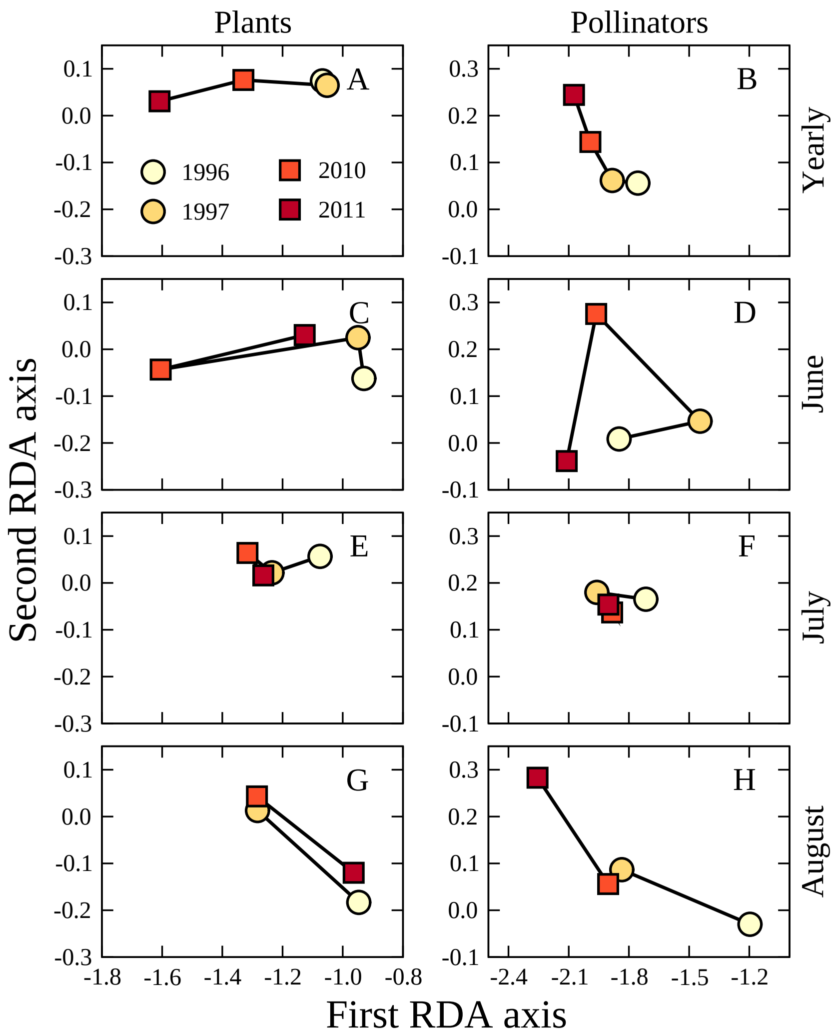

Figure 17: The median roles of plants and their insect pollinators differed between years. A-B) The median roles of both plants and pollinators in yearly plant-pollinator networks moved towards more negative values along the first axis of a redundancy analysis (RDA) of species' roles against year. B) The median roles of pollinators also moved towards more positive values of the first RDA axis. For both plants and pollinators, moving from negative to positive values along the first RDA axis represented a shift towards higher frequencies of positions representing generalists, while the same transition along the second axis represented a shift from small to large motifs (Fig. *S4.6*). C-H) The median roles of plants and pollinators in monthly networks showed more variable trends. C-D) In the June networks, the median roles of plants showed similar patterns to those in the yearly networks while the median roles of pollinators showed no clear trend over time. E-F) In the July networks, the median roles of both groups were similar across years, although the roles of plants again showed a more similar trend to that in the yearly networks. G-H) In the August networks, the median roles of plants differed much more between years in the same decade than across decades while the roles of pollinators showed a similar trend to that in the yearly networks.

change in monthly roles across decades varied between months for both plants and pollinators ($F_{2,451}$=3.75, p<0.001 and $F_{2,226}$=2.80, p<0.001, respectively, for the interaction term in a PERMANOVA of monthly roles against decade, month, and their interaction). This means that not only are species' roles changing over time (supporting Hypothesis 4; Table 6), this change is unevenly distributed across species that are active in different months.

For both plants and pollinators, moving from negative to positive along the first CAP axis represents a shift from positions representing specialists who interact with generalists to positions representing generalists interacting with other generalists, although the exact motifs involved differed between species groups. The second axis, meanwhile, represented a shift from positions in small motifs to positions representing generalists in large motifs. It is noteworthy that, although plants and pollinators were analysed separately, both groups' roles diverged along similar axes. As the roles of plants and pollinators moved towards more negative values along the first axis in 2010 and 2011, both groups participated more frequently in specialist positions. Combining these results with those for species' degrees, described above, it is clear that while plants' roles shifted towards more specialised positions the addition of more pollinators to the community has meant that their numbers of interaction partners have remained stable. Pollinators, on the other hand, appeared in more specialised positions and interacted with fewer plants in 2010-2011.

The roles of plants and pollinators in monthly networks, however, showed different trends. The roles of plants in June networks followed the same trend as the yearly networks, as did the July networks (albeit to a lesser extent). Plants' median roles in the August networks, meanwhile, showed much greater differences within each decade than across decades. From the pollinators' perspective, species' roles in June varied widely while roles in July were very similar in all networks. Only in the August networks did pollinators' roles follow the same pattern as in the yearly networks. These differing patterns suggest that, in a network context, plants and pollinators are not responding to climate change in the same ways.

Did species' roles vary with dates of first interaction?

As well as varying across decades, plants' and pollinators' yearly roles varied systematically with their dates of first interaction ($F_{1,124}$=16.1, p=0.004 and $F_{1,285}$=37.6, p<0.001, respectively, for the date term in PERMANOVAs of yearly roles against decade, date, and their interaction). For both groups, the relationship between yearly roles and date did not vary between decades ($F_{1,124}$=0.796, p=0.843 and $F_{1,285}$=1.38, p=0.233, respectively, for the interaction term in the above PERMANOVAs). Moreover, date remained a significant predictor even after controlling for changes to network structure between years ($F_{1,123}$=14.9, p<0.001 and $F_{1,284}$=33.8, p<0.001, respectively) in CAPs of species' roles against date, conditioned by network structure. Our results for plants' and pollinators' yearly roles therefore support Hypothesis 5 but not Hypothesis 6 (Table 6).

Support for these two hypotheses from the monthly roles, however, was mixed. Plants monthly roles did not vary with their dates of first interaction or with the interaction between date and decade ($F_{1,228}$=7.68, p=0.159 and $F_{1,228}$=1.02, p=0.382, respectively). After controlling for changes to network structure, however, date of first interaction did significantly predict plants' monthly roles ($F_{1,219}$=7.92, p<0.001). Thus, plants' roles did vary with their dates of first interaction, although this variation could be obscured by contrasting changes in network structure. There was, therefore, some support for Hypothesis 5 but none for Hypothesis 6 from the plants' monthly roles (Table 6).

Pollinators' monthly roles, in contrast, did vary with their dates of first interaction ($F_{1,453}$=17.9, p<0.001), and this relationship remained significant after accounting for network structure ($F_{1,444}$=26.8, p<0.001). Unlike pollinators' yearly roles, this relationship varied between decades ($F_{1,453}$=4.78, p=0.004), offering strong support for Hypotheses 5 and 6. When the roles of pollinators in monthly networks from the 1990's and the 2010's were analysed in separate PERMANOVAs, date of first interaction was a significant predictor of pollinators' roles in 1996 and 1997 ($F_{1,200}$=14.0, p=0.011) but not in 2010 and 2011 ($F_{1,253}$=9.08, p=0.092). This suggests that pollinators' roles may once have been predictable by their dates of first interaction, but that changes to the community have since undermined this trend.

Was change in dates of first interaction related to change in roles?

The magnitudes of changes in plants' yearly roles were not related to changes in their dates of first interaction for plants with advancing or retreating phenologies (R^2=0.117, p=0.459 and R^2=0.008, p=0.462, respectively). For pollinators, in contrast, the relationship between the change in date of first interaction and change in pollinators' yearly roles differed depending on whether the pollinators' phenologies advanced or retreated. For pollinators which became active earlier in 2010 or 2011 than in 1996 or 1997, the amount of dissimilarity in the pollinators' yearly roles was not related to the amount of change in date of first interaction (R^2=0.110, p=0.143). For pollinators which became active later in 2010 or 2011, dissimilarity in yearly roles increased slightly with increasing differences between dates of first interaction (R^2=0.086, p=0.048). In both cases, the range of dissimilarities was large for all values of change in date of first interaction (Fig. 18A). Our yearly results, therefore, offer very limited support for Hypothesis 7 (Table 6).

As with yearly roles, the amount of change in plants' monthly roles was not related to the amount of change in plants' phenologies

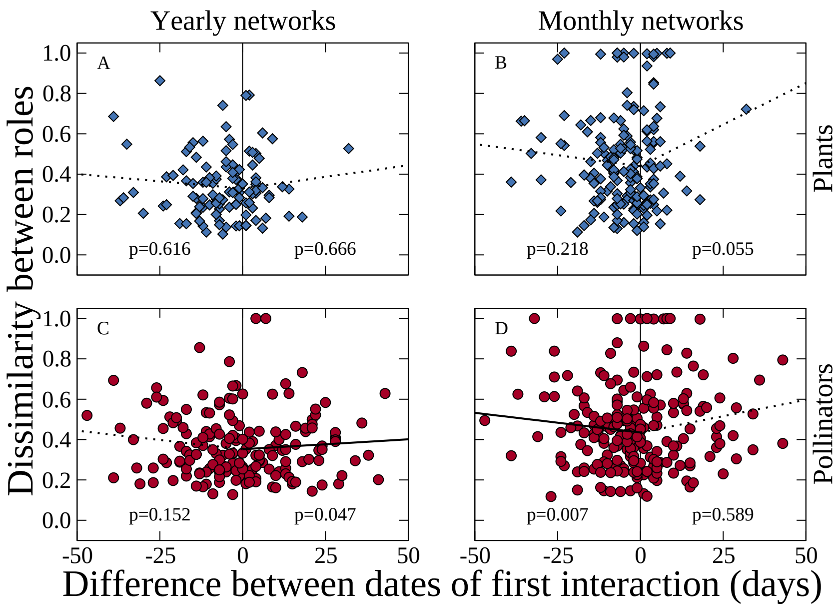

Figure 18: The relationship between the magnitude of change in species' roles between decades and the magnitude of change in species' dates of first interaction between decades differed between plants and their pollinators. A-B) There was no relationship between the amount of change in plants' roles and the amount of change in their dates of first interactions in either yearly or monthly networks. C-D) Pollinators with greater changes to their date of first interaction also showed greater dissimilarities between roles. C) In yearly networks, this relationship held for pollinators which became active later in the year but not those which became active earlier. D) The reverse was true in monthly networks. In all panels, change in roles was measured using Bray-Curtis dissimilarity, and difference in dates of first interaction is measured in days. The *p*-values were determined using Mantel tests of Bray-Curtis dissimilarity between roles against absolute difference in dates of first interaction. Plants and pollinators were analysed separately, as were species becoming active earlier and later within each species type. Lines are based on linear regressions of the dissimilarity between roles against change in emergence date, sign of change, and their interaction, and are indicative only.

for plants which became active earlier in the year in 2010-2011 (R^2=0.117, p=0.264), but change in roles was related to change in phenology for plants which became active later in the year in 2010-2011 (R^2=0.104, p=0.025, respectively). Pollinators' monthly roles, meanwhile, showed the opposite relationship with the amount of change in dates of first interaction to that in the yearly networks. Specifically, for pollinators with retreating phenologies, the amount of dissimilarity in the pollinator's monthly roles was not related to the size of the change in its date of first interaction (R^2=0.046, p=0.549; Fig. 18B) while for pollinators with advancing phenologies the amount of dissimilarity increased with the size of the change in date of first interaction (R^2=0.190, p=0.005). Once again, this constitutes weak support for Hypothesis 7 (Table 6).

Simulating dates of first interaction

One other potential explanation for our unexpected results related to Hypothesis 7 (Table 6) is that our estimates of species' dates of first interaction may not be entirely accurate. As our networks were assembled by observing the visitors to focal plants, it is particularly likely that pollinators' true dates of first interaction may be different than we observed. To determine how robust our results are to noise in estimations of species' dates of first interaction, we repeated our tests for Hypotheses 5-7 (i.e., those which depend upon dates of first interaction; Table 6) using 1000 sets of simulated dates (see *S4.3, Supporting Information S4* for details). In nearly all cases, our results for plants using simulated dates were similar to those obtained using the observed dates. This indicates that these results are generally robust to noise in our estimates of date of first interaction and means we can be quite confident in them. For the pollinators, however, our results for Hypotheses 5 and 6 using the observed dates were significantly more extreme than those obtained using the simulated dates. This suggests that our results for the pollinators were more susceptible to noise in our estimates of dates of first interaction, even though the results for Hypothesis 7 were similar using the observed and similar datasets.

Discussion

We found evidence in support of most of the hypotheses we tested in this study, although the degree of support varied between types of species and network time scales. Testing the hypothesis that there would be substantial species turnover during 15 years of warming (Hypothesis 1), we found support from the pollinator community but not the plants. This may be because the lifecycles of plants and pollinators occur on different timescales— the plant community at Zackenberg is perennial while the insects live for only one year. Plants may also be space-limited such that new species cannot grow in the study site until a plant present in the previous year dies.

On the surface, it appeared that there was no support for the idea that dates of first interaction would change between decades (Hypothesis 2), as mean dates of first interaction were not significantly different between decades for plants or for pollinators. However, when examining the patterns at higher resolution we found that the dates of first interaction for plants which persisted in the community between years *did* shift earlier between decades, while the few plants which were first observed in 2010-2011 had substantially later dates of first interaction. It therefore appears that dates of first interaction among resident plants are changing in line with previously reported changes to flowering phenology (Høye et al., 2013; Schmidt et al., 2016). Neither the dates of first interaction for persistent pollinators nor those of new arrivals differed significantly between decades, indicating that changes in pollinators' emergence dates are not reflected in their interaction phenologies. While it is possible that the high turnover in the pollinator community makes it difficult to obtain a clear signal of changing phenology, these results contrast with known changes to pollinators' emergence dates (Høye et al., 2007; Høye and Forchhammer, 2008b). The lack of change in pollinators' dates of first interactions also suggests that plants and their pollinators may indeed be becoming uncoupled as proposed in earlier studies (Høye et al., 2013; Gezon et al., 2016; Hua et al., 2016; Schmidt et al., 2016).

The possibility that plants' and pollinators' phenologies are diverging is strengthened by our results for Hypothesis 3, where we found that network structure changed consistently between decades (Table 6). Specifically, the network structure shifted towards higher frequencies of motifs representing plants sharing few pollinators with each other. This indicates that the network became more open and loosely connected over time, and that the trend was likely driven by changes to plants' roles. Examining the networks for each month separately, we found that this trend was evident in the August networks but not those for June or July. Given the relatively constant size of the plant community over time, these monthly results indicate that pollinators that are active later in the year were not able to visit as many plants in 2010-2011 as they were in 1996-1997. This may be because the dates of first interaction for plants have advanced while those of pollinators have not. Whatever their cause, these changes in network structure are likely cause for concern, as loosely connected networks tend to be less robust to species loss (Dunne et al., 2002; Gilbert, 2009; Kaiser-Bunbury et al., 2010).

We also found support for Hypothesis 4, that species' roles would change between decades. In line with the changes in network structure, we found that the roles of both plants and pollinators shifted towards higher frequencies of motifs representing specialists interacting with generalists and lower frequencies of motifs representing generalists. These changes to species' roles were significant even after controlling for changes to network structure. As plants' mean degrees did not change between decades, these changes in roles suggest that newly-arrived pollinators in 2010-2011 tend to interact with relatively few plants and that some of the persistent pollinators have lost interaction partners such that the increasing size of the pollinator community did not result in more interactions per plant. Because all of the positions in plants' roles which showed the strongest declines describe generalists interacting with other generalists, it seems likely that pollinators with many interaction partners in 1996-1997 lost more interactions in 2010-2011 than did more specialised pollinators. From the pollinators' perspective, motifs describing generalists interacting with other generalists and motifs describing generalists interacting with large sets of specialists showed large declines. This is consistent with our picture of specialist pollinators arriving at Zackenberg and persistent pollinators losing some of their interactions, as is the increase in motifs describing pollinators sharing few plants with many other pollinators. As specialists are more vulnerable to extinction following a perturbation to their community (Burkle et al., 2013; Tylianakis, 2013), the changes to species' roles we have observed suggest plants and pollinators may be more vulnerable to continued climate change at Zackenberg. Based on changes to species' roles in the monthly networks, it appears that the species most likely to bear the brunt of future changes are plants that are most active early in the summer and pollinators that are most active late in the summer.

After establishing that network structure and species' roles both changed between decades, we then tested whether species' roles were related to their dates of first interaction and therefore likely to be affected by changes in phenology (Hypothesis 5). Plants' and pollinators' roles in the yearly networks were significantly associated with their dates of first interactions whether or not we controlled for network structure, as were pollinators' roles in monthly networks. Plants' roles in monthly networks only varied with their dates of first interaction after we controlled for network structure. We therefore conclude that species' dates of first interaction are indeed related to their structural roles in the plant-pollinator networks. For plants, this relationship did not vary between decades (i.e., there was no support for Hypothesis 6; Table 6). This suggests that, as plants' dates of first interaction advanced, they merely shifted into roles that had previously been occupied by other plants. For pollinators, the relationship between species' roles and their dates of first interaction did not vary between decades in the yearly networks but seemed to be stronger in 1996-1997 in the monthly networks. As pollinators' roles changed between decades but their dates of first interaction did not, it is understandable that the relationship between roles and phenology is breaking down. Since this breakdown was not detected in pollinators' yearly roles, however, it may be quite a subtle effect (and only detectable with the finer-grained monthly networks).

Lastly, we found limited support for Hypothesis 7 for both plants and pollinators (Table 6). For plants, there was no relationship between the change in roles and the change in their dates of first interaction in the yearly networks, and a significant relationship in the monthly networks for plants which became active later in 2010-2011 in the monthly networks. For pollinators the situation was more complex. In the yearly networks, there was a significant relationship for pollinators whose dates of first interaction retreated between decades, while in the monthly networks there was a significant relationship for pollinators whose dates of first interaction had advanced between decades. From these results, we must conclude that the amount of change in the dates when species become active is not a good predictor of the amount of change in their roles. Other elements of species' phenologies, such as emergence or flowering dates, may be better predictors of species' roles within their communities, but testing this was beyond the scope of the current study. In addition, the results of our analyses using simulated dates of first interaction suggest that our results for pollinators may be more susceptible to noise than those for plants. In future studies at Zackenberg, this discrepancy could be reduced by complementing observations of focal plants with analyses of pollinators carrying pollen are first caught, as opposed to pollinators which have emerged but not yet visited a plant. In the absence of such information, we are obliged to place more weight upon our results for plants than those for pollinators.

Putting all of our results together, we have shown that the plant-pollinator community at Zackenberg has experienced a great deal of turnover in pollinator species and changes to the timing of interactions. This is consistent with earlier findings showing that plants' flowering dates have advanced (Høye et al., 2013; Schmidt et al., 2016) and that species' ranges are shifting as the climate warms (Buisson et al., 2008; Flenner and Sahlén, 2008). Along with these changes, we have shown that the structure of the plant-pollinator network at this site has changed over time, as have the roles of species within it. In general, the Zackenberg pollination network appears to be unravelling, with fewer plants sharing pollinators and pollinators becoming more specialised. This is especially true for plants and pollinators active late in the summer. These species may have difficulty finding enough open flowers to feed upon (for pollinators) or obtaining sufficient pollination service (for plants). As feeding and reproduction are both essential to the maintenance of a population, some of these species may be lost if these trends continue for long enough. Moreover, both plants and pollinators are tending to share fewer interaction partners over time, leading to less redundancy in pollination services and food resources, respectively. Redundant sets of interaction partners are believed to provide an 'insurance policy' that can sustain species if their most important interactions are disrupted (Yachi and Loreau, 1999; Memmott et al., 2004; Potts et al., 2010). With severe weather and other perturbations becoming increasingly likely as climate change continues (Hassol, 2004; Adger et al., 2007; Steiner et al., 2015; Benestad et al., 2016), the plant-pollinator communities at high-arctic sites like Zackenberg may therefore be increasingly vulnerable if species' roles continue to change in the same ways. Moreover, as arctic communities have been warming faster than temperate sites (IPCC, 2013; Settele et al., 2014), they can be seen as the "canary in the coal mine", predicting future changes at lower latitudes.

Acknowledgements

The staff at Zackenberg Research Station provided access and assisted with logistics in Greenland. Heidi Elberling and Jesper B. Mosbacher also collected data. Insect identifications were done by Elisabeth Stur and Torbjørn Ekrem (Chironomidae), Verner Michelsen (all other flies), Ole Karsholt (Lepidoptera), Gergely Várkonyi (wasps), Paul H. Williams (bumble bees), and The Barcode of Life Data System (BOLD), Canada, sequenced all species to confirm identifications. We are grateful to the Natural Sciences and Engineering Research Council of Canada (ARC), INTERACT (projects QUANTIC) under the European Community's Seventh Framework Programme (TR), the Academy of Finland (grant numbers 1276909 and 1285803 to TR), the Ella and Georg Ehrnrooth Foundation (TR), the Carlsberg Foundation (CR), the Danish Research Council (JMO), Marsden Fund grant UOC-1101, administered by the Royal Society of New Zealand (DBS), and a Rutherford Discovery Fellowship (DBS) for financial support.

Supporting information

S4.1: Simulated dates of first interaction
S4.2: Tentatively-dated observations: methods
S4.3: Tentatively-dated observations: results
S4.4: Supplemental figures

Bibliography

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Chapter 5: Concomitant predation on parasites is highly variable but constrains the ways in which parasites contribute to food-web structure.

Alyssa R. Cirtwill[1] & Daniel B. Stouffer[1]

[1]Centre for Integrative Ecology
School of Biological Sciences
University of Canterbury
Private Bag 4800
Christchurch 8140, New Zealand

Published in *Journal of Animal Ecology* (2015).
DOI: 10.1111/1365-2656.12323.

This chapter has been modified from its original version. It has been formatted to match this thesis.

Summary

1. Previous analyses of empirical food webs (the networks of who eats whom in a community) have revealed that parasites exert a strong influence over observed food-web structure and alter many network properties such as connectance and degree distributions. It remains unclear, however, whether these community-level effects are fully explained by differences in the ways that parasites and free-living species interact within a food-web.

2. To rigorously quantify the interrelationship between food-web structure, the types of species in a web and the distinct types of feeding links between them, we introduce a new methodology to quantify the structural roles of both species and feeding links. Roles are quantified based on the frequencies with which a species (or link) appears in different food-web motifs– the building blocks of networks.

3. We hypothesised that different types of species (e.g., top predators, basal resources, parasites) and different types of links between species (e.g., classic predation, parasitism, concomitant predation on parasites along with their hosts) will show characteristic differences in their food-web roles.

4. We found that parasites do indeed have unique structural roles in food webs. Moreover, we demonstrate that different types of feeding links (e.g., parasitism, predation, or concomitant predation) are distributed differently in a food-web context. More than any other interaction type, concomitant predation appears to constrain the roles of parasites. In contrast, concomitant predation links themselves have more variable roles than any other type of interaction.

5. Together, our results provide a novel perspective on how both species and feeding link composition shapes the structure of an ecological community, and vice-versa.

Keywords

network motifs, species roles, interaction roles, role dispersion, role diversity

Introduction

Food webs– the networks of who eats whom in an ecosystem – provide ecologists with tools to analyse the structure of ecological communities (Cohen, 1978; Pascual and Dunne, 2007) and compare them across space and time (Thompson and Townsend, 2005b; Shurin et al., 2006; Olesen et al., 2008). Food webs also connect biodiversity to ecosystem functions by integrating patterns and processes from individual to community scales (Thompson et al., 2012). In particular, the overall structure of food webs has been directly tied to ecosystems' responses to environmental change (Thompson and Townsend, 2010, 2005a; Tylianakis et al., 2008) and robustness to species loss (Dunne et al., 2002b, 2004; Estrada, 2007; Srinivasan et al., 2007; Gilbert, 2009; Rezende et al., 2009).

The vast majority of food web studies, however, have focused on networks of predator-prey interactions between free-living species (Combes, 1996; Huxham et al., 1996; Marcogliese and Cone, 1997; Lafferty et al., 2006), prompting calls for a broader and more comprehensive food-web theory (Marcogliese and Cone, 1997; Lafferty et al., 2006; Fontaine et al., 2011; Kéfi et al., 2012), especially where parasites are concerned (Marcogliese and Cone, 1997; Lafferty et al., 2006; Dobson et al., 2008; Lafferty et al., 2008). Although typically small and difficult to observe, parasites can exert a strong influence on their communities (e.g., Huxham, Beaney & Raffaelli, 1996). They participate in a large proportion of feeding links (henceforth "links") (Lafferty et al., 2006; Dunne et al., 2013b) and exhibit comparable diversity and biomass to free-living species (Dobson et al., 2008; Kuris et al., 2008). Moreover, parasites' complex life histories, which commonly involve different sets of hosts for different life stages, render them vulnerable to secondary extinctions and therefore decrease network robustness (Lafferty and Kuris, 2009).

Parasites are also of interest because of the many ways in which they could potentially influence food-web structure– the organisation of links between species (Combes, 1996; Thompson et al., 2005; Lafferty et al., 2006; Dunne et al., 2013b; Thieltges et al., 2013, Fig. 19). Like generalist predators, many parasites have multiple potential hosts which may each support different life stages (Marcogliese and Cone, 1997; Lafferty et al., 2006; Rudolf and Lafferty, 2011). Parasites may also have one or more free-living stages which can be important prey for free-living predators (Combes, 1996; Kuris et al., 2008). Further, parasites vary in the ways in which they are transmitted between hosts: they can actively infect new hosts, be ingested as eggs

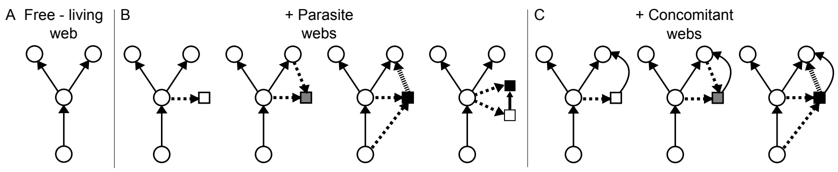

Figure 19: Parasites can be incorporated into food webs in several different ways, each of which increases the complexity of the web. **(A)** Food webs are typically composed of free-living species (circles) and the predator-prey links between them (arrows indicate the direction of energy flow). **(B)** In "+ parasite" webs, parasites (squares) parasitize free-living hosts (dotted line). They may parasitize one host for their entire life cycle (white square), different hosts (grey square), or be target prey to free-living predators (black square, hatched line). Where two parasites infect the same host (black and white square), one may kill the other, usually consuming it (thick black line). **(C)** "+ concomitant" webs also include links between parasites and the predators of their hosts (curved lines). In these links, the parasite may simply be digested (white square), or it may infect the predator and parasitize it as well (grey square). In some cases, a parasite (black square) may be consumed by the same predator both as concomitant prey and as target prey.

or cysts, or be ingested as concomitant prey along with the current host (Kuris et al., 2008; Thieltges et al., 2013).

Because of their plethora of life-history strategies, small body sizes, and unusual mode of life, it would appear that the ecological roles of parasites are completely distinct from those of more "traditional" predators and prey (Marcogliese and Cone, 1997; Rudolf and Lafferty, 2011). Indeed, at least one study has concluded that parasites tend to have broader and less-contiguous prey ranges than free-living species (Dunne et al., 2013b). Despite these important differences, however, that same study has suggested that parasites and free-living species can appear to have similar effects on food-web structure. For example, when parasites are added to a food web without including concomitant predation, species richness and number of links necessarily increase, and connectance, link density, and degree distributions are altered (Dunne et al., 2013b). Nevertheless, these structural changes are similar to the trends that emerge when comparing webs with different numbers of free-living species (Dunne et al., 2013b) and follow known patterns of scaling with species richness (Riede et al., 2010).

In contrast, the addition of concomitant predation links resulted in greater structural changes. First, by adding more links but no additional species, link density and connectance must necessarily increase (Dunne et al., 2013b). Importantly, this increase in connectance was observed even when the connectance of webs excluding concomitant predation was adjusted to account for the exclusion of this class of links and did not fit the scaling pattern observed in free-living webs (Dunne et al., 2013b). The higher connectance of food webs including concomitant links may in turn drive other trends in food-web structure, especially in properties such as nestedness which have been observed to increase when parasites are added to food webs (Lafferty et al., 2006) and are known to positively correlate with connectance (Dunne et al., 2002a). In addition to changing connectance, the addition of concomitant predation altered the frequencies with which different configurations of interactions among species occurred. In particular, the overlay of host-parasite and predator-prey interactions changed the frequencies of two-way feeding interactions (A eats B and B eats A), reflecting an effect of the intimacy between host and parasite on network structure (Dunne et al., 2013b).

This increase in connectance and the trickle-down effects on food-web structure attributable to higher connectance suggest that parasites may have their most unique effects on food-web structure as concomitant prey (Dunne et al., 2013b). This notion was most strongly supported by an analysis of three-species food-web motifs from the same study. A food-web motif represents a unique interaction pattern such as three-species food chains, apparent competition, or trophic loops (Milo et al., 2002; Kashtan et al., 2004; Stouffer et al., 2007, 2012), and the frequencies with which different motifs occur can be used to characterise fine-scale food-web structure (Stouffer et al., 2007). These frequencies were similar for webs composed solely of free-living species and webs including parasites but not concomitant links (Dunne et al., 2013b). This implies that the roles of free-living species serving as hosts are structurally similar to those of free-living species serving as prey, and that parasites as consumers have similar roles to free-living consumers (Dunne et al., 2013b). When concomitant links were added, the frequencies of motifs including at least one two-way link changed. This appeared to be driven by the increase in intraguild predation (predation between two species that share a common prey/host) as parasites are eaten along with their host (Dunne et al., 2013b), suggesting that parasites have different structural effects as resources than free-living species.

Comparisons of whole-network structure such as these, however, can mask the mechanisms behind the trends they uncover (Stouffer, 2010) since knowledge of a network-level pattern does not unambiguously determine how different species contribute to that pattern (Saavedra et al., 2011; Stouffer et al., 2012). For example, network-level measures such as connectance are a useful first step to predict predicting overall community stability (Dunne et al., 2002b); but connectance alone is a poor predictor of variation in species' degrees (Dunne et al., 2002a) or which species is most critical to maintain that stability (Dunne et al., 2002b; Olesen et al., 2011). One way to overcome this drawback is to examine network structure directly from the perspective of the building blocks of networks: species and the links between them (Stouffer, 2010; Baker et al., 2015).

Here we use an extension of food-web motifs to quantify species' "structural roles"– which provide holistic summaries of how they interconnect with the rest of the web (Stouffer et al., 2012, Fig. *S5.1*)

–and hence to compare the different ways in which parasites and free-living species are thus embedded in their communities. This definition of role is rigorously defined by the relative frequencies with which species appear across different motifs like apparent competition, omnivory, or trophic loops. As such, our definition of roles incorporates information on a species' predators and prey, as well as how that species is indirectly linked to more distant species. Roles can therefore also be conceptualised as summaries of the "shape" of species' biotic niches within a food web. As a consequence, we can estimate the degree to which species' contributions to network structure (and hence to energy flows and other ecosystem functions) are redundant by identifying species with similar roles. Such species can likely compensate for each other in the face of disturbances, increasing the network's robustness (Naeem, 1998; Rosenfeld, 2002).

To understand how roles can vary between species, consider three hypothetical top predators: one which is a strict specialist that acts as the top of only one food chain; a second, generalist predator that acts as the top of several food chains; and a third predator which forms the top of several food chains *and* engages in omnivory. The roles of the first two predators are very similar– despite having different numbers of prey species, both predators only ever appear in one position in the food web: at the top of a food chain. The third predator, which is involved in motifs describing omnivory, as well as three-species food-chains, has a more complex role. One could therefore argue that the first two species make similar structural contributions to the network while the third predator has a distinct effect. Moreover, these species likely make different contributions to the stability and functioning of the community (Stouffer, 2010; Stouffer et al., 2012).

This argument rests upon the fact that species' structural roles describe the ways a species directly and indirectly influences biomass and energy flows through a food web. Therefore, the hypothesis that parasites and free-living species interact with other species in fundamentally different ways can be directly tested by comparing their structural roles. Here we focus on the comparison of the roles of parasites to those of free-living species interacting only with other free-living species. When concomitant predation is excluded, parasites have many prey but few consumers and are usually considered to be the tops of their food chains (Thompson et al., 2005). We therefore expect the structural roles of parasites excluding concomitant predation to be similar to the roles of free-living species with no free-living predators (hereafter "top predators") or to intermediate consumers with few free-living predators. When concomitant predation is taken into account, however, parasites have both prey and many consumers. If parasites have similarly-shaped niches to those of free-living species, we would then expect the structural roles of parasites including concomitant predation to be similar to those of free-living intermediate consumers.

In a similar way, we can examine food webs from the perspective of the links within them. Just as a species' structural role summarises the ways in which it is connected to other species, a link's structural role summarises the ways in which an energy transfer between two species is embedded in the larger food web (Fig. *S5.3*). The roles of links, like those of species, vary depending on how many connections a link has to the rest of the web, and the nature of species involved in those connections. A link between an unpalatable basal resource, its specialist herbivore, and a specialist consumer of that herbivore, for example, would have a role summarised by a single dimension describing its single position. In contrast, a link between two generalist intermediate consumers would have a role with many dimensions corresponding to the many disparate positions that link appears in across food-web motifs. Note that, as with species, roles describe the relative frequencies with which a link occupies different positions rather than the raw count. Thus a link which appeared in the same position 10 times would have the same role as a link which only appeared in that position once, and both would have very different roles to a link which appeared once in each of 10 different positions. By comparing link roles in this way, we can determine whether feeding links involving parasites are organised differently within a food web regardless of whether the roles of parasites themselves are different. This alternative view is hinted at by the observation that food-web structure is altered more by the inclusion of concomitant links than by the simpler addition of parasites without concomitant predation (Dunne et al., 2013b).

It is more difficult to generate intuitive hypotheses about differences between the roles of types of links because of a dearth of previous studies that have directly characterised their roles in food webs. Nevertheless, predation, parasitism, and concomitant predation all involve different types of species and have different functional consequences for the two interacting species. We therefore expect significant differences in the structural roles of these links. Since adding concomitant predation links changed the motif structures of food webs (Dunne et al., 2013b), we expect that these links

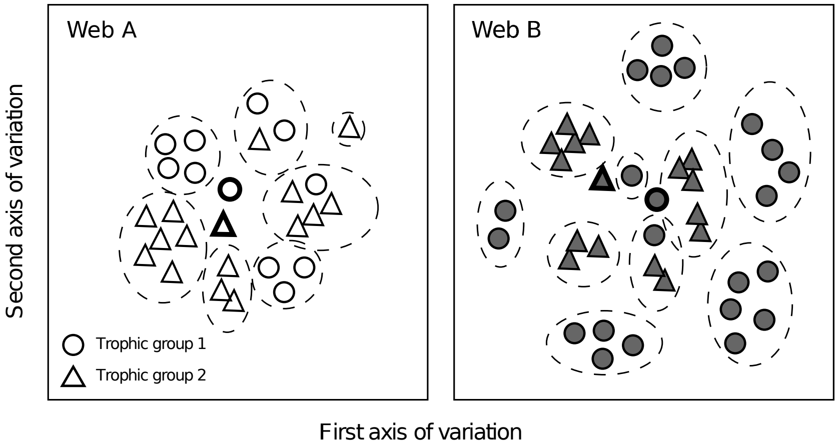

Figure 20: Visualising the distribution of species roles within two hypothetical food webs. In panels **(A)** and **(B)**, the roles of two trophic groups (e.g., top predators and intermediate consumers) are indicated by circles and triangles, respectively. Because our definition of roles is multidimensional, they are most easily represented using a correspondence analysis in which roles are compared along major axes of variation rather than axes based on particular motifs. Axis one might represent, for example, the tendency for roles to contain motifs involving two-way interactions, while axis two might represent the tendency for roles to contain motifs representing trophic loops. Under this representation, dispersion and diversity provide complimentary measures of the distribution of roles within communities. Dispersion measures the spread of roles about the median role for a trophic group (indicated by shapes with thick outlines), while diversity measures the number of statistically identifiable role "phenotypes" (indicated by dashed ovals). In hypothetical web **A**, the roles of the two types of species have similar levels of dispersion and diversity despite greater numbers of species in trophic group 2 being present in the community. In hypothetical web **B**, the roles of species in trophic group 1 are more widely-dispersed and more diverse than those of trophic group 2.

will have different roles from those of links between free-living species. Conversely, because adding links describing parasitism and predation among parasites to food webs does not change motif structure of food webs, we expect that these links will have similar roles to those of links between free-living species.

As well as comparing roles of different types of species and links across communities, we aimed to study the variability of different roles within communities. Measuring this variability provides a more rigorous analysis of the potential overlap or redundancy among the structural roles of species within a type. Specifically, we quantified the within-community dispersion and diversity of roles for each group of species and links. The dispersion of a type of roles is its within-group variance– that is, how similar the role of each group of species or links is to the median role for that group in its community (see *Materials and methods*). A high role dispersion for a group of species indicates that each species' role has limited overlap with those of other species in the same group. Role diversity, in contrast, quantifies the observed number of statistically unique role "phenotypes"– characteristic multidimensional shapes into which roles can be grouped –occupied by species or links from a particular group in a community (see *Materials and methods*). Role diversity therefore offers a perspective on how different types of species or links contribute to the overall role diversity of a food web. A high diversity of roles for a group of species means that these species occupy a wider range of the potential roles available to all species in all food webs. Importantly, these two measures are complimentary, such that a group of species whose roles have high dispersion might exhibit high or low role diversity (Fig. 20).

Once the distributions of species and link roles have been quantified within communities, we are able to compare these distributions across communities. Similar patterns of distribution across communities can point to general rules in food-web structure such as the scaling of many food-web properties with species richness and connectance (Havens, 1992; Dunne et al., 2002a; Riede et al., 2010). Here we are particularly interested in whether role dispersion and diversity exhibit scaling relationships with species richness (or link richness, in the case of link roles). If, for example, dispersion and diversity increase with species richness, this would suggest that species roles are increasingly variable in larger webs and that adding more species does not create redundancy within the food-web. Such a situation would recall May's "devious strategies" by which communities persist, with none acting in the exact same way as the next (May, 2001). It is also possible that role dispersion and diversity do not increase with species or link richness; such saturation of role distributions would indicate high redundancy and create a community that is robust to perturbations (Petchey et al., 2008).

Materials and methods

Empirical Data

The food webs studied here describe seven temperate coastal communities (Huxham et al., 1996; Hechinger et al., 2011b; Mouritsen et al., 2011; Thieltges et al., 2011a,b, Tables S1-S3) that included both free-living species and parasites (see S1 for the full definition of 'parasite'). Since we were interested in particular species rather than whole-network characteristics, we did not aggregate species with the same predator and prey sets into trophic species as is common elsewhere (Martinez, 1991; Vermaat et al., 2009; Dunne et al., 2013b). The links in these food webs describe several different classes of interaction: predation among free-living species, parasitism of free-living species, predation among parasites, and target and concomitant consumption of parasites (Hechinger et al., 2011b).

Using these different link types, we constructed three food webs describing different interactions among the species in each community (Fig. 19). The first, "free-living" web contains only free-living species and the predator-prey links between them. The second, "+ parasite" web includes every species and link in the free-living web as well as parasites, parasitism of free-living species, intraguild predation between parasites, and predation by free-living species upon parasites in which the parasite is target prey (e.g., when a fish consumes trematode cercariae). The third, and most complex, "+ concomitant" web contained all of the species and links in both of the previous webs as well as concomitant links where parasites are consumed together with their hosts. For each of the seven communities we therefore have a free-living, parasite, and concomitant web (giving a total of 21 food webs).

Quantifying Species Roles

We then analysed the role of each species within its community by quantifying the ways in which the focal species participates the set of 13 unique three-species building blocks that make up a food web – network motifs (Milo et al., 2002; Kashtan et al., 2004; Stouffer et al., 2007, 2012). Of the three-species motifs, five contain only one-way interactions (A eats B, B does not eat A) and the remaining eight contain at least one two-way interaction (A eats B and B eats A). The two types of motifs tend to occur with different frequencies (Stouffer et al., 2007) and, by definition, have different effects on energy flow throughout a food web. The frequency with which a species appears in each motif summarises the organisation of its feeding links, as both predator and prey. Mathematically, the number of times a focal species i in community s (e.g., the Ythan estuary) in web type w (e.g., the "+ parasite" web) appears in each of the 30 unique positions across the 13 three-species motifs gives a multidimensional vector $\overrightarrow{f_{si}^{w}}$ that robustly quantifies the species' role within the food web (Stouffer et al., 2012, *S5.2, Fig. S5.1; Supporting Information S5*).

Given a dataset composed of roles for all species in all webs for each community, we first compared the roles for species in different trophic groups. We divided free-living species into top predators (T), basal resources (B), and intermediate consumers (I) based on their interactions with other free-living species (see S1 for more details). Since food webs have traditionally been composed only of free-living species and the roles of species have been understood in this context, we used the roles of free-living species in the free-living webs as a baseline against which to compare the roles of parasites with (P_c) and without (P) concomitant links. Although using the free-living species web as a baseline means comparing the roles of parasites in a larger web to free-living species in a smaller web, network-level results suggest that motif frequencies do not change systematically after the addition of more species, including parasites (Bascompte and Melián, 2005; Stouffer et al., 2007; Dunne et al., 2013b). We therefore do not expect network size to greatly influence parasites' roles compared to those of free-living species. We included the roles of the same parasite species in both the "+ parasite" and "+ concomitant" webs in order to determine whether parasites have different roles when concomitant links are excluded or included. All five groups of species were represented in each of the seven webs, giving a sample size of $n = 35$ for analysis of species roles.

Quantifying Link Roles

Following an analogous methodology to that used in the determination of species roles, each link k in web type w at community s was assigned a role vector $\overrightarrow{f_{sk}^{w}}$ based on the frequency with which it occurred in each of the 24 unique "link positions" that make up the 13 three-species motifs (*S5.2, Fig. S5.2; Supporting Information S5*). As with the roles of species, we used links between free-living species (F→F) in the free-living webs to set the *de facto* baseline since these are the links current food-web theory is based upon. For consistency with the analysis of species roles, we included the roles of all other types of links from the least complex web in which they appeared. That is, we used the roles of parasitism (F→P), intraguild predation (P→P), and target predation on parasites ($P\xrightarrow{t}F$) as calculated in the "+ parasite" webs and the roles of concomitant predation ($P\xrightarrow{c}F$) links from the "+ concomitant" webs. $P\xrightarrow{c}F$ links include those in which the ingested parasite can infect its predator (i.e., trophic transmission) and those in which the parasite is digested and killed. Note that predation among parasites and target predation on parasites were not recorded in the Ythan estuary web. This means that while analyses of species roles had a sample size of $n = 35$ (seven sites, 5 types of species roles), analyses of link roles had a sample size of only $n = 33$ (seven sites for most link types, six sites for predation among parasites and target predation on parasites).

Quantifying differences in the Distribution of Roles

Median Roles

We first visualised the median roles of parasites with and without concomitant predation alongside of those of the three free-living trophic groups. To do this, we performed a correspondence analysis using the function cca from the package vegan (Oksanen et al., 2014) in R (R Core Team, 2014). Using correspondence analysis of species roles also allowed us to examine the axes along which most variation between roles occurred. We used the same procedure to visualise the median roles of different types of links, and the axes along which link roles varied.

To compare median roles, we used a non-parametric permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) across the full set of species (or link) roles. Recall that as we have defined them here, roles are multidimensional descriptions; the spatial median of the roles in a given group thus describes the "typical" role for that group. For species, we compared median roles across trophic groups (T, I, B, P, and P_c). We conducted a similar PERMANOVA analysis comparing median roles across link types ($F\rightarrow F$, $F\rightarrow P$, $P\rightarrow P$, $P\xrightarrow{t}F$, and $P\xrightarrow{t}F$). All comparisons of median roles were conducted using the adonis function from the vegan package (Oksanen et al., 2014) in R (R Core Team, 2014).

Like the traditional ANOVA, the PERMANOVA first calculates the distance between all pairs of observations and then compares among-group distances to within-group distances following a pseudo-*F* statistic (Anderson, 2001). Importantly, a PERMANOVA does not assume that the data follow any particular distribution. Instead, a *p*-value for the test statistic is calculated by directly permuting the raw data (Anderson, 2001). Since we were most interested in differences between types of species (or links) and not between different communities, we stratified permutations by community. That is, roles were shuffled randomly within a community but the complete set of roles for that community was not changed by the permutation process. In this way, we compared observed distances only to those that could be randomly generated from the same community, controlling for possible effects of changes in species richness or other properties between communities.

The distance metric used in a PERMANOVA helps to define the null hypothesis being tested (Anderson, 2006). We used Bray-Curtis dissimilarity between roles as our distance metric because it has proven useful for other ecological questions (Legendre and Legendre, 2012) and also has specific properties that make it well suited for our purposes. In particular, Bray-Curtis dissimilarity measures differences between the roles based only on positions in which at least one of the species (or links) appears and hence is not affected by "double zeros" in the data (Legendre and Legendre, 2012). This means that species (or links) that appear in few positions are not considered more similar to each other due to the large number of shared zero frequencies. In addition, we wished to avoid a situation in which two species involved in different numbers of links would be considered to have different roles even if they occurred with the same frequencies across all motif positions. We therefore calculated dissimilarities based on relative positional frequencies rather than absolute frequencies (that is, the number of times a species or link appeared in each position divided by the number of times it appeared in any position).

Role Dispersion

In addition to comparing median roles across communities, we explored the dispersion of roles about these median roles using the function betadisper from the package vegan (Oksanen et al., 2014) in R (R Core Team, 2014). As when comparing median roles, we used Bray-Curtis dissimilarity to measure the dispersion of roles within a community around their group median. We were then able to compare the scaling relationships between role dispersion and species or link richness across communities. We hypothesised that role dispersion of a given type of species or link could increase with the number of those species or links observed at an individual community, indicating that each species and link fills a novel structural role. To determine the relationships between the number of species (or links) of a type at a community and the mean dispersion of roles for that species type at that community, we used a linear regression, fit using the function lm in R (R Core Team, 2014).

Role Diversity

We also measured the diversity of unique roles within a community for each group of species or links. To do this, we used a heuristic optimisation method to identify clusters of species (or links) that appear in the same motif positions more often than one would expect by chance (Guimerà et al., 2007; Sales-Pardo et al., 2007; Stouffer et al., 2012, *S5.3, Supporting Information S5*). Each cluster was interpreted as a unique role phenotype.

As with dispersion, we then compared the scaling relationships between role diversity and species or link richness across communities. We expected diversity to increase with species or link richness, implying that each species or link adds to the niche space of its food web. To quantify this possible relationship between the number of species or links and the number of roles in a community, we used a generalised linear model with a Poisson distribution and

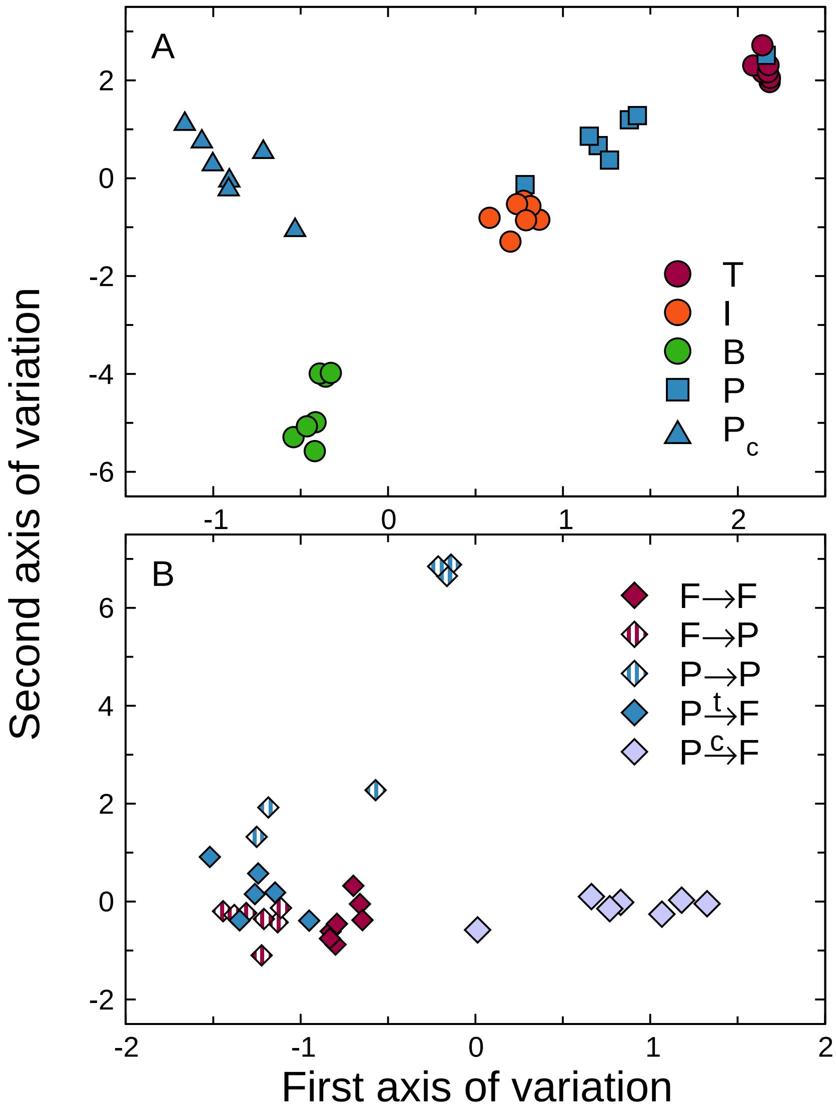

Figure 21: The median roles of species and links vary predictably by type. **(A)** Within the seven different communities, the different types of species have different median roles, shown here with respect to their location along their first two correspondence analysis axes. The first correspondence analysis axis for species roles described 64.9% of their total variance, and the second axis described 13.0%. When concomitant links are excluded, parasites (P) tend to have roles similar to those of top predators (T). When concomitant links are added, however, parasites' (P_c) roles are much more similar to those of basal resources (B). Intermediate species' (I) roles were between those of B and T species. **(B)** Different types of links also have different median roles, again shown with respect to their first two correspondence analysis axes. The first correspondence analysis axis for links described 60.7% of their total variance, and the second axis described 15.2%. While there is some overlap between roles, concomitant predation links and predation between parasites mainly varied along the first axis while predation between free-living species, parasitism, and target predation on parasites mainly varied along the second axis.

logarithm link function, fit using the function glm in R (R Core Team, 2014).

Results

Median Roles

We found that both different trophic groups and different link types have different median roles (see S4 for more details). Both P_c roles and the roles of $P \xrightarrow{c} F$ links were separated from the roles of other types of species or links, respectively, along the first correspondence analysis axis (Fig. 21). This axis corresponded to a division between motifs that include only one-way interactions and those that include at least one two-way interaction (Fig. *S5.3*), with P_c roles and the

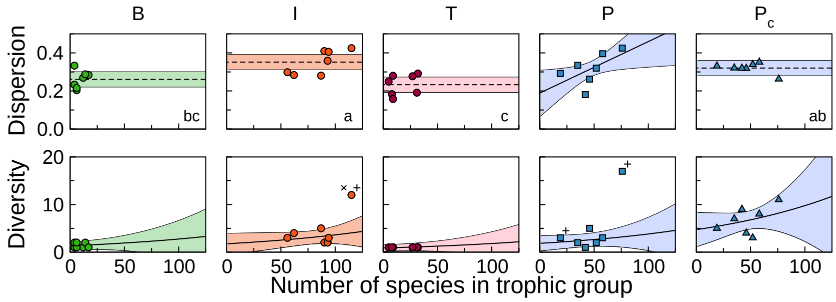

Figure 22: The influence of the number of species in a trophic group on the dispersion and diversity of species roles differed between free-living species and parasites. **(Top row)** Role dispersion increased with number of species for parasites without concomitant links ($p = 0.036$). The dispersion of the roles of all other species types did not vary with species richness (dashed lines). The roles of intermediate consumers were most dispersed, followed by those of parasites with concomitant links, basal resources, and top predators. Letters in the lower right of each panel indicate groups based on mean dispersions of each type of role (Tukey's HSD test with critical value = 4.11, α=0.05, and df=29). Roles with the same letter do not have significantly different mean dispersions. **(Bottom row)** Role diversity increased with increasing species richness for all types of species ($p = 0.003$), and the estimated rate of increase was the same for all species types. For any given species richness, parasites with concomitant links had more diverse roles than any other type of species, followed by intermediate consumers, parasites without concomitant links, basal resources, and top predators (Tukey's HSD test with critical value = 4.14, α=0.05, df=26). In both rows, shaded regions represent 95% confidence regions for the predicted dispersion or diversity after the removal of statistical outliers (indicated by '+'s) where applicable. Refer to *S5.5, Supporting Information S5* for details on the regressions.

roles of $P\xrightarrow{c}F$ links being found more often in motifs including at least one two-way interaction.

Dispersion & Diversity of Species Roles

Comparing the underlying variation of species roles, we found that dispersion was not affected by species richness for B, I, T, and P_c roles ($t_{28} = 1.563$, $p = 0.129$; Fig. 22; for details of the regression see *S5.5, Supporting Information S5*). P_c roles were significantly more dispersed than T roles but had similar dispersion to other types of roles (Tukey's HSD test with critical value = 4.11, α=0.05, and df=29). Unlike all other types of species roles, dispersion of P roles increased with species richness ($t_{29} = 2.195$, $p = 0.036$; *S5.5, Supporting Information S5*).

The diversity of distinct roles in a trophic group increased with the number of species in that group, but the strength of this relationship did not vary across groups (Fig. 22). For any given number of species, P_c roles were significantly more diverse than those of other types of species ($z = 5.632$, $p < 0.001$; *S5.5, Supporting Information S5*). P roles were significantly more diverse than T roles but their diversity overlapped with those of I and B roles (Tukey's HSD with critical value 4.14, α=0.05, and df=26).

Dispersion & Diversity of Link Roles

Dispersion of the roles of P→P links was positively related to the number of those links in a community ($t_{27} = 4.195$, $p < 0.001$; Fig. 23B; *S5.6, Supporting Information S5*) and was independent of the number of links for all other link types. Of those, the roles of $P\xrightarrow{c}F$ links were the most widely-dispersed, followed by those of F→F links, F→P links, and $P\xrightarrow{t}F$ links (Tukey's HSD test with critical value 4.13, α=0.05, and df=27; Fig. 23A). In contrast to the diversity of species roles, the diversity of unique link roles did not vary with

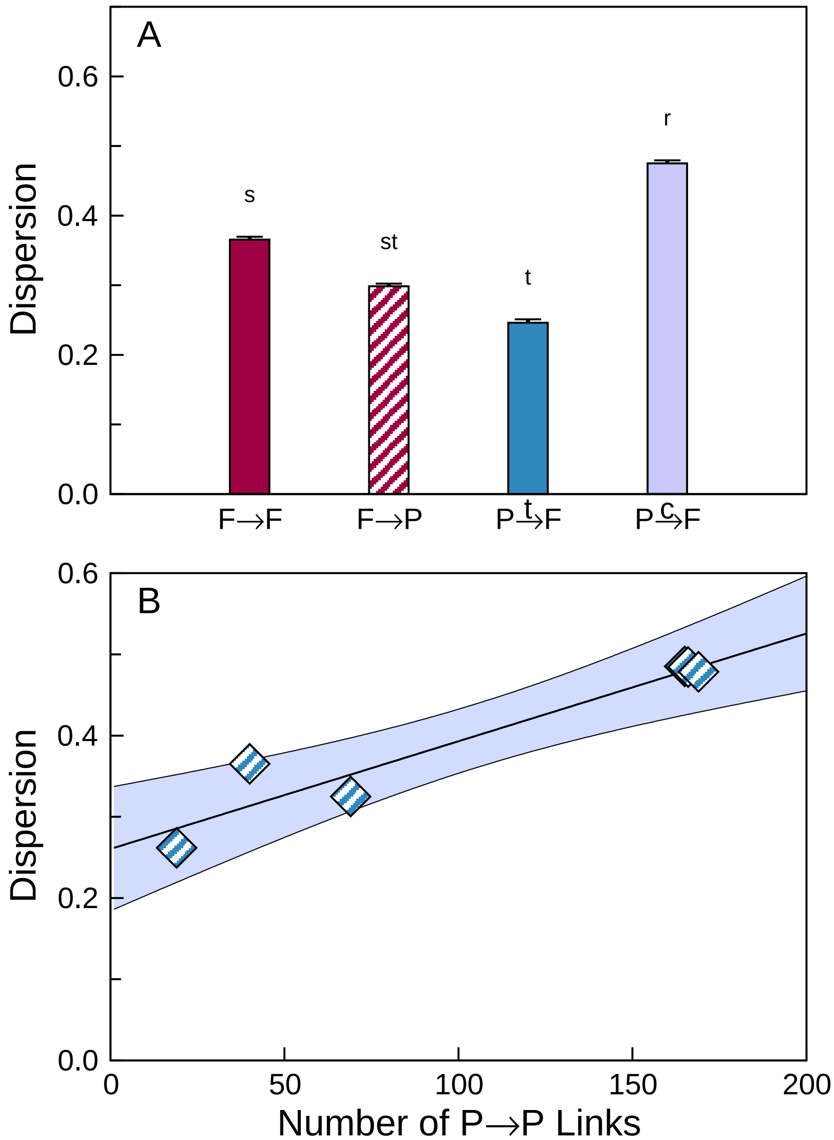

Figure 23: Dispersion of link roles varied across link types while diversity did not. **(A)** The roles of concomitant predation links (P$\xrightarrow{c}$F) were most dispersed followed by those of predation among free-living species (F→F), parasitism (F→P), and target predation on parasites (P$\xrightarrow{t}$F). For these link types, the dispersion of link roles was not related to the number of links in a community. **(B)** Dispersion of the roles of links describing predation between parasites, on the other hand, increased with the number of such links in a community. In **(A)**, the different letters indicate significantly different dispersions and the error bars depict 95% confidence intervals about the mean. Letters above each bar indicate groups based on mean dispersions, and types of link with different letters have significantly different dispersions (Tukey's HSD test with critical value 4.13, α=0.05, df=27). In **(B)** the shaded region represents a 95% confidence region for predicted dispersion. See *S5.5*, *Supporting Information S5* for details about the regressions.

the number of links of that type in a community (Fig. *S5.6*), nor did it differ across types of links (Tukey's HSD test with critical value 4.10, α=0.05, and df=28).

Discussion

Parasites' unique life histories and ways of feeding suggest that they should interact with other species differently than free-living species (Marcogliese and Cone, 1997; Lafferty et al., 2006, 2008; Warren et al., 2010; Thieltges et al., 2013). Despite these important morphological and behavioural differences, a previous study comparing versions of food webs including and excluding parasites found that webs including both types of species but not concomitant predation have similar structural properties to similarly-sized webs composed of free-living species only (Dunne et al., 2013b). This indicates that differences between free-living species and parasites as consumers do not translate to the network level (Dunne et al., 2013b). Nevertheless, webs including free-living species, parasites, and concomitant predation links do indeed have different structures from other webs, suggesting that it is parasites' unique positions as concomitant resources that have the greatest effects on network structure, including effects on properties such as connectance which have been linked to robustness (Dunne et al., 2002b, 2013b). In order to examine this inference in greater detail, here we have examined food-web structure from the perspective of species and the links between them. We have thus been able to systematically uncover the ways in which free-living species, parasites, and the multiple types of links between them differ in the broader food-web context.

At the species level, our results reaffirmed the impact of links in which parasites are concomitant resources on network structure (Poulin et al., 2013; Thieltges et al., 2013). The roles of parasites excluding concomitant predation were most similar to those of top predators and intermediate consumers. One potential explanation for the similarity of parasites' roles to those of free-living intermediate consumers could be the aggregation of parasite life stages. While free-living intermediate consumers may experience predation during any time of life, parasites have very few consumers except during free-living life stages. Although a stage-specific analysis is beyond the scope of the present work, this suggests that the structural roles of different parasite life stages could range from those of free-living basal resources (for non-feeding stages with consumers) through to those of free-living top predators (for parasitic stages that are not affected by other parasites in the same host). Nevertheless, when concomitant predation was included, the roles of parasites were distinct from those of any other type of free-living species. This suggests that the network-level effects of concomitant predation may truly be due to changes in the roles of parasites themselves.

In addition to affecting the median roles of parasites, the inclusion of concomitant predation greatly altered the distribution of parasites' roles. Specifically, adding concomitant predation increased role variability in parasite-poor communities to a similar level as that of parasite-rich communities, such that parasites' roles appeared saturated when concomitant predation was included and unsaturated when they were not. This apparent homogenising effect of concomitant predation may arise from the fact that these links bind the roles of parasites to those of their hosts, creating intimate structural similarities. In parasite-poor communities, it is likely that few parasites share common hosts and therefore common concomitant predation links. As parasites "inherit" role variability from their hosts via concomitant predation, less overlap in host ranges among parasites may lead to greater dispersion of their roles.

Unlike role dispersion which was saturated for most trophic groups, role diversity increased with number of species for all groups. This implies that, while species roles are similarly predictable on the basis of species type regardless of the size of the food web, roles overall do not become more redundant as the number of species in the web increases. This observation fits in well with the suggestion that there is no single way to configure a stable community (May, 2001). Contrary to models of stable ecosystems where greater diversity begets greater niche overlap in order to use resources as efficiently as possible, in unstable systems each species' niche may have to be distinct if it is to withstand disturbances (May, 2001). Beyond this overall lack of saturation, P_c roles were more diverse than other types of roles for a given number of species in the trophic group. Lower redundancy in P_c roles despite their similar dispersion to other role types could be a result of the different potential outcomes of concomitant predation for the parasite. While concomitant predation is always fatal for the host, the parasite may, for certain predators, be able to infect the predator and use it as its next host. For many parasites, such "trophic transmission" is an essential part of the life cycle (Thieltges et al., 2013), and it is possible that the roles of such links differ from those of concomitant predation links in which the parasite is destroyed. This lack of redundancy, coupled with the increase in role dispersion resulting from including concomitant predation, means that parasites should have widely varying effects on network structure. This in turn implies that parasites can generate a variety of effects on population dynamics and energy flows through their communities. In particular, lack of redundancy means that any effects of fluctuations in the population of one parasite (e.g., on host mortality) are unlikely to be compensated for by another parasite with a similar role.

To further clarify the impact of different types of links, we considered the roles of links directly. The dispersion of link roles generally appeared to be saturated–that is, independent of the number of a given type of links present in a network. This suggests that there were sufficient links in each community to occupy the entire role space for most types of links. Given the saturation of role dispersion for most types of species, this is not surprising. The only type of link for which role dispersion was not saturated was predation among parasites. This type of link includes hyper-parasitism, predation among free-living stages of parasites, and attack by one parasite on others within the same host, with or without consumption (Hechinger et al., 2011b). This variety of types of feeding and interaction locations might explain the apparent tendency for links describing predation among parasites to be increasingly distinct from the group median. Surprisingly, this variability in link roles does not appear to be linked to a greater diversity of unique role phenotypes.

Dispersion, conversely, differed among link types with the roles of concomitant predation links being the most variable. While concomitant predation ties the roles of parasites to those of their hosts, the roles of these links are non-trivially tied to the roles of the predation links that lead to them. Alternatively, it is possible that the wide variety of outcomes of concomitant predation for both parasite and consumer (Thieltges et al., 2013) leads to these links having inherently more variable roles. Were that the case, however, we could expect a greater diversity of unique roles for these links as well as greater diversity, which we did not observe. It therefore appears that, by combining predation with parasitism, concomitant predation is simply less predictable than other types of interactions. This may mean that the consequences of concomitant predation for energy flows or population dynamics are similarly unpredictable.

Conclusions

Our species-centric and link-centric perspectives allow us to robustly identify how and where the contributions of parasites to network structure differ from those of different types of free-living species. Within a complex food web, it is common to characterise species' structural roles in terms of the organisation of their direct and indirect links with other species (Luczkovich et al., 2003; Olesen et al., 2007; Allesina and Pascual, 2009). As we show here, the structural roles of links can also be characterised by the pair of species that make them up and, by extension, all other links those species participate in. Though both perspectives build from the same fundamental information, our analysis demonstrates that they are not equivalent and instead provide a complementary picture of the building blocks of food-web structure.

Overall, our results reinforce the idea that concomitant predation plays a disproportionately important part in determining the structure of food webs (Dunne et al., 2013b; Poulin et al., 2013) and that it places considerable constraints on the median roles of parasites while simultaneously increasing the variability about these median roles. This implies that concomitant predation not only affects the ways in which parasites in general affect community functions and stability but that it decreases the redundancy of each species' contribution to those effects. Historically, concomitant predation has often been omitted from food webs, either because it is assumed to be energetically insignificant (Thieltges et al., 2013) or because it is inherently difficult to directly observe (Marcogliese and Cone, 1997). The structural implications of these links as shown here, as well as their prevalence within food webs (Thieltges et al., 2013), potential energetic implications (Lafferty et al., 2006; Hechinger et al., 2011a; Thompson et al., 2013), and importance as sources of either mortality or trophic transmission (Lafferty et al., 2006; Thieltges et al., 2013) for parasites mean that they should no longer be ignored. Finally, as concomitant predation links reveal the deep intimacy between hosts and parasites, they provide a critical lens through which to examine the many ways in which parasite-host and predator-prey interactions are linked.

Acknowledgements

We thank Jennifer Dunne, Nicolas Mouquet, Dominique Gravel, Robert Poulin, Nick Baker, Katie Bowron, Carol Frost, Camille Coux, Guadalupe Peralta, and Simon Litchwark for comments on the manuscript. This research was supported by an NSERC-PGS-M graduate scholarship and a University of Canterbury Doctoral Scholarship (to ARC) and a Marsden Fund Fast-Start grant (UOC-1101), administered by the Royal Society of New Zealand (to DBS). We are thankful to the BlueFern University of Canterbury Super Computer for computing facilities.

Data accessibility

Food webs used in this study are publicly available via Dryad data package (Dunne et al., 2013a, http://dx.doi.org/10.5061/dryad.b8r5c).

Supporting Information

S5.1 Additional References and Description of Food Webs
S5.2 Supplemental methods: quantifying species' and links' roles.
S5.3 Supplemental methods: role dispersion & diversity

S5.4 DSupplemental results: median roles.

S4.5 Model selection for analysis of dispersion and diversity of species roles.

S4.6 Model selection for analysis of dispersion and diversity of link roles. Figure showing link role diversity.

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Chapter 6: Taking the scenic route: trophic transmission of parasites and the properties of links along which they travel.

Alyssa R. Cirtwill[1], Clement Lagrue[2], Robert Poulin[2], Daniel B. Stouffer[1]

[1]Centre for Integrative Ecology
School of Biological Sciences
University of Canterbury
Private Bag 4800
Christchurch 8140, New Zealand

[2]Department of Zoology
University of Otago
340 Great King Street
PO Box 56
Dunedin 9054, New Zealand

Manuscript prepared in the style of *Ecology*.

This chapter has been modified from its original version. It has been formatted to match this thesis.

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. As feeding links among free-living species have different dynamic and structural properties, it seems plausible that these links will vary in their effectiveness as transmission routes. Moreover, most parasites are restricted to certain host taxa at each life stage, so not all links will be possible transmission routes. Here we test this possibility for parasites and their hosts in four New Zealand lakes. We use three dynamic properties and one structural property to measure differences among feeding links and then test whether each property can predict whether or not a link will transmit parasites. In each test, we use both an unrestrictive and a taxonomically-informed null model, allowing us to determine the extent to which the taxonomy of free-living species affects parasites' transmission routes. Contrary to our expectations, we found that parasites tend to be transmitted along dynamically weak links (i.e., links that make small contributions to the diets of predators, transmit little biomass, and involve rare prey). However, the structural properties of links that transmit parasites reveal that they are likely to be particularly important to the community because they are highly central and can therefore affect many free-living species. By comparing our results against our two null models, we also found that several of the trends we identify are largely determined by the restriction of parasites to particular host taxa. This means that the host specificity of parasites is a key determinant of their transmission routes. As a whole, our results suggest that parasites follow transmission routes that are particularly unlikely to have a destabilising effect on the community. Dynamically weak links, like those that transmitted parasites in this study, tend to stabilise food webs by dissipating perturbations to the community. Structurally important links, conversely, can have a large impact on food webs. Parasites therefore appear to strike a balance between the highway and the scenic route and are transmitted along links that bind their communities together.

Keywords

concomitant predation, food-web dynamics, network motifs, food-web structure, interaction roles

Introduction

Parasites are increasingly recognised 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 for free-living species 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). Many parasites in turn rely on the structure of 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. 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 links which contribute a particularly large proportion of a a predator's diet might be more likely to occur and therefore be a better component of a transmission route than a link which contributes less to the diet of the predator. This is especially true for definitive hosts, which often experience only minor effects from infections (Lafferty, 1992). Because the cost of infection is low and infected prey are often easier to catch and kill (Lafferty, 1992), 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 killed. 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 a 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 are likely to be critical to the overall functioning of the community and therefore may be more reliable than other links.

In addition to their dynamic properties, a link might be important because of its *structural properties*— the ways in which the link contributes to the structure of the food web. In particular, links which are highly "central"— that is, those which lie on the shortest paths between many pairs of species (Newman, 2010)—could be good transmission routes. These links are considered important because they indirectly affect many species (Jordán et al., 2007; Lai et al., 2012). As such, variability in central links would have a large effect on the rest of the web and destabilise the community (Lai et al., 2012). Central links may therefore be less variable and more reliable than other links. Supporting this hypothesis, previous research has shown that highly-central species tend to host more parasite species than do other free-living species (Chen et al., 2008; Thompson et al., 2013). Highly central hosts also 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.

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. For example, most trematodes use molluscs 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 rather than because these hosts are the best "stops" for parasites to visit on their transmission routes. 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 parasites tend to be transmitted along feeding links that are particularly important to the food web. We also test which dynamic or structural properties of feeding links most parsimoniously explain trends in parasite transmission. 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, and (iv) be more central than other links. We also expect that the influence of these properties will depend on the restriction of parasites to particular host taxa. To investigate this last question, we test each of the above hypotheses using both an unrestrictive null model and a more conservative, taxonomically informed null model that explicitly incorporates the effects of the host specificity of parasites.

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. Together, these webs included 2160 links between 110 free-living species. The lake communities also contained 49 parasite life stages, 13 of which were trophically transmitted. For a detailed description of sampling methods and reconstruction of feeding links, see *S6.1, Supporting Information s6* 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 predicted the outcome of a link for parasites. 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 gut contents accounted for by that link. For this and other properties, we took the average across all individuals in a species within the same lake and sampling period. 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 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 parasite transmission separately from the relationship between contribution to diet and transmission. We defined abundance as the number of prey individuals per m^2 in each lake. 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 *S6.1, Supporting Information S6* 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 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.

Thirdly, 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 independently of the other properties. We estimated the biomass transfer ω_{ilm} for each link i in lake l during sampling period m as

$$\omega_{ilm} \approx \kappa_{lm}^{3/4} \rho_{ilm}, \qquad (5)$$

where κ_{lm} is the mean biomass of the consumer from link i in lake l during sampling period m, 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.

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. Because we dealt with the dynamic properties of links separately, we calculated centrality using unweighted links. Although central links are generally thought to be particularly important to the structure and functioning of a community, from the parasite perspective these "highways" are a double-edged sword. Depending on the broader structure of the network, a central link has the potential to expose the parasite to many free-living species that are not suitable hosts. Highly-central links could therefore either promote transmission or result in losses for the parasite. To get an idea of these broader structures, and how they affect the outcomes of links for parasites, we also characterised links' structural roles using motifs— unique patterns of interacting species that can be understood as the building blocks of networks (see *S6.2*, *Supporting*

Information S6 for details). The results for these structural roles were qualitatively similar to those for centrality and so are not presented here.

Outcomes of links for parasites

Next, we categorised the outcomes of feeding links for each 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. 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 life stage of the focal parasite 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.

A link was categorised 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. 24). 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 categorised as a "loss". This includes cases where the parasite is digested along with its host by the predator (e.g. trematode sporocysts inside a snail host that is eaten by a fish) as well as cases where the parasite is killed in an indigestible cyst form (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, categorise 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 categorised as

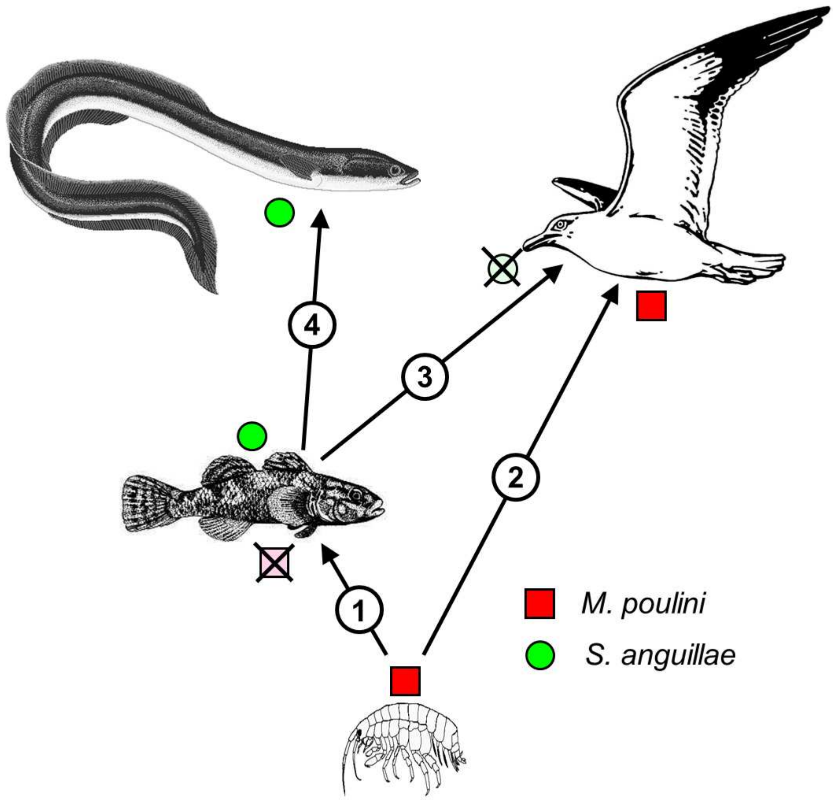

Figure 24: The small subset of species represented here (taken from the dataset used in this study) is used to illustrate the different outcomes of feeding links for parasites. *Maritrema poulini* uses amphipods and *Stegodexamene anguillae* uses small fish as intermediate host prey. These parasites 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*.

"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 dynamic and structural properties

We began by testing what combination of the five properties we consider (contribution to predator's diet, abundance of prey, biomass of prey, amount of biomass transferred, and centrality) provided the most parsimonious explanation for the outcome of a link for a parasite. To do this, we performed a series of canonical correspondence analyses (CCAs) using the cca function in the R (R Core Team, 2014) package vegan (Oksanen et al., 2014). Each CCA relates a matrix of dummy variables describing the outcomes of links for parasites to a constraining matrix composed of different combinations of link properties. We performed a CCA for each of the 31 unique linear combinations of predictors. In each case, we scaled and centred all properties. To provide a baseline, we also performed a "null" CCA which related the matrix describing outcomes of links to a unit vector. For each model, we obtained the AIC score using the function extractAIC, again from vegan (Oksanen et al., 2014). We then compared these AIC values to find the combination of predictors that most parsimoniously explains the outcomes of links for parasites (Table *S3*). To supplement this analysis, we also tested whether any of the properties were strongly correlated. Clear linear relationships between properties would mean that they provide redundant information, potentially biasing our results.

Based on the results of these preliminary tests (see *Appendices S6.3 & S6.4*), we chose to explore the relationships between outcomes of links for parasites and each property (i.e., hypotheses i-iv) separately. To do this, we began by comparing the mean values of each property for links with different outcomes using a modified Tukey's Honest Significant Difference test. Rather than assuming equal variances in all links, we used pooled variances for each pair of outcomes. We then tested whether each property was a significant predictor of links' outcomes using a modified ANOVA. Rather than assume that each property was normally distributed, we obtained the null distribution of the F statistic by permuting values of the focal property across the set of links 999 times. In order to control for the different numbers of intermediate (prey) and definitive (predator) hosts for different parasite life stages, we restricted our permutations to occur within the interaction-outcome combinations for each parasite.

At first, we imposed no further restrictions on the permutations to control for the host specificity of parasites. Such an unrestrictive null model, however, can re-assign transmission links to physiologically and ecologically inappropriate hosts. As noted previously, parasites are often restricted to hosts from a particular taxonomic group (Table *S6.2, S6.1; Supporting Information S6*). To control for these restrictions, we compared our results to those obtained using a taxonomically-informed, restrictive null model where links with a given outcome for a parasite (e.g., transmission) were only shuffled within the set of predator-prey interactions that could conceivably have that outcome, as determined based on expert knowledge. Specifically, we limited the substitution of dynamic properties for "transmission" links to interactions where the prey was a potential intermediate host of the parasite— based on the taxonomy of known intermediate hosts — and the predator was a potential definitive host (again based on taxonomy). Similarly, we restricted the substitution of properties for "loss" links to interactions where the prey was a potential intermediate host of the parasite but the predator was not a potential definitive host. Thirdly, we restricted the substitution of properties for "unused" links to interactions where the prey was not a potential intermediate host (regardless of the predator). 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.

Results

Outcomes & contribution to predator's diet

The contribution of a feeding link to the predator's diet was significantly associated with the outcome of the link for parasites when the host specificity of parasites was ignored ($F_{2,42019}$=13.62, P<0.001), but not when we used the taxonomically-informed null model ($F_{2,42019}$=13.62, P=0.999). Surprisingly, transmission links made up a smaller proportion of predators' diets than did unused links ($\Delta_{Transmission-Unused}$=-0.072, P<0.001 for a Tukey's HSD test; Fig 25A) and made similar contributions to loss links ($\Delta_{Transmission-Loss}$=-0.062, P=0.391). Comparing these results to each of our null models, we found that transmission links contributed a much lower proportion of predators' diets than expected based on the unrestrictive null model, but made similar contributions to those expected under the taxonomically-informed null model (Fig. 25A). Loss links, meanwhile, contributed similar proportions of predators' diets to those predicted by the unrestrictive null model but higher proportions than expected based on the taxonomically-informed null model. Unused links made similar contributions to those predicted by both null models.

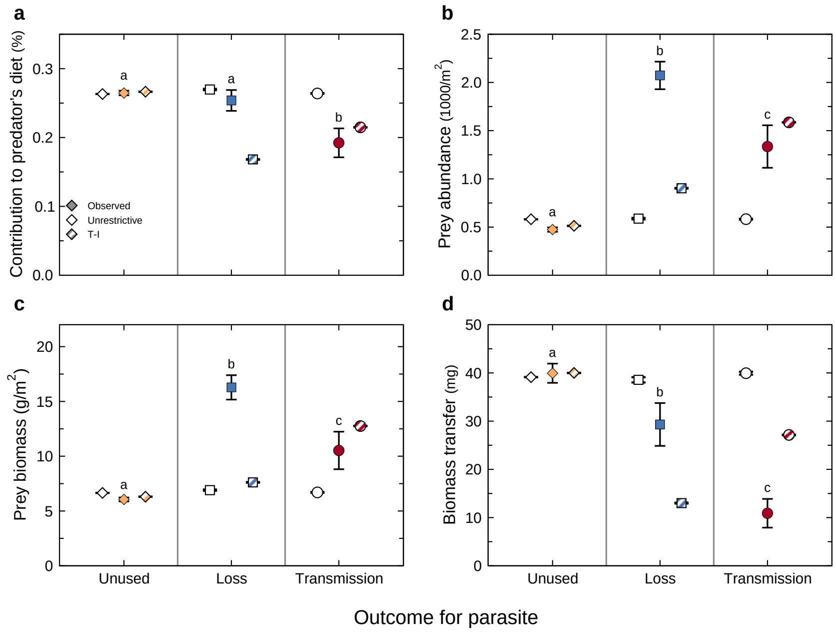

Figure 25: The dynamic properties of feeding links among free-living species affect the consequences of these links for parasites. **a)** The contributions of feeding links to the predator's diet varied across links with different outcomes for parasites, but this trend was not significant when the host specificity of parasites was taken into account. **b-c)** The local abundance and local biomass of the prey species, however, varied significantly among links with different outcomes whether or not host specificity was acknowledged. **d)** The amount of biomass transferred along a link showed the same trend as we observed for the contribution of a link to the predator's diet. For each property, we show the mean observed value (± 2 SE; circles). Different letters above the observed values represent significant differences in Tukey's HSD tests for each property. Empty symbols (to the left of the observed values) represent the mean value (± 2 SE) expected using our unrestrictive null model while symbols with striped fill (to the right of the observed values) represent the mean value (± 2 SE) expected under our taxonomically-informed (T-I) null model.

Outcomes & prey abundance

As with the contribution of links to predators' diets, the abundance of prey was significantly associated with the outcome of a link for the parasite under the unrestrictive null model ($F_{2,28793}$=392.875, P<0.001). Abundance was also significantly associated with different outcomes when the host specificity of parasites *was* taken into account in the taxonomically-informed null model ($F_{2,28793}$=392.875, P<0.001). Contrary to our expectations, transmission links involved prey with lower abundances than did loss links ($\Delta_{Transmission-Loss}$=-737, P<0.001 for a Tukey's HSD test; Fig. 25b). However, transmission links *did* involve prey with higher abundances than unused links ($\Delta_{Transmission-Unused}$=861, P<0.001). Comparing these observed values with those in the null models, we found that unused links involved prey with similar abundances to those expected under both null models while transmission and loss links behaved differently than expected (Fig. 25b). Specifically, transmission links involved prey with higher abundances than expected based on the unrestrictive null model, but slightly lower than expected based on the taxonomically-informed null model. Loss links, in contrast, involved prey with higher abundances than predicted by either null model.

The relationship between the biomass of prey and the outcomes of links for parasites was qualitatively identical to that between abundance and the outcomes of links. The local biomass of the prey species was significantly associated with different outcomes of the link for parasites, whether the host specificity of parasites was ignored or taken into account ($F_{2,31832}$=257.9, p<0.001 and $F_{2,31832}$=257.9, p<0.001, respectively). Transmission links involved prey with lower biomasses than did loss links, ($\Delta_{Transmission-Loss}$=-5.76g, p<0.001 for a Tukey's HSD test; Fig. 25c). Both transmission and loss links involved prey with higher biomasses than did unused links ($\Delta_{Transmission-Unused}$=4.47g, p<0.001 and $\Delta_{Loss-Unused}$=10.2g, p<0.001, respectively). The observed biomass values for unused links were similar to those expected under both null models (as with all other link properties we tested). As with prey abundance, transmission links involved prey with higher biomasses than expected based on the unrestrictive null model, but slightly lower than expected based on the restrictive null model. Loss links, in contrast, involved prey with higher biomasses than predicted by on either null model.

Outcomes & biomass transfer

Again as with the contribution of links to predators' diets, the amount of biomass transferred along a link was correlated with outcomes for parasites when the unrestrictive null model was used, but not under the taxonomically-informed null model ($F_{2,42019}$=8.169, P=0.001; $F_{2,42019}$=8.169, P=0.643, respectively). Surprisingly, transmission links transferred less biomass than did loss or unused links ($\Delta_{Transmission-Loss}$=-18.4mg, P=0.002 and $\Delta_{Transmission-Unused}$=-29.0mg, P<0.001, respectively, for a Tukey's HSD test; Fig. 25d). Again like the contribution to predators' diets, and similar to prey abundance, the amount of biomass transferred by unused links was similar to what was expected under either null model (Fig. 25d). Both transmission and loss links transferred less biomass than expected under the unrestrictive null model, but transmission links also transferred less biomass than expected under the taxonomically-informed null model. Loss links, in contrast, transferred more biomass than expected based on the taxonomically-informed null model.

Outcomes & centrality

Like prey abundance, link centrality was significantly correlated with different outcomes for parasites, whether or not host specificity was taken into account ($F_{2,42019}$=527.5, P<0.001 in both cases). Both transmission links had much higher centralities than unused links

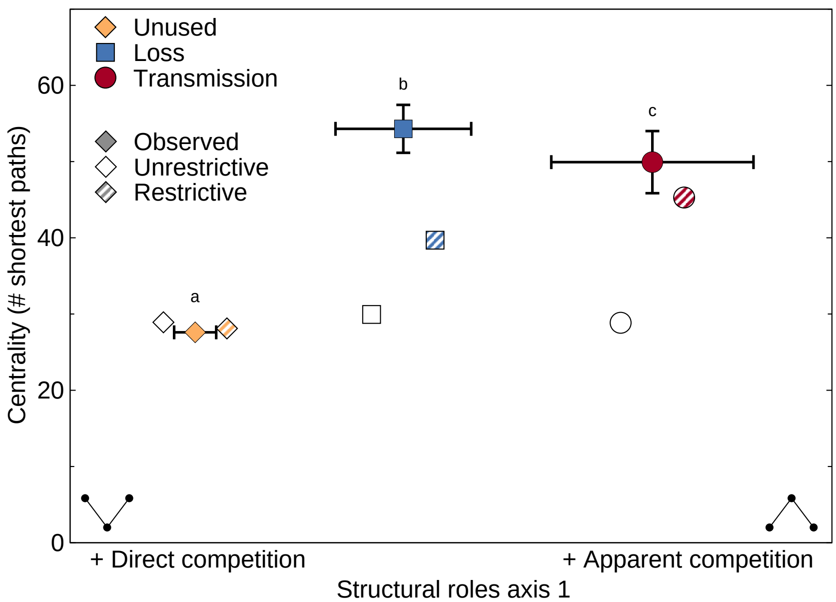

Figure 26: The outcomes of feeding links for parasites vary with their structural properties. Transmission links (circles) and loss links (squares) had significantly higher centralities than unused links (diamonds). Solid fill indicates the observed centralities, no fill represents the mean value expected using our unrestricted null model, and striped fill the mean value expected using our taxonomically-informed null model. For the observed and expected values, we show the mean centrality (±2 SE), although in some cases the error bars are very small. Note that although transmission and loss links had similar centralities, they had significantly different structural roles. Here we show the median role for each link along the RDA axis that explains the most variation in links' roles, as well as the motifs most strongly associated with the axis. Transmission links appeared more frequently in the apparent competition motif (two predators with one prey) and less frequently in the apparent competition motif (two prey with one predator) than did loss links. For a more detailed discussion of links' structural roles, see *Supporting Information S6*. Note that, for visual clarity, the expected values for centrality have been staggered along the x-axis and only their vertical positions are meaningful.

($\Delta_{Transmission-Unused}$=22.3, P=0.011 and for a Tukey's HSD test; Fig. 26). Loss links, however, were more central than transmission links ($\Delta_{Transmission-Loss}$=-4.37, P<0.001). As with the dynamic properties described above, the centralities of unused links were very similar to those expected under either null model (Fig. 26). Transmission and loss links, meanwhile, were both more central than expected under either null model, although when using the taxonomically-informed null model transmission links were only slightly (but still significantly) more central than expected based on the null model.

Discussion

Taken together, our results clearly show that the dynamic and structural properties of links among free-living species affect the links' likelihoods of transmitting parasites. The contribution of a link to the predator's diet, the abundance of the prey, the amount of biomass it transfers, and the centrality of a link all significantly predicted whether or not a link would transmit a parasite. However, these relationships did not always run in the direction we expected. In particular, parasites tended to be transmitted along links that would appear to be *less* important than other links in terms of their dynamic properties.

Transmission links tended to contribute less to predators' diets than other types of links. Predators therefore appear to be "avoiding" prey species which contain parasites that can infect the predator. After taking the host specificities of parasites into account, however, transmission links make similar contributions to predators' diets to what would be expected at random. This suggests that taxa which are potential intermediate hosts for the parasites in this system are not particularly important prey for parasites' definitive hosts. Loss links, meanwhile, make much greater contributions to predators' diets than expected based on the taxonomically-informed null model. This suggests that consuming infected prey is a common strategy for predators which are not suitable hosts for the focal parasite. This has previously been observed in other aquatic systems where, for example, cockles infected with trematodes are mainly consumed by fish and whelks and only rarely by the parasites' bird definitive hosts (Mouritsen and Poulin, 2003). The parasite induces changes in its host that limit burrowing ability and make the cockle more vulnerable to predation by birds, but other predators also take advantage of the increased availability of this prey (Mouritsen and Poulin, 2003). As morphological and behavioural changes that make parasites' intermediate hosts more vulnerable to predation are common (Ness and Foster, 1999; Miura et al., 2006; Mouritsen and Poulin, 2003; Lefèvre et al., 2009), it is likely that exploitation of these modifications by predators other than the definitive host are also common.

Our results for prey abundance were quite similar to those for the contribution of links to predators' diets. Transmission links involved more abundant prey than unused links, but less than loss links. Moreover, prey abundances for transmission links were similar to (but lower than) what was expected under the taxonomically-informed null model. This once again suggests that parasites use abundant intermediate hosts largely because they are restricted to these host taxa. We expected that abundant hosts might promote transmission because these species tend to be encountered frequently and therefore involved in many feeding interactions (Wootton, 2005; Canard et al., 2014). For parasites, however, this means that an abundant intermediate host is likely to be consumed by many predators that are not suitable definitive hosts (Canard et al., 2014). By infecting rarer intermediate hosts where possible, parasites may be using prey that are actively sought by their definitive hosts (Wootton, 2005), improving the odds of transmission. Without knowledge of predators' preferences for different prey, however, testing this possibility is beyond the scope of this study.

Transmission links also transferred less biomass than any other link type. Unlike the other properties we considered, transmission links transferred less biomass than expected based on either null model. Based on this result, predators appear to be obtaining most of their food either from prey infected with parasites that cannot infect the predator (i.e., loss links) or uninfected prey (i.e., unused links). This provides a counterpoint to Thompson et al. (2013)'s finding that parasites tend to accumulate in species which participate in many high-biomass food chains. Thompson et al. (2013) did not, however, find any relationship between parasite diversity and the amount of biomass flowing *into* a species— a closer equivalent to our measure of biomass transfer. It therefore appears that while parasites may "go with the flow" to the extent that they enter food chains which transmit large amounts of biomass, they are more often killed than transmitted to their definitive hosts along such chains. As loss links in particular transferred more biomass than expected under the taxonomically-informed null model, predators may even preferentially consume infected prey as long as they are not suitable hosts for the parasite. This is consistent with previous work suggesting that infected prey are easier to find and/or capture, reducing foraging costs for a predator (Lafferty, 1992; Mouritsen and Poulin, 2003).

Although transmission links tended to be less important than other links in terms of their dynamic properties, our results for centrality supported our hypothesis that transmission links would be structurally important. Notably, loss links were also highly central. This is consistent with earlier research that found that more parasite species infect highly central hosts (Chen et al., 2008) or hosts with many links to other species (Thompson et al., 2013). Despite loss and transmission links having similar centralities, our use of motifs to examine links' structural properties in more detail indicates that transmission and loss links tended to be embedded in the food web in different ways. In particular, it seems that generalist predators are frequently "dead ends" for parasites while links involving prey species with many predators more commonly result in transmission. This demonstrates that, while transmission and loss links are both structurally important, they nevertheless play different roles within the food web, just as suggested by our results for links' dynamic properties.

Overall, our results highlight the critical importance of taking host specificity into account. This outcome may be particularly striking since we address host specificity at a relatively coarse level (i.e., classes) when some parasites are known to be specialised to particular families or genera. It is therefore possible that our null model may not fully capture the restrictions on some parasite species. Nevertheless, the dramatic differences in the interpretation of our results after including even coarse measures of host specificity in our analyses demonstrate that, to truly understand trophic transmission of parasites, host specificity *must* be taken into account.

Beyond emphasising the importance of host specificity, our results make it clear that parasite transmission is affected by the structure and dynamics of the free-living community. In particular, several of our results suggest that weak links— links that make relatively small contributions to the predator's diet, transfer little biomass, etc. —may be the most important for parasites' transmission through food webs. Intriguingly, weak links have also been touted as critical for community stability (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, stabilising it (McCann et al., 1998; Wootton and Stouffer, 2016).

Weak links' contribution to community stability might also explain why they are common transmission routes for parasites. 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). Parasites can also cause such perturbations by altering the population dynamics of their hosts (Marcogliese and Cone, 1997) or affecting the strength of interactions among free-living species (Lefèvre et al., 2009). If parasites were transmitted along dynamically strong links, the effects of parasites on their hosts could exacerbate any environmental perturbations the community experienced. This could lead to dramatic fluctuations in host populations and the loss of the parasite. This scenario seems especially likely given our result that parasites tend to be transmitted along highly central links. As described above, perturbations to these links are likely to have substantial effects on the community (Jordán et al., 2007). It may well be that parasites can only be transmitted along links that are structurally *or* dynamically important without destabilising their hosts' populations. More work is necessary to determine whether the long-term persistence of parasites in a community is indeed related to the community's overall stability, but our results suggest that this is an avenue worth following.

Acknowledgements

We thank Anne Besson, Isa Blasco-Costa, Manna Warburton, and Kim Garrett for assistance with field collection and laboratory processing of samples. We thank Bernat Bramon Mora, Melissa Broussard, Carla Gomez Creutzberg, Matthias Dehling, and Matt Hutchinson for productive discussions about this study. This study was funded by two separate Marsden Fund grands, administered by the Royal Society of New Zealand, to RP and to DBS, and by an NSERC PGS-D scholarship and UC Doctoral Scholarship to ARC.

Supporting information

S6.1: Detailed methods for data collection

S6.2: Supplemental methods and results for links' structural properties

S6.3: Results of model selection

S6.4: Testing for correlations between link properties

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General discussion

Summary of results

Over the course of this thesis, I have demonstrated several ways in which species' roles in ecological networks can be used to connect their natural histories to the structure and function of the communities in which they are embedded. In Chapter 1, my co-authors and I reviewed several common definitions of species' roles and highlighted the similarities and differences among them. We framed each definition of species' roles in terms of their niches, and suggested that discussing species' roles in a niche context will avoid confusion between role definitions. Although the remaining chapters in this review use only a few of the role concepts included in the review, Chapter 1 also illustrates the range of potential applications of species' roles.

Chapters 2 through 6 present original research. In Chapter 2, we tested whether knowledge about arthropods' roles in a mainland food web could be used to improve the predictions of models based on the Theory of Island Biogeography. In this case we defined roles as simply the set of prey and arthropod predators for each species. We found that incorporating information about species' roles significantly improved the predictions of models for both immigration and extinction. Arthropods' roles as consumers were especially informative. This could be because the presence of prey for a given species was much more variable than the presence of predators across our dataset, or because the dataset only included information on arthropods and neglected other taxa (e.g., birds) that could have large impacts on the arthropods' ability to colonise islands.

In Chapter 3, we again defined species' roles as their sets of interaction partners. This time, we used plants' roles in pollination and herbivory networks to test whether closely-related species have more similar roles. In general, this was indeed the case. In both network types, dissimilarity in species' interaction partners increased as phylogenetic distance increased. Within families, however, there was a great deal of variability. In some families more closely-related species had more similar roles, as expected, while in others the opposite trend emerged. Our results therefore suggest a complex history of convergent and divergent evolution among plants and their interaction partners.

In Chapters 4-6, we defined roles more abstractly. Specifically, we used motifs to categorise the roles of species (Chapters 4 and 5) and interactions between species (Chapters 5 and 6). In Chapter 4, we tested whether the roles of plants and their insect pollinators in a high-Arctic community changed after 15 years of climate change. Both groups' roles did indeed change, as did the structure of the network overall. In particular, our results suggest that phenological uncoupling may be occurring in this system. This suggests that, under continuing climate change, some plants may not receive adequate pollination services and some pollinators may not find sufficient food.

In Chapter 5, we compared the roles of parasites and free-living species in order to establish whether parasites' roles are similar to those of any free-living group and whether including different types of interactions (i.e., parasitism, antagonism among parasites, and concomitant predation on parasites inside their hosts) affect parasites' roles. When concomitant predation was not included in parasites' roles, they were similar to those of free-living top predators and intermediate consumers. When concomitant predation was included in parasites' roles, however, these roles were unlike those of any group of free-living species. By analysing their roles in this way, we demonstrated that parasites are important both as consumers of free-living species and as a food source for them.

In this chapter, we also expanded the concept of species' structural roles to interactions between species. We showed that different types of interaction (predation between free-living species, parasitism, antagonism among parasites, and concomitant predation) had different roles, and that the roles of concomitant predation interaction were particularly variable. This interaction is contingent upon parasitism and predation interactions already taking place, and so likely inherits variation in roles from both of these interaction types. Concomitant predation is also interesting because it can have a variety of consequences for parasites. For trophically-transmitted parasites, consumption of the current host by a suitable host for the parasite's next life stage is required for the parasite to complete its life cycle (Marcogliese and Cone, 1997). If the current host is consumed by an inappropriate predator, however, or if the parasite is not trophically-transmitted, then the parasite dies. It seems likely that interactions which have different outcomes for the parasites have different structural roles. While testing this possibility was beyond the scope of Chapter 5, it formed the focus of Chapter 6.

In Chapter 6, we defined the roles of feeding links between free-living species based on their motifs (as in Chapter 5), but also based on other structural and dynamic properties. Specifically, we measured each link's centrality (the number of times it appears on the shortest path between two species), its importance to the predator (i.e., the proportion of the predator's diet that the link contributes), the amount of biomass transferred along the link, and the abundance and biomass of the prey involved in the link. These measures combined give a comprehensive picture of the way each link fits into the overall network. We then tested whether any of our measures of links' roles were related to the outcome of these links for parasites. We expected that parasites would tend to be transmitted along links that were very important to the structure and dynamics of the network (i.e., highly central, transferring large amounts of biomass, etc.). We did indeed find that parasites tended to be transmitted along highly central links, transmission links also tended to be dynamically (e.g., transmit less biomass) weaker than links resulting in the death of the parasite. As such weak links are believed to promote community stability by dissipating perturbations to any one species, while highly central links are believed to transmit perturbations and could thereby destabilise the community, it appears that parasites are transmitted along routes that are unlikely to strongly disrupt the community.

Implications

As the implications of each chapter have been discussed within the chapters themselves, I will now consider the impact of this thesis as a whole. In Chapters 2 through 6, my co-authors and I demonstrated a variety of contexts in which species' roles can be used to gain a deeper understanding of ecological communities. In each case, we used species' roles either as a bridge between the overall network structure and species' traits or to tailor community-level ecological theory to particular species. Thus, this thesis demonstrates how species roles can be used to make network ecology directly applicable for parasitologists, island biogeographers, etc. Over the course of this thesis, I have become firmly convinced that this type of applicability is essential for network ecology to achieve its full potential.

Network ecology began as an offshoot of graph theory and, like other extensions of graph theory in linguistics, neurology, and sociology, has remained strongly interdisciplinary (Dunne, 2006). Some of the methods I used to determine species' roles in Chapters two through six, for example, were first developed in the context of sociology (Jordán et al., 2007; Lai et al., 2012) or statistical physics (Guimerà et al., 2007). This history has shaped network ecology into a highly versatile discipline, able to address any type of interaction in any system one might wish. However, because they borrow so many terms and methodologies from outside of ecology, studies of networks can be difficult for non-specialists to understand and connect to their own work. As demonstrated by Chapters four and six (which were collaborations with empirically-grounded researchers), roles are one way to overcome this dilemma.

Because roles are species-level properties, they are easy to associate with other knowledge about species in a way that analyses of network structure are not. For example, we can identify the most central species in a lake food web and determine whether they are fish, invertebrates, or algae and how their morphologies and behaviours differ from less-central species. Thus, a species' importance to the rest of the network can be explained in typically ecological terms. A network-level metric like connectance, in contrast, is more difficult to connect to the particulars of the study system because it summarises all species and interactions into a single measure. Instead, network metrics have been studied in the context of site characteristics like latitude and ecosystem type (Briand, 1983; Riede et al., 2011; Baiser et al., 2012; Cirtwill et al., 2015) or spatial scale (Martinez and Lawton, 1995; Thompson and Townsend, 2005). Similarly, while network-level properties have been used to gauge the stability of different ecological communities (May, 1972; Dunne et al., 2002; Gilbert, 2009; Fortuna et al., 2010; Plank and Law, 2012), such studies are not concerned with the persistence of any particular species of interest. Roles could be used to fill this gap in the future.

Although species' roles are easier to integrate with natural history than network-level metrics, they can still be unintuitive and difficult to interpret. This is particularly true for high-dimensional role concepts like structural roles. The length of the vectors used to define structural roles is undoubtedly part of the problem; even a 24-dimensional vector is difficult for a human to grasp, and in studies of bipartite networks structural role vectors may be over 100 entries long. This issue is easily solved by comparing roles statistically and interpreting differences between them with respect to the motifs which explain the most variation. However, the motifs themselves can also be a challenge to interpret. Few of the motifs used to define species' structural roles have been empirically studied (Bascompte and Melián, 2005). Those that have are small (3-4 species) and include only one-way interactions. Although one-way feeding links are more common than two-way links overall, for some types of species (e.g., fish whose diets change depending on their age and size [Rudolf and Lafferty, 2011]) two-way interactions may be both more common than expected and quite important to the population dynamics of both species involved. Motifs including two-way interactions therefore merit further study, particularly in empirical systems rather than simulations.

One-dimensional conceptions of species' roles are often easier to interpret, but can be just as difficult to connect with particular ecological traits if the traits in question were not the focus of the study. We saw this in Chapter 3, where our ability to interpret the changing trends in conservation of plants' roles across families was limited by a dearth of information about relevant traits of these families. This highlights the benefits of collaborations between network ecologists and researchers with expertise in the study system being examined. Such collaborations can suggest which of species' traits are most relevant to their network roles, but also tend to suggest interesting questions that might not develop in a group comprised of only network specialists. I believe that the work in this thesis argues strongly for intradisciplinary but inter-speciality collaborations, and I intend to continue along this line in my future work.

Next steps

Over the course of this thesis we use several different definitions of species and link roles. Going forward, it would be useful to understand which role definitions are strongly correlated and which provide unique information about species' relationships to their communities. As well as understanding the relationships between different role concepts in relatively stable communities, it would also be interesting to investigate how different definitions of species' roles change as interaction networks are altered. Interactions can be lost long before the species involved go extinct (Aizen et al., 2012) and some definitions of species roles may be better than others at tracking the effects of interaction loss across the network. Moreover, different definitions of roles may vary in their ability to predict species' impact on their community and species' persistence in the face of perturbations. Identifying which role concepts are best suited to these types of questions will make roles a much more useful tool for conservation ecologists and other working on similar questions.

As well as working to understand the relationships between role concepts, it would be fruitful to investigate the spatial and temporal variation in species' roles. Roles are likely to vary over both large and small scales, but there may be consistent "archetypal" roles in different ecosystem types, for different taxa, etc. Comparing species' roles across smaller scales, meanwhile, would indicate how variable interactions are across species. Those with highly variable roles might be more able to adapt to climate change or other perturbations, but they might also be more likely to become invasive if introduced to a new community. Where spatially replicated communities can be combined with information about the assembly of the community, it would also be interesting to test whether the order in which species colonise a site affects their roles. In addition to comparing the roles for a single species across sites, it would be interesting to compare the roles of large collections of species. Specifically, it may be possible to group species roles into a small number of 'archetypal' roles that are particularly common across sites. Examining which species share similar roles could provide a great deal of insight into how similar network structures develop from disparate communities.

As with spatial variation, temporal variation in species' roles can also indicate how species are responding to global change. As my co-authors and I showed in Chapter 4, comparing species' roles across decades can show how communities are responding to global change. This approach could be used in many other systems to investigate the effects of different perturbations on entire communities. It would also be interesting to investigate how species' roles change throughout a year. A species that acts as a relatively minor component of a food web for the majority of the year might assume a keystone role during one season. This variation is obscured in most food webs which are either snapshots of a single time period or aggregated over long time scales, but has the potential to dramatically alter our understanding of the structure and functioning of ecological communities.

Combining food webs with phylogenies is yet another area ripe for exploration. Earlier work has shown that species' roles tend to be conserved across phylogenies (Stouffer et al., 2012), but my co-authors and I also show (Chapter 3) that there can be substantial variation about this general trend. Identifying the conditions under which species' roles tend to be phylogenetically conserved has the potential to illuminate the interplay between evolution and ecology in structuring biological communities. The cases in which species' roles are not conserved are likely to be particularly interesting as non-conservation of interactions could be due to convergent or divergent evolution, the loss of interaction partners, or a number of other causes. Species with unique roles based on their phylogenies could also be of particular conservation concern, as these species are unlikely to be replaced by a relative if they are lost.

Moving from species roles to interaction roles, it would also be interesting to test whether strong and weak interactions tend to have different structural roles in networks. The distribution of interaction strengths within a network has been linked to stability in several studies (McCann et al., 1998; Emmerson and Yearsley, 2004; Banašek-Richter et al., 2009; Tang et al., 2014; Nilsson and McCann, 2016), but a link's structural role may modulate the effects of its strength on the rest of the community. In particular, because concepts like motif roles describe meso-scale or global network structures they have the potential to capture different links' abilities to affect species other than the two that are directly involved.

Apart from continuing to build on role concepts as described above, studies of species' roles have much to gain from collaborations between network ecologists and those with more empirical expertise. As ecologists find more ways in which species' traits are related to their roles in ecological networks, species' roles will become more and more valuable tools with which to understand their ecology. In particular, understanding the roles of introduced species in their native communities may help us to predict which species will become invasive and how they will spread by helping us to predict likely interaction partners for the introduced species in its novel range. Similarly, gaining a better understanding of the roles of species that are economically important to humans (e.g., food fish, crop plants, bees) will help policymakers to manage the impact of human activities across whole ecological communities rather than from the perspective of a single metric at a time. These are only a few ways in which concepts of species roles may be used in the near future; such a versatile toolkit will surely come to be applied in myriad ways and in a plethora of systems.

Conclusion

I hope that the body of work that this thesis represents is a convincing argument that species' roles are a valuable addition to network ecology; particularly as a bridge between network structure and species' traits. Connecting network-level and species-level information has been named as one of the 100 outstanding fundamental questions in ecology (Sutherland et al., 2013), and I am pleased to have made a contribution to solving it. Moving forward, more attention should be given to the biological meaning of different role concepts. The studies which make up this thesis offer several ways to do this— by collaborating with specialists in the study system from which the web is drawn, by making an important ecological trait like parasitism the focus of the study, or by drawing on an extensive literature about the system. I am certain that I and other researchers will find more links between network ecology and other subdisciplines in the future.

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Appendix: Are parasite richness and abundance linked to prey species richness and individual feeding preferences in fish hosts?

Alyssa R. Cirtwill[1], Daniel B. Stouffer[1], Robert Poulin[2], & Clement Lagrue[2]

[1]Centre for Integrative Ecology
School of Biological Sciences
University of Canterbury
Private Bag 4800
Christchurch 8140, New Zealand

[2]Department of Zoology
University of Otago
340 Great King Street
PO Box 56
Dunedin 9054, New Zealand

Published in *Parasitology* (2016).
DOI: 10.1017/S003118201500150X

Summary

Variations in levels of parasitism among individuals in a population of hosts underpin the importance of parasites as an evolutionary or ecological force. Factors influencing parasite richness (number of parasite species) and load (abundance and biomass) at the individual host level ultimately form the basis of parasite infection patterns. In fish, diet range (number of prey taxa consumed) and prey selectivity (proportion of a particular prey taxon in the diet) have been shown to influence parasite infection levels. However, fish diet is most often characterised at the species or fish population level, thus ignoring variation among conspecific individuals and its potential effects on infection patterns among individuals. Here, we examined parasite infections and stomach contents of New Zealand freshwater fish at the individual level. We tested for potential links between the richness, abundance and biomass of helminth parasites and the diet range and prey selectivity of individual fish hosts. There was no obvious link between individual fish host diet and helminth infection levels. Our results were consistent across multiple fish host and parasite species and contrast with those of earlier studies in which fish diet and parasite infection were linked, hinting at a true disconnect between host diet and measures of parasite infections in our study systems. This absence of relationship between host diet and infection levels may be due to the relatively low richness of freshwater helminth parasites in New Zealand and high host-parasite specificity.

Keywords

fish diet, helminth parasites, infection levels, individual host, transmission mode.

Introduction

Parasites are both important agents of natural selection and factors contributing to the dynamics of host populations (Ebert et al., 2000; Albon et al., 2002; Marcogliese, 2004). Within a population, variation in the degree of parasitism incurred by individual hosts underpins the importance of parasitism as an evolutionary or ecological force. Identifying which processes influence parasite distribution among hosts, and make some hosts more susceptible to infection than others, is thus a central question in parasite ecology (Carney and Dick, 1999; Poulin, 2000; González and Poulin, 2005). Factors influencing parasite richness (number of parasite species) and abundance (number of conspecific parasite individuals) at the individual host level ultimately form the basis of parasite infection patterns (Carney and Dick, 2000). Several ecological factors and host attributes can influence the number and diversity of parasites infecting hosts at the individual level. In fish, these factors may include age/size, the number of different prey consumed as well as prey selectivity, habitat, etc. (Poulin, 2000; Johnson et al., 2004b; Locke et al., 2014). Many helminth parasites have complex life cycles that are embedded within food webs, relying on trophic transmission (i.e. consumption of an infected prey by the predator host) to reach their next host (Simková et al., 2001). For example, richness and abundance of trophically transmitted parasites in fish can thus be largely explained by the diversity of the prey/intermediate host community upon which different fish feed (Carney and Dick, 2000; Bolnick et al., 2003; Klimpel et al., 2006). Fish with a broad diet, feeding on more species of prey, may thus have more diverse trophically transmitted adult parasites (i.e. higher parasite richness) than those with more narrow, specialised diets (Kennedy et al., 1986; Lo et al., 1998; Locke et al., 2014). At the same time, a selective diet may not preclude fish hosts from accumulating large numbers of parasites (i.e. high parasite abundance). Trophically transmitted parasites usually utilise limited numbers (often only 1 or 2) of intermediate host prey taxa, and parasite abundance in fish hosts therefore depends on the importance of these few species in the fish diet rather than the absolute number of prey groups consumed; i.e. a fish feeding mostly on the parasite's intermediate host is more likely to accumulate parasites than a fish feeding equally on all prey species forming its diet (Kennedy et al., 1986; Marques et al., 2011). The degree of diet selectivity and the type/taxa of prey favoured by fish hosts may thus influence parasite infection levels, even in fish with qualitatively broad diets (Kennedy et al., 1986; Marques et al., 2011). Shifts in dietary preference with age/size can also be important determinants of adult helminth richness and abundances in fish hosts (Johnson et al., 2004b; Poulin et al., 2011). Prey selection is largely gape-limited, both within and among fish species, and the diversity of prey consumed usually increase with gape size, itself strongly linked to fish body size (Wainwright and Richard, 1995; Hyndes et al., 1997; Marcogliese, 2002; Klimpel et al., 2006). Overall, variability in feeding preferences may thus strongly affect parasite richness and abundance among sympatric, conspecific fish hosts (Knudsen et al., 1997).

On the contrary, prey diversity should have little effect on parasites that infect fish directly (Simková et al., 2001). Many larval trematodes infect fish through skin penetration and use fish as intermediate rather than definitive hosts (Locke et al., 2013, 2014). Larval trematodes directly penetrating fish skin subsequently enter a dormant stage and wait for the fish to be consumed by the appropriate definitive host predator. Trematode larvae can accumulate in fish hosts over time, unlike adult helminths in the gastrointestinal tract which are shorter lived (Carney and Dick, 2000; Locke et al., 2014). As a result, larger fish are expected to have higher richness and abundances of skin-penetrating trematode larvae (Zelmer and Arai, 1998; Carney and Dick, 2000; Poulin, 2000). Overall, among conspecific fish, larger individuals may harbour higher adult and larval helminth richness and abundances because they tend to consume a greater number of prey; they should be exposed to an increasing variety of potential intermediate hosts, being less gape-limited, and have been accumulating more larval parasites than their smaller conspecifics (Bell and Burt, 1991; Poulin, 1995; Morand et al., 2000; González and Poulin, 2005; Dick et al., 2009; Zelmer, 2014).

Phylogenetic effects relating to host specificity can also structure parasite communities among fish species that have similar diets but are phylogenetically distinct (Poulin, 1995). A broad diet may bring a fish into contact with a wide diversity of parasite species, though only a small subset of these may infect the host for evolutionary reasons (e.g. host-parasite compatibility; Kennedy et al., 1986). Ingestion of larval helminths by fish is frequent in most fish species due to the abundance and diversity of these parasites in aquatic ecosystems (Marcogliese, 2002; Parker et al., 2003). However, while different, co-occurring fish species can be exposed to the same helminths, host-parasite compatibility may subsequently modulate parasite infection patterns among fish host species (Lagrue et al., 2011). Overall, similarities or differences in parasite richness and abundance among sympatric fish species should be largely influenced by the combination of host diet and species-specific host-parasite compatibility (Lile, 1998; Knudsen et al., 2008; Lagrue et al., 2011).

Despite the potential for effects on parasite infection patterns, fish diet is most often characterised at the species or population level, thus ignoring potential variation among individuals (Fodrie et al., 2015). Diet variation and 'individual specialisation' among conspecific individuals is common in natural populations, including fish (Bolnick et al., 2002, 2003; Araújo et al., 2011; Layman et al., 2015; Rosenblatt et al., 2015). Species assumed to be dietary generalists and exhibiting broad population-level diets can actually specialise at the individual level, inducing intraspecific differences in risk of parasitism (Curtis et al., 1995; Wilson et al., 1996). Combining data on individual fish stomach contents (number of prey groups and relative abundance in fish diet) and parasites (richness and specific abundances) may therefore provide a more accurate picture of the link between host diet and infection levels. Numerous fish species are considered opportunistic omnivores consuming a wide variety of prey taxa, though as individuals, fish can display contrasting dietary preferences that may yield differences in parasite richness and abundance among conspecific hosts. An individual host typically harbours a small sample of the local parasite community that reflects its individual diet range (i.e. number of prey groups consumed) and prey selectivity (Locke et al., 2013). Usually, parasites are aggregated among available hosts (Poulin, 2007; Poulin et al., 2013). This is often due to differences in the rate of parasite acquisition among hosts. For trophically transmitted helminths, differences in diet among conspecific hosts can generate heterogeneity in exposure to parasites and ultimately produce such aggregated distributions (Knudsen et al., 2004; Poulin, 2007).

Here, we used field sampling to quantify and analyse the richness and abundance of all helminth parasites as well as stomach contents of individual fish of 11 species. Stomach contents reflect short-term feeding patterns, but may still capture the causal link between diet and helminth richness and abundance among but also within fish species (i.e. among conspecific fish individuals; (Johnson et al., 2004b). Individual fish feeding preferences are likely consistent over time, at least seasonally, and even a single stomach content sample should reflect fairly accurately individual fish diet. Strong overlap in parasite infection (richness and abundance), or lack thereof, among unrelated fish species may reflect similarities or differences in diet, habitat and host specificity (or a combination of these factors) that are sometimes difficult to tease apart due to phylogenetic effects (Carney and Dick, 1999). Here, by comparing parasite richness and abundance among sympatric conspecifics, we eliminated these potential phylogenetic and geographical effects. Our main goal was to determine whether differences in parasite richness and abundance among fish species and among conspecific fish individuals can be linked to variations in the number of prey groups consumed, feeding preferences and/or fish size. These factors should have contrasting influences on trophically compared with directly transmitted parasites. We thus tested the potential effects of diet range and selectivity on parasite infection levels in individual fish host separately for the 2 parasite categories. Trophically transmitted parasite richness should increase with diet range in fish diet and specific parasite abundance be more influenced by individual fish feeding preferences. In contrast, directly transmitted parasites should not be influenced by fish host diet. Overall, differences in feeding preferences among individuals may be reflected in differences in parasite infections. Ideally, individual feeding preferences would be assessed at multiple time points; however, for obvious reasons (the need to sacrifice fish to recover gut contents and parasites), this is not possible, and we must rely on a single measurement.

Material and Methods

Data collection

Field sampling

Fish were sampled in 4 lake ecosystems. 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; South Island, New Zealand) were selected to provide a variety of lake types (size, depth and altitude), freshwater communities (coastal vs alpine, trophic state and tidal or not; see Table SA.1 for details). Within each lake, 4 sampling sites were selected along the littoral zone to cover all microhabitat types (substrate, macrophytes, riparian vegetation, etc.) present within each lake. The 4 lakes were sampled in early spring, summer and late autumn (austral seasons: September 2012, January and May 2013). Fish were captured at each site and in each lake to assess potential spatial variability within and among lakes in fish gut contents (prey richness and selectivity) and infection levels (parasite richness and abundance). We used a combination of fish catching gear types so that accurate cross-sections of fish species and size classes were sampled from each site. Two fyke nets and 10 minnow traps were set overnight in each site, when some fish species are more active (i.e. eels and common bully), as they are passive sampling methods relying on fish to willingly encounter and enter traps (Hubert, 1996). The next day, trapped fish were recovered and set aside for later dissection. Sampling was then complemented using two 15m long multi-mesh gillnets. Gillnets were benthic-weighted sets with top floats, 1.5m high and comprised 3 panels of 25, 38 and 56mm meshes, each 5m long. Gillnets covered the whole water column and were used to capture highly mobile, mainly diurnal fish (i.e. trout, perch and mullet). Fish caught in the nets were removed immediately to avoid excessive accumulation and the potential visual deterrence to incoming fish (Lagrue et al., 2011). Finally, fish sampling was completed using a standard, fine-mesh (5mm mesh size) purse seine net. As an active sampling method, seine netting captures small and/or sedentary fish species (i.e. galaxiids, smelt and juvenile fish of most species) that are not captured by passive gear like fyke nets or gillnets (Thorogood, 1986). All fish were killed immediately to inhibit the digestion process and stored on ice to preserve internal tissues, stomach contents and parasites for future identification, count and measures. In the laboratory, fish were identified to species, measured to the nearest millimetre (fork length), weighed to the nearest 0.01g and then dissected. The gastrointestinal tract, from oesophagus to anus, and all internal organs (heart, liver, gall bladder, gonads, swim bladder, etc.) of each fish were removed and preserved in 70% ethanol for later diet and parasite analyses. Fish bodies were frozen separately for later parasite analyses as ethanol preservation renders muscle tissues difficult to screen for parasites.

Parasites

Complete necropsies of all fish were conducted under a dissecting microscope. The head, gills, eyes, brain and spine of each fish were examined using fine forceps to pull apart fish tissues and obtain an accurate, total parasite count for all helminth species in each individual fish. Soft tissues (muscle and skin) were removed from the spine, crushed between 2 glass plates and examined by transparency to identify and count parasites. Internal organs and the gastrointestinal tract were first rinsed in water to wash off the ethanol. The digestive tract was then separated from other organs. Liver, swim bladder, gall bladder, gonads and other organs and tissues from the body cavity (fat, mesentery, kidneys, heart, etc.) were all screened for parasites. Finally, the digestive tract was dissected. Stomach and intestine contents were removed, screened for parasites and then set aside for later diet examination. Oesophagus, stomach, pyloric caeca (when present), intestine and rectum were then examined for gastrointestinal parasites. All parasites were identified and counted. For each fish individual, helminth parasite richness (total number of species) and specific abundances (total number of individuals per parasite species) were determined. The life stage (adult or larval) and infection mode (directly or trophically transmitted) of all individuals was also recorded. Note that no external parasite (copepods, monogeneans or leeches) were recovered from any of the fish examined and are thus not considered here.

Fish diet contents

Food items from the stomach and intestine of all fish were identified under a dissecting microscope to determine the diet range of each individual (number of different prey taxa). Prey items were also counted to estimate the relative importance of each prey taxa in individual fish gut contents. Relative importance of each prey (number of a specific prey divided by the total number of prey items in the fish diet contents) was used as an estimate of diet selectivity of individual fish hosts.

Analyses

Parasite richness

As different mechanisms are expected to affect the number of directly and trophically transmitted parasite species acquired by a given fish host, we first divided the parasite community within each fish based on transmission mode (considering each life stage separately for parasites with complex life cycles). We then tested for a potential relationship between the richness of each group of parasites and host diet range (here defined as the number of prey taxa found in the fish host's gut contents), size (log of weight in grams) and their interaction. To account for the possibility that the richness of a host's parasite community was lower or higher because of its environment, we also included nested random effects of lake and site within lake. These random effects allow us to control for additional variation in parasite richness that can be explained by lake and site-within-lake without sacrificing the degrees of freedom that would be lost if they were fixed effects. This gave us the model:

$$\Sigma_i = \beta_0 + \beta_{0t} + (\beta_1 + \beta_{1t})\omega_i + (\beta_2 + \beta_{2t})\rho_i + (\beta_3 + \beta_{3t})\omega_i\rho_i + L_i + S_i + \epsilon_i \quad (6)$$

where Σ_i is the number of parasite species with a given transmission mode (direct or trophic) in an individual host i, ω_i is the log of the weight of the fish host, ρ_i is the host's diet range, L_i is a random effect of lake, S_i is a nested random effect of site within lake, and ϵ_i is a residual error term. Note that β_0, β_1, β_2, and β_3 refer to directly-transmitted parasites while β_{0t}, β_{1t}, β_{2t}, and β_{3t} are 'adjustments' to these β's when considering trophically transmitted parasites. As we were not interested in seasonal variations in this study, we analysed data from all 3 seasons together.

As richness, defined here as the number of parasite species per fish host, can take integer values only, and because many potential hosts did not contain any parasites, we fit these models as zero-inflated Poisson processes where the fixed effects described above applied to the Poisson components of the model only. That is, the zero-inflated component consisted of a fixed probability of having a parasite richness of zero, modulated by different random effects of lake and site within lake. In addition to having separate random effects, separate variance terms were fit to the zero-inflated and Poisson components of the model with no covariance between them. Because the number of parasites infecting a host varied among fish species, we fit separate models for each host species. We also restricted our analyses to fish host species in which at least 1 individual was infected with at least 1 parasite and to host species represented by at least 11 individuals (to give the necessary degrees of freedom to fit the model above). Individuals of *Anguilla australis* and *Anguilla dieffenbachi* were pooled under *Anguilla* spp. to increase sample size and fit a single model at the genus level. Both species are biologically and functionally similar, feeding on the same prey and acquiring the same parasites, and often co-exist (McDowall, 1990). We fit all models using the function MCMCglmm in the R (R Core Team, 2014) package of the same name (Hadfield, 2010).

Abundance and biomass of trophically transmitted parasites

We next tested whether feeding preferences of individual fish hosts showed any relationship with the abundance and biomass of trophically transmitted parasites with which they were infected. For each fish host species and each trophically transmitted parasite species found in that host, we determined the proportion η_{iq} of host i's gut contents (by abundance) accounted for by intermediate host q. We used abundance (rather than biomass or volume) to determine proportions because, while prey species deliver different amounts of energy to the predator depending on their size, each intermediate host acts as a single 'packet' of parasites delivered to the definitive host. While addressing the richness of fish parasite communities, we fit separate models for each observed combination of fish host and parasite species.

Using these data, we constructed parallel models for the abundance of each parasite species in each individual fish host. When a host i had 2 intermediate host preys q and r, we fit the model:

$$Y_{ij} = \beta_0 + \beta_{0t} + \beta_1\omega_i + \beta_2\eta_{iq} + \beta_3\eta_{ir} + \beta_4\omega_i\eta_{iq} + \beta_5\omega_i\eta_{ir} + L_i + S_i + \epsilon_{ij} \quad (7)$$

where Y_{ij} is the number of individuals of parasite species j observed in a fish host i and all other symbols are as in equation 6 or as defined above. Where only 1 intermediate host prey taxon was observed for a given fish host-parasite combination, β_3 and β_5 were omitted from the model. We then fit an equivalent model for the total biomass of parasites,

$$M_{ij} = \beta_0 + \beta_{0t} + \beta_1\omega_i + \beta_2\eta_{iq} + \beta_3\eta_{ir} + \beta_4\omega_i\eta_{iq} + \beta_5\omega_i\eta_{ir} + L_i + S_i + \epsilon_{ij} \quad (8)$$

where M_{ij} is the biomass of parasite species j observed in host species i and all other symbols are as above.

We fit both of these models to each fish host-parasite combination with sufficient sample size (the minimum required sample size varied depending on the number of intermediate hosts and levels of random effects). We also excluded combinations where none of the parasite's potential intermediate hosts were observed in the diet of fish hosts as the effect of diet could not be measured in these cases. As parasite abundances were integer values, we fit the models of parasite abundances as Poisson processes, and we fit the model of parasite biomass as a Gaussian process. We therefore fit equation 7 using the function glmer in the R (R Core Team, 2014) package lme4 (Bates et al., 2014) and fit equation (Venables and Ripley, 2002) using the function lmer in the R package lmerTest (Kuznetsova et al., 2014) (Kuznetsova et al. 2014). After fitting the full models, we fit the suite of all possible reduced models for each full model using the R (R Core Team, 2014) function dredge from package MuMIn (Bartón, 2014) and then averaged across all models (weighting by AIC) using the function model.avg, also from the package MuMIn.

Results

Across all samples, 614 fish representing 11 species were examined, and 12 species of parasites were identified (see Table A1 for details). A total of 309 546 parasites with different transmission modes (direct vs trophic) and prey hosts were recovered (see Table A2 for details). Note that the trematodes *Stegodexamene anguillae* and *Telogaster opisthorchis* use fish, albeit different species, as both intermediate and definitive hosts and were found as either directly transmitted metacercariae (i.e. trematode parasites larval stage) or trophically transmitted adults (Table A2). The different life stages of these 2 parasite species were thus considered separately in the models.

Table A1: Details of the fish species, status, life-history strategy and numbers examined for our study with the parasite species identified from each fish species.

Fish species	Status	L.S.	n_{Tot}	n_1-n_2-n_3-n_4	Parasite species
Aldrichetta forsteri	Nat.	M.v.	15	0-0-15-0	*H. spinigera*
Anguilla spp.	Nat.	Cat.	38	4-11-15-8	*Anguillicola* sp., *C. parvum*, *H. spinigera*, *S. anguillae*, *T. opisthorchis*, Nematoda sp.
Galaxias argenteus	Nat.	Amp.	1	0-0-1-0	
Galaxias maculatus	Nat.	Amp.	70	0-12-15-43	*A. galaxii*, *Eustrongylides* sp., *S. anguillae*, *T. opisthorchis*
Gobiomorphus cotidianus	Nat.	F.r.	268	60-24-68-116	*Apatemon* sp., *C. parvum*, *Deretrema* sp., *Eustrongylides* sp., *S. anguillae*, *T. opisthorchis*, *Tilodelphys* sp., Cestoda sp.
Onchorhynchus mykiss	Int.	F.r.	4	0-0-0-4	
Perca fluviatilis	Int	F.r.	179	50-46-47-36	*A. galaxii*, *C. parvum*, *Eustrongylides* sp., *H. spinigera*
Retropinna retropinna	Nat.	Amp.	23	0-10-13-0	*Eustrongylides* sp., *H. spinigera*, *Cestoda* sp.
Rhombosolea retiaria	Nat.	Amp.	2	0-0-2-0	*A. galaxii*, *C. parvum*, *H. spinigera*
Salmo trutta	Int.	F.r.	14	3-1-10-0	*A. galaxii*, *C. parvum*, *Eustrongylides* sp.

Nat., native; Int., introduced; L.S., life-history strategy; M.v., marine visitor; Cat., catadromous; Amp., amphidromous; F.r., freshwater resident; nTot, total number of fish examined; number of fish examined from lakes Hayes (*n*1), Tuakitoto (*n*2), Waihola (*n*3) and Tomahawk Lagoon (*n*4).

Table A2: Details of the parasite phylum/class, numbers, life stage, transmission mode, and prey host species used for transmission for each parasite species.

(B) Parasite				Transmission	
Species	Phylum/class	Life stage	n_{Total}	Mode	Prey host(s)
Acanthocephalus galaxii	Acanthocephala	Cyst.	26	Trophic	Amphipod sp.A
Anguillicola sp.	Nematoda	Ad.	9	Trophic	Copepod sp.
Apatemon sp.	Trematoda	Mc.	270 666	Direct	
Coitocaecum parvum	Trematoda	Ad.	721	Trophic	Amphipod spp.A,B
Deretrema sp.	Trematoda	Ad.	14	Trophic	Decapod sp.
Eustrongylides sp.	Nematoda	L.	231	Trophic	Oligochaete sp.
Hedruris spinigera	Nematoda	Ad.	645	Trophic	Amphipod sp.B
Stegodexamene anguillae	Trematoda	Mc.	28 469	Direct	
S. anguillae	Trematoda	Ad.	1791	Trophic	Fish
Telogaster opisthorchis	Trematoda	Mc.	5029	Direct	
T. opisthorchis	Trematoda	Ad.	1112	Trophic	Fish
Tilodelphys sp.	Trematoda	Mc.	600	Direct	
Unnamed sp.	Cestoda	L.	4	Direct	
Unnamed sp.	Nematoda	Ad.	229	Unknown	

Cyst., cystacanth; Ad., adult; Mc., metacercaria; L., larva; Prey host(s): Paracalliope fluviatilis (Amphipoda sp.A), *Paracorophium excavatum* (Amphipoda sp.B), *Tenagomysis chiltoni* (Decapod sp.), *Gobiomorphus cotidianus* and *Galaxias maculatus* (Fish).

Overall, 2 224 096 prey items belonging to 53 different taxa were found in stomach contents of fish, identified and counted.

Parasite richness

We were able to fit our models in 6 fish taxa: *Aldrichetta forsteri* (n=15), *Anguilla* spp. (n= 38), *Gobiomorphus cotidianus* (n=268), *Perca fluviatilis* (n=179), *Galaxias maculatus* (n=70) and *Salmo trutta* (n=14). As hypothesised, there was no significant effect of host diet range on the richness of directly transmitted parasites in *A. forsteri* (β_2=2.30, P=0.165), *Anguilla* spp. (β_2=1.21, P=0.106), *G. maculatus* (β_2=-1.74, P=0.182), *G. cotidianus* (β_2=0.101, P=0.459), *P. fluviatilis* (β_2=-0.299, P=0.454) or *S. trutta* (β_2=-3.25, P=0.221). In *G. maculatus*, there was a significant interaction between diet range and host size (β_3=2.61, P<0.001), but in all other fish species the interaction was non-significant (β_3=-0.194, P=0.967; β_3=0.727, P=0.518; β_3=-0.209, P=0.133; β_3=-0.062, P=0.761; and β_3=1.24, P=0.649 for *A. forsteri*, *Anguilla* spp., *G. cotidianus*, *P. fluviatilis* and *S. trutta*, respectively). There was thus no overall effect of fish gut contents on directly transmitted parasite richness in any of the 4 fish taxa mentioned above; in the case of *G. maculatus* the effect of the interaction between host mass and diet range was small relative to the variability between MCMCglmm fits (Fig. A1; Table A3).

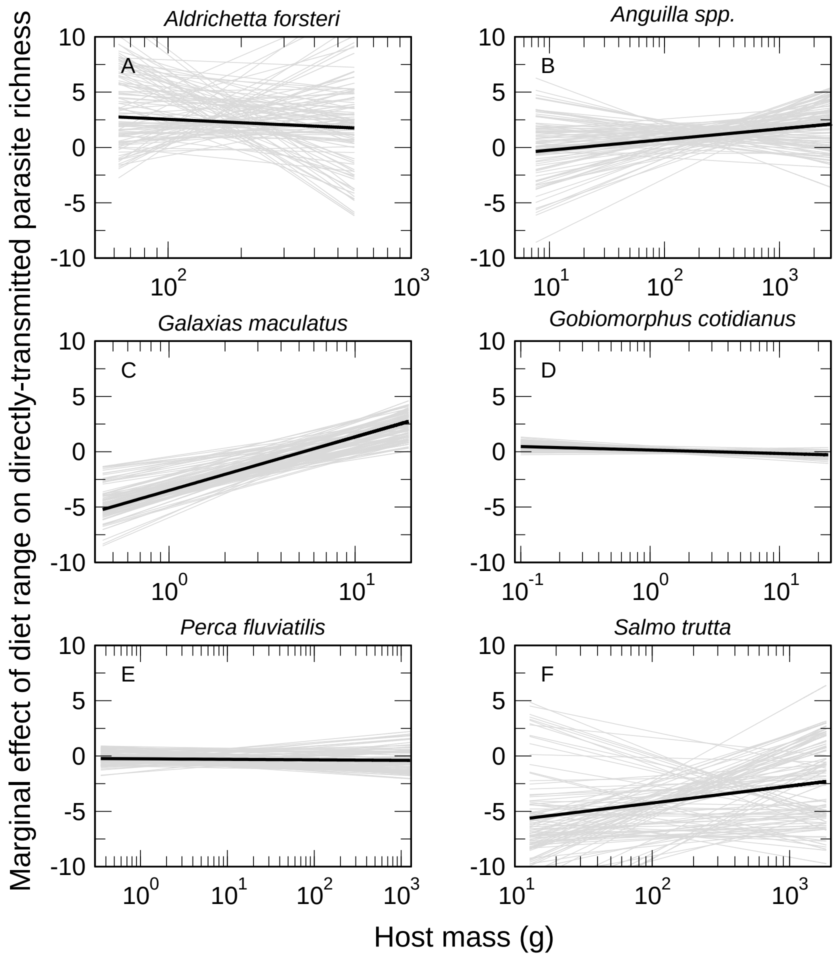

Figure A1: Marginal effects of fish host diet range on the richness of directly transmitted parasites found in the 6 fish taxa for which models could be fitted; (A) *Aldrichetta forsteri*, (B) *Anguilla* spp., (C) *Galaxias maculatus*, (D) *Gobiomorphus cotidianus*, (E) *Perca fluviatilis* and (F) *Salmo trutta*. Marginal effects are obtained by summing the effect of host diet range with the effect of the interaction between host mass and diet range across the observed range of fish host masses. A marginal effect of zero indicates that there is no overall effect of host diet range on parasite richness. Marginal effects greater than zero indicate that parasite richness increases with increasing host diet range, and marginal effects below zero indicate that parasite richness decreases as host diet range increases. Horizontal lines indicate that the effect of host diet range does not vary with host size, while sloped lines indicate that the effect of host diet range differs among hosts of different sizes. We show mean marginal effects (mean over 10 000 MCMCglmm iterations; black line) along with the marginal effects estimated in 100 of the MCMCglmm iterations with below-average deviances (grey lines).

Contrary to our expectations, there was no effect of host diet range on the richness of trophically transmitted parasites in *A. forsteri*, *Anguilla* spp., *G. maculatus*, *G. cotidianus*, *P. fluviatilis* and *S. trutta* ($\beta_2 + \beta_{2t}$=-0.227, P=0.780; $\beta_2 + \beta_{2t}$=0.291, P=0.651; $\beta_2 + \beta_{2t}$=-0.779, P=0.445; $\beta_2 + \beta_{2t}$=-0.268, P=0.175; $\beta_2 + \beta_{2t}$=-0.267, P=0.436; and $\beta_2 + \beta_{2t}$=1.61, P=0.437, respectively). Furthermore, there was no significant interaction between host size and diet range in any of the above fish ($\beta_3 + \beta_{3t}$= 0.044, P=0.928; $\beta_3 + \beta_{3t}$=-0.615, P=0.524; $\beta_3 + \beta_{3t}$=-0.622, P=0.532; $\beta_3 + \beta_{3t}$= 0.279, P=0.089; $\beta_3 + \beta_{3t}$=-0.242, P=0.778; and $\beta_3 + \beta_{3t}$=-0.154, P=0.957, respectively). There was therefore no overall effect of diet range on the richness of trophically transmitted parasites at any host size in these fish (Fig. A2; Table A3).

Table A3: Estimated fixed effects in equation 6 (with P-values in parentheses). β_1, β_2 and β_3, represent the effects of host mass, diet range and their interaction (respectively) on the richness of directly transmitted parasites, while β_{1t}, β_{2t} and β_{3t} are adjustments to these effects for trophically transmitted parasites. β_1 + β_{1t} therefore represents the main effect of host mass acting on the richness of trophically transmitted parasites. Effects are means over 1000 MCMC iterations.

Species	β_1	$\beta_1 + \beta_{1t}$	β_2	$\beta_2 + \beta_{2t}$	β_3	$\beta_3 + \beta_{3t}$
Aldrichetta	-0.718	0.118	2.30	-0.227	-0.194	0.044
forsteri	(0.366)	(0.582)	(0.165)	(0.780)	(0.967)	(0.928)
Anguilla	-0.324	1.67	1.21	0.291	0.727	-0.615
spp.	(0. 532)	(<0.001)	(0.106)	(0.651)	(0.518)	(0.524)
Galaxias	-1.68	0.971	-1.74	-0.779	2.61	-0.622
maculatus	(0.303)	(0.474)	(0.182)	(0.445)	(<0.001)	(0.532)
Gobiomorphus	0.332	0.101	0.067	-0.268	-0.209	0.279
cotidianus	(0.005)	(<0.001)	(0.459)	(0.175)	(0.133)	(0.089)
Perca	0.390	0.846	-0.299	-0.267	-0.062	-0.242
fluviatilis	(0.590)	(0.025)	(0.454)	(0.436)	(0.761)	(0.778)
Salmo	2.42	-0.870	-3.25	1.61	1.24	-0.154
trutta	(0.429)	(0.483)	(0.221)	(0.437)	(0.649)	(0.957)

Abundance and biomass of trophically transmitted parasites

We were able to fit our models to the abundance and biomass of 3 trophically transmitted parasites in 3 fish host taxa: *Hedruris spinigera* in *A. forsteri*, *Coitocaecum parvum* in *P. fluviatilis*, and both *Eustrongylides* sp. and *C. parvum* in *G. cotidianus*. In the first 3 cases, only 1 prey species is used by the parasite as an intermediate host. *Hedruris spinigera* uses the amphipod *Paracorophium excavatum* for transmission to *A. forsteri*, *C. parvum* uses the amphipod *Paracalliope fluviatilis* only for transmission to *P. fluviatilis* and *Eustrongylides* uses oligochaete sp. to reach *G. cotidianus*. Two prey species, the amphipods *P. excavatum* and *Pa. fluviatilis* are used as intermediate hosts by *C. parvum* to be transmitted to and infect *G. cotidianus*. As expected, the abundance of *H. spinigera* in *A. forsteri* (i.e. number of parasites per individual fish host) tended to increase as the proportion of the intermediate host *P. excavatum* in the diet of an individual fish increased (β_2=15.6, P=0.005). This effect interacted negatively with host mass (β_4=-10.7, P<0.001) such that in smaller *A. forsteri* (roughly <300mm) the abundance of *H. spinigera* increased sharply with the proportion of *P. excavatum* in the diet but in the largest *A. forsteri* the abundance of *H. spinigera* decreased (Fig. A3-A; Table A4). Note that 'small' and 'large' here refer to opposite ends of the continuum of *A. forsteri* lengths and not to explicit groups.

The abundances of *C. parvum* in *P. fluviatilis* and *Eustrongylides* sp. in *G. cotidianus* did not vary with the proportion of intermediate hosts (the amphipod *Pa. fluviatilis* and an unnamed oligochaete, respectively) in the diets of the fish hosts (β_2=0.010, P=0.989 and β_2=0.006, P=0.723, respectively). There was no significant interaction between fish host size and the proportion of intermediate hosts in fish host diets (β_4=0.025, P=0.966 and β_4=0.002, P=0.839, respectively). As such, there was no overall effect of the proportion of intermediate hosts in fish diet contents on parasite abundance for these 2 parasite-host combinations (Fig. A3-B, C; Table A4).

Likewise, the abundance of *C. parvum* in *G. cotidianus* did not vary with the diet of fish hosts. Parasite abundance was not significantly associated with the proportion of either intermediate host (the amphipods *Pa. fluviatilis* and *P. excavatum*; β_2=-0.087, P=0.383 and β_3=-0.127, P=0.283, respectively). Further, there were weak interactions between the proportions of each intermediate host in the diet and fish host size (β_4=-0.034, P=0.955 and β_5= 0.307, P=0.610,

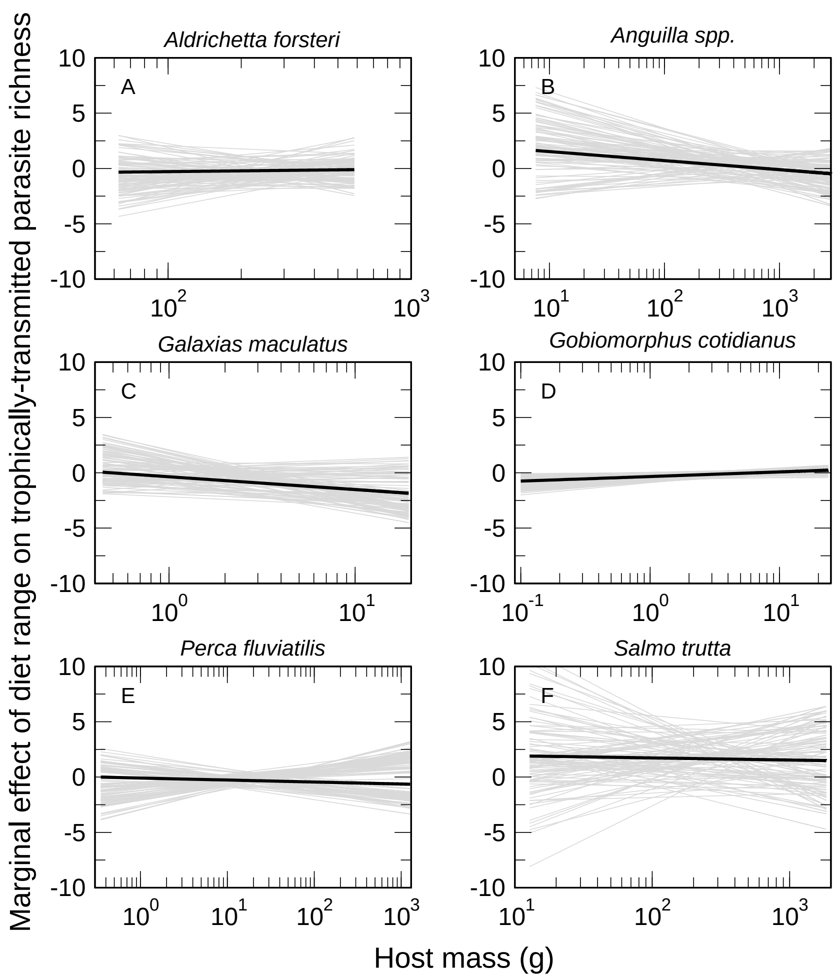

Figure A2: Marginal effects of fish host diet range on the richness of trophically transmitted parasites found in the 6 fish taxa for which models could be fitted; (A) *Aldrichetta forsteri*, (B) *Anguilla* spp., (C) *Galaxias maculatus*, (D) *Gobiomorphus cotidianus*, (E) *Perca fluviatilis* and (F) *Salmo trutta*. Marginal effects are obtained by summing the effect of host diet range with the effect of the interaction between host mass and diet range across the observed range of fish host masses. We show mean marginal effects (mean over 10 000 MCMCglmm iterations; black line) along with the marginal effects estimated in 100 of the MCMCglmm iterations with below-average deviances (grey lines). See Fig. A1 for details about the interpretation of marginal effects.

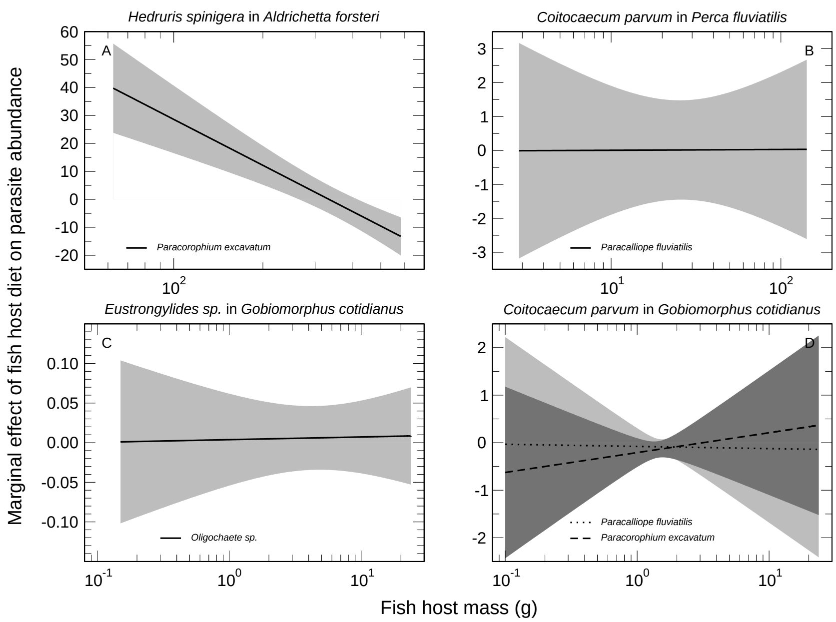

Figure A3: Marginal effects of the proportion of intermediate hosts in fish stomach contents on the abundance of trophically transmitted parasites in individual fish hosts in the 4 parasite-fish host taxon combinations for which models could be fitted; (A) *Hedruris spinigera* in *Aldrichetta forsteri*, (B) *Coitocaecum parvum* in *Perca fluviatilis*, (C) *Eustrongylides* sp. in *Gobiomorphus cotidianus* and (D) *C. parvum* in *G. cotidianus*. Intermediate host prey taxa are also identified within each panel. Marginal effects are obtained by summing the effect of proportion of intermediate host with the effect of the interaction between fish host mass and proportion of intermediate hosts across the observed range of fish host masses. We show mean marginal effects (black lines) with 95% confidence intervals (grey). See Fig. A1 for details about the interpretation of marginal effects.

respectively). Overall, the abundance of *C. parvum* did not vary significantly with the diet of *G. cotidianus* (Fig. A3-D; Table A4). In general, relationships between parasite biomass and proportions of intermediate hosts in the diet of fish hosts were similar to the relationships with parasite abundances described above (see *Supplementary Material* for details).

Discussion

Conspecific individuals are often treated as ecologically equivalent although individual specialisation in habitat or resource use is a

Table A4: Estimated fixed effects in equation 7 (with *P*-values in parentheses). β_1 indicates the effect of fish host mass on the abundance of the parasite, β_2 and β_3 the effects of the proportions of 2 intermediate hosts in the diet of the fish host, and β_4 and β_5 the effects of the interaction between proportion of intermediate host and fish host mass. NA indicates that only 1 intermediate host was found in the gut contents of the fish host. Estimates are based on averages over the full equation 7 and all possible reduced models, weighted by AIC.

Fish host	Parasite	β_1	β_2	β_3	β_4	β_5
Aldrichetta forsteri	*Hedruris spinigera*	0.257 (<0.001)	15.6 (0.005)	NA	-10.72 (<0.001)	NA
Perca fluviatilis	*Coitocaecum parvum*	0.119 (0.862)	0.010 (0.989)	NA	0.025 (0.966)	NA
Gobiomorphus cotidianus	*Eustrongylides* sp.	0.441 (<0.001)	0.006 (0.723)	NA	0.002 (0.839)	NA
Gobiomorphus cotidianus	*Coitocaecum parvum*	0.375 (<0.001)	-0.087 (0.383)	-0.127 (0.283)	-0.034 (0.955)	0.307 (0.610)

widespread phenomenon with potentially broad ecological implications (Bolnick et al., 2003). Inter-individual variation in diet can influence infection risk among conspecific fish when exposure to parasites varies with prey type (Curtis et al., 1995; Wilson et al., 1996). Fish that consume more species of prey should have more diverse trophically transmitted parasites (Locke et al., 2014). Comparatively, exposure to directly transmitted parasites should not depend on host diet (Simková et al., 2001; Locke et al., 2013, 2014). We indeed found no clear relationship between fish gut contents and the richness of directly transmitted parasites in individual hosts. Results indicate that infection levels of directly transmitted helminth larvae are highly variable among fish species, indicating high host specificity and potential phylogenetic constraints in these parasites.

In contrast with our predictions, we also did not find clear relationships between host diet range and the richness of trophically transmitted parasites in fish hosts. Although broader diet range has been linked with higher parasite richness in fish, this pattern is only observed when a wide variety of prey species is utilised by a diverse array of parasite species for transmission (Carney and Dick, 1999). If only a few species in the ecosystem are actually used by local parasites for trophic transmission, then parasite richness in fish host is unlikely to increase with diet range (Kennedy et al., 1986). In lakes sampled here, the number of fish parasite species using trophic transmission is relatively low (8 species overall with a maximum of 7 in any 1 lake/season combination) and the overall number of prey taxa used by these parasites limited to 7, divided into only 3 groups (fish, crustaceans and oligochaetes). Comparatively, 53 different prey taxa were found in fish gut contents with a maximum of 26 prey taxa in any 1 site/lake/season combination. It is thus possible that, as long as the few prey taxa used by parasites are consumed by fish, a broader diet range does not further increase the richness of parasites found in individual hosts (Kennedy et al., 1986). Usually, larger fish harbour higher parasite diversities because large individuals have a higher feeding rate and are also less gape-limited (and thus less restricted in prey choice) than small fish (Poulin and Cribb, 2002; González and Poulin, 2005). Generally, our results indicate that individual fish size did not have major effects on the relationship between host diet range and parasite richness in fish species captured in the present study.

Interspecific differences in diet range and host-parasite compatibility among fish species may add extra layers of complexity to the factors determining parasite richness in individual fish hosts (Knudsen et al., 1997, 2008; Lagrue et al., 2011). Fish species sampled here have contrasting life-history strategies, varying from freshwater resident to marine visitors, potentially affecting their parasite fauna (Bouillon and Dempson, 1989; Kristoffersen et al., 1994). However, apart from *A. forsteri*, all other fish species examined in our study are permanent freshwater residents as adults (McDowall, 1990). Although the larvae of the catadromous and amphidromous fish sampled here are oceanic, their freshwater parasite fauna could not have been influenced by different life-history strategies. *Aldrichetta forsteri* is a marine fish that migrates inland into freshwater during the summer months and usually remains freshwater bound for several months, feeding exclusively on freshwater prey. However, it is possible that recently immigrated fish individuals may lack freshwater parasites due to their recent arrival from the sea, potentially influencing diet-parasite links. Unfortunately, this cannot be determined from our data as we cannot determine residence time of fish in freshwater.

Parasites can also be highly host-specific and may never be found in some fish species even though prey taxa used for transmission are consumed by that particular fish species. Alternatively, some parasite-carrying prey may never be consumed by a given fish species, further reducing parasite richness in any particular host (Kennedy et al., 1986; Lagrue et al., 2011); for example, parasites transmitted through fish prey consumption can only infect large piscivorous fish predators. Finally, gut contents may also provide a biased representation of individual diet range (Svanbäck et al., 2015). Apparent differences in diet among individual fish may reflect short-term foraging activities, with observed diets being only snapshots of actual diet ranges; all fish within a population may actually be feeding on the same range of available prey (Curtis et al., 1995). Comparatively, parasites likely remain in fish for longer than the prey used for transmission and thus provide a clearer signature of prey consumed over extended time periods than stomach contents (Johnson et al., 2004a; Valtonen et al., 2010). For example, in our study, prevalence of *H. spinigera* in *A. forsteri* was 100% although only 40% of fish were found with the intermediate host prey *P. excavatum* in their gut contents, indicating that all fish individuals were feeding on *P. excavatum* even though the prey was not found in stomach contents. Similarly, only around 10% of *G. cotidianus* individuals infected with *Eustrongylides* sp. larvae had eaten oligochaetes recently. However, on the other end of the spectrum, only around 10% of *G. cotidianus* individual infected by *C. parvum* had not consumed the host *Pa. fluviatilis*, while all infected *P. fluviatilis* had the prey intermediate host in their stomachs. These differences are likely explained by the specific persistence time (i.e. lifespan) of each parasite in fish hosts. *Eustrongylides* sp. larvae remain in the fish until transmission to the bird definitive host and thus potentially for the life time of the fish. *Hedruris spinigera* is a large nematode that attaches to the stomach epithelium of the fish host, needing to achieve significant growth and to find a mate before reproduction, and likely remain in the fish for longer than the small, fast maturing, hermaphrodite *C. parvum* adult (Lagrue et al., 2011). On the other hand, although intestinal parasites were found in introduced fish host species (Table A1), a previous study on the same system showed that their abundance and size are significantly lower in introduced hosts (Lagrue et al., 2011). Despite feeding heavily on intermediate host prey, these fish harboured low abundances of small parasites, hinting at a quick turnover with parasites remaining in fish host for a short amount of time due to host-parasite incompatibility. As a result, infection levels in introduced species may be more closely linked to recent, short-term fish host diet. Overall, stomach content data represent only a very limited window of time unless stomach contents are repeatedly sampled from the same individual using non-lethal methods like stomach flushing (Araújo et al., 2011). However, this is logistically very difficult to achieve and cannot document parasite richness and abundance simultaneously as parasite identification and count require host dissection. Overall, the utility of the stomach contents data when assessing fish diet range and selectivity and their link with parasite richness and abundance will likely be influenced by species-specific host-parasite characteristics.

While diet range did not seem to influence parasite richness, diet specialisation among fish individuals may still influence their exposure to trophically transmitted parasites (Bolnick et al., 2003). Among individuals, variation in diet is common in natural populations (Svanbäck et al., 2015). Intraspecific differences in diet preferences (i.e. individual diet specialisation; Layman et al., 2015; Rosenblatt et al., 2015) should thus translate in abundance variations of trophically transmitted parasites among conspecific fish hosts (Curtis et al., 1995; Wilson et al., 1996). Diet range may be limited, but fish feeding intensively on the few prey taxa used by local parasites for transmission should carry heavy parasite loads, and vice versa for fish feeding preferentially on prey taxa devoid of parasites (Kennedy et al., 1986; Dick et al., 2009). Differences in prey selectivity among sympatric fish should thus cause differences in parasite acquisition, and potential patterns of parasite segregation and aggregation among hosts (Crofton, 1971; Knudsen et al., 1997, 2004, 2008). However, our results showed no clear link between the proportion of prey intermediate hosts in individual fish diet contents (i.e. individual diet preference) and the abundance of parasites in fish hosts. Furthermore, relationships between diet preferences and parasite abundance were differentially influenced by fish size and species as well as prey and parasite species. In particular, the relationship between the abundance of *H. spinigera* in *A. forsteri* and the proportion of the intermediate host in the diet of *A. forsteri* was stronger in smaller fish. It is important to note, however, that feeding observations over short time frames (e.g., stomach content analyses) may overestimate the degree of diet specialisation and thus influence documented relationship between parasite loads and host diet (Novak and Tinker, 2015). As mentioned previously, the temporal scale of study, as well as the number of independent observations, can greatly influence estimates of the degree and persistence over time of diet range and preferences (Curtis et al., 1995; Fodrie et al., 2015). Dietary variations among individuals can also be caused by temporal or spatial patchiness in prey distribution rather than individual specialisation and may not be reflected in parasite loads if individual hosts are mobile enough to move among prey patches (Rosenblatt et al., 2015). Again, potential links between feeding specialisation and variation in parasite loads among individual fish hosts should be confirmed through repeated diet and parasite sampling, if at all feasible.

Overall, there was no clear relationship between diet range, estimated as the number of prey taxa in fish stomach contents, and parasite richness or between diet preferences (i.e. the proportion of prey species used for parasite transmission in individual fish diet contents) and parasite loads among individual fish hosts. Whether this lack of clear patterns was due to stomach sampling method limitations or accurately represents host-parasite relationships in the study systems is a question that should be tested further, but is technically and logistically challenging. Sampling repeatedly and concomitantly stomach contents and parasite abundances overtime in the same fish individuals would be ideal but is difficult if not impossible in wild fish. Although the methods used here are only a proxy of overall fish diet and parasite surveys, our results are roughly consistent across several host and parasite species, and contrast with those of earlier studies using similar methods in which diet and parasite infection were linked (Curtis et al., 1995; Knudsen et al., 1997, 2003; Bertrand et al., 2008). This pattern hints at a true disconnect between host diet (at least as measured here) and measures of parasite infections although host-parasite species-specific patterns may vary. Inherent characteristics of New Zealand lake systems (low parasite species richness, limited numbers of prey species used for trophic transmission, high host-parasite specificity) likely limit the influence of diet range and individual diet specialisation on parasite richness and abundance patterns. Repeated diet sampling over a longer time period, by maintaining fish in enclosure and using non-lethal stomach flushing to document individual fish diet for example, would help confirm or invalidate the utility of gut content data as well as the role of variation among individuals in diet specialisation and its effects on parasite loads among sympatric fish. Our results and those of previous studies confirm that, although parasite acquisition is obviously related to host diet, other factors that vary widely among ecosystems, hosts and parasites likely influence how parasite richness and load are linked to host diet.

Acknowledgements

We thank Anne Besson, Isa Blasco-Costa, Manna Warburton and Kim Garrett for assistance with field collection. Animal sampling was carried out in accordance with Otago University animal ethics guidelines (Animal Ethics Approval ET 10/12) and under the New Zealand Department of Conservation and Fish and Game fishing permit (NZ - DOC permit OT-34204-RES). We thank three anonymous referees and the editor for positive and constructive comments on previous versions of the manuscript.

Financial Support

This study was funded by a grant from the Marsden Fund (Royal Society of New Zealand) to RP. ARC was supported by an NSERC PGS-D Doctoral Scholarship and a University of Canterbury Doctoral Scholarship, and DBS by a Marsden Fund grant (UOC-1101) and a Rutherford Discovery Fellowship (both administered by the Royal Society of New Zealand).

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Supporting Information S2

Supporting information for Chapter 2:

Knowledge of predator-prey interactions improves predictions of immigration and extinction in island biogeography

Alyssa R. Cirtwill & Daniel B. Stouffer

6958 *S2.1: Full models*

Table S2.1: Main effects included in the full initial immigration, repeat immigration, and extinction models. Note that each model also included *all* possible interaction terms between the fixed effects indicated below, plus random effects of census and source population. These models were simplified to give the models in Tables S2.4–S2.6. See Tables S2.2-S2.3 for a complete list of terms included in each model. TIB refers to models based on the Theory of Island Biogeography–that is, excluding any trophic interactions.

Model	Main effects						
(a) Initial and repeat immigration	Distance from mainland	Island diameter	Interval between censuses	Species richness	Presence of predators	Ability to consume basal resources	Presence of animal prey
Null							
TIB	X	X	X				
Species-richness	X	X	X	X			
Top-down	X	X	X		X		
Top-down & Species-richness	X	X	X	X	X		
Bottom-up	X	X	X			X	X
Bottom-up & Species-richness	X	X	X	X		X	X
Top-down & Bottom-up	X	X	X		X	X	X
(b) Extinction							
Null							
TIB		X	X				
Species-richness		X	X	X			
Top-down		X	X		X		
Top-down & Species-richness		X	X	X	X		
Bottom-up		X	X			X	X
Bottom-up & Species-richness		X	X	X		X	X
Top-down & Bottom-up		X	X		X	X	X

Table S2.2: Symbols used in mathematical description of the statistical models.

Symbol	Description
C_{ijk+1}	Probability of immigration for species i on island j between census k and census $k+1$
X_{ijk+1}	Probability of extinction for species i on island j between census k and census $k+1$
δ_j	Distance of island j from the mainland (meters)
λ_j	Diameter of island j (meters)
τ_{k+1}	Time between census k and census $k+1$ (days)
Σ_k	Species richness during census k
ρ_{ijk}	Presence of predators of species i on island j during census k:
	ρ_{ijk}=1 if predators of species i were observed on island j during census k, ρ_{ijk}=0 otherwise
η_i	Ability of species i to eat plants:
	η_i=1 if species i is able to eat basal resources, η_i=0 otherwise
α_{ijk}	Presence of animal prey for species i on island j during census k:
	α_{ijk}=1 if prey of species i were observed on island j during census k, α_{ijk}=0 otherwise
E_{k+1}	Random effect of period between censuses k and $k+1$
S_i	Random effect of species i
W_{ijq}	Random effect of source population (i.e., the interaction between species i, island j, and event window q)
ϵ_{ijk+1}	Residual error for species i on island j between census k and census $k+1$

Table S2.3: Mathematical structure of the full initial immigration models. Mathematical structure of the repeat immigration models was identical except that the random effect of species (S_i) was replaced with a random effect of the interaction between species, island, and colonisation interval (W_{ijq}) as in the full extinction models (Table S2.4). All symbols are as in Table S2.2. TIB refers to a model based on the original Theory of Island Biogeography, without any trophic effects.

Model	Mathematical structure
Null	$C_{ijk+1} = E_{k+1} + \epsilon_{ijk+1}$
TIB	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \delta_j\lambda_j + \delta_j\tau_{k+1} + \lambda_j\tau_{k+1} + \delta_j\lambda_j\tau_{k+1} + E_{k+1} + S_i + \epsilon_{ijk+1}$
Species-richness	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \Sigma_k + \delta_j\lambda_j + \delta_j\tau_{k+1} + \delta_j\Sigma_k + \lambda_j\tau_{k+1} + \lambda_j\Sigma_k + \tau_{k+1}\Sigma_k + \delta_j\lambda_j\tau_{k+1} + \delta_j\lambda_j\Sigma_j + \delta_j\tau_{k+1}\Sigma_j + \lambda_j\tau_{k+1}\Sigma_j + \delta_j\lambda_j\tau_{k+1}\Sigma_j + E_{k+1} + S_i + \epsilon_{ijk+1}$
Top-down	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \rho_{ijk} + \delta_j\lambda_j + \delta_j\tau_{k+1} + \delta_j\rho_{ijk} + \lambda_j\tau_{k+1} + \lambda_j\rho_{ijk} + \tau_{k+1}\rho_{ijk} + \delta_j\lambda_j\tau_{k+1} + \delta_j\lambda_j\rho_{ijk} + \delta_j\tau_{k+1}\rho_{ijk} + \lambda_j\tau_{k+1}\rho_{ijk} + \delta_j\lambda_j\tau_{k+1}\rho_{ijk} + E_{k+1} + S_i + \epsilon_{ijk+1}$
Top-down & Species-richness	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \Sigma_k + \rho_{ijk} + \delta_j\lambda_j + \delta_j\tau_{k+1} + \delta_j\Sigma_j + \delta_j\rho_{ijk} + \lambda_j\tau_{k+1} + \lambda_j\Sigma_j + \lambda_j\rho_{ijk} + \tau_{k+1}\Sigma_k + \tau_{k+1}\rho_{ijk} + \Sigma_k\rho_{ijk} + \delta_j\lambda_j\tau_{k+1} + \delta_j\lambda_j\Sigma_k + \delta_j\lambda_j\rho_{ijk} + \delta_j\tau_{k+1}\Sigma_k + \delta_j\tau_{k+1}\rho_{ijk} + \delta_j\Sigma_k\rho_{ijk} + \lambda_j\tau_{k+1}\Sigma_k + \lambda_j\tau_{k+1}\rho_{ijk} + \tau_{k+1}\Sigma_k\rho_{ijk} + \delta_j\lambda_j\tau_{k+1}\Sigma_k + \delta_j\lambda_j\tau_{k+1}\rho_{ijk} + \delta_j\lambda_j\Sigma_k\rho_{ijk} + \delta_j\tau_{k+1}\Sigma_k\rho_{ijk} + \lambda_j\tau_{k+1}\Sigma_k\rho_{ijk} + \delta_j\lambda_j\tau_{k+1}\Sigma_k\rho_{ijk} + E_{k+1} + S_i + \epsilon_{ijk+1}$
Bottom-up	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \eta_i + \alpha_{ijk} + \delta_j\lambda_j + \delta_j\tau_{k+1} + \delta_j\eta_i + \delta_j\alpha_{ijk} + \lambda_j\tau_{k+1} + \lambda_j\eta_i + \lambda_j\alpha_{ijk} + \tau_{k+1}\eta_i + \tau_{k+1}\alpha_{ijk} + \eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1} + \delta_j\lambda_j\eta_i + \delta_j\lambda_j\alpha_{ijk} + \delta_j\tau_{k+1}\eta_i + \delta_j\tau_{k+1}\alpha_{ijk} + \delta_j\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\eta_i + \lambda_j\tau_{k+1}\alpha_{ijk} + \lambda_j\eta_i\alpha_{ijk} + \tau_{k+1}\eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1}\eta_i + \delta_j\lambda_j\tau_{k+1}\alpha_{ijk} + \delta_j\lambda_j\eta_i\alpha_{ijk} + \delta_j\tau_{k+1}\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1}\eta_i\alpha_{ijk} + E_{k+1} + S_i + \epsilon_{ijk+1}$
Bottom-up & Species-richness	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \Sigma_k + \eta_i + \alpha_{ijk} + \delta_j\lambda_j + \delta_j\tau_{k+1} + \delta_j\Sigma_k + \delta_j\eta_i + \delta_j\alpha_{ijk} + \lambda_j\tau_{k+1} + \lambda_j\Sigma_k + \lambda_j\eta_i + \lambda_j\alpha_{ijk} + \tau_{k+1}\Sigma_k + \tau_{k+1}\eta_i + \tau_{k+1}\alpha_{ijk} + \Sigma_k\eta_i + \Sigma_k\alpha_{ijk} + \eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1} + \delta_j\lambda_j\Sigma_k + \delta_j\lambda_j\eta_i + \delta_j\lambda_j\alpha_{ijk} + \delta_j\tau_{k+1}\Sigma_k + \delta_j\tau_{k+1}\eta_i + \delta_j\tau_{k+1}\alpha_{ijk} + \delta_j\Sigma_k\eta_i + \delta_j\Sigma_k\alpha_{ijk} + \delta_j\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\Sigma_k + \lambda_j\tau_{k+1}\eta_i + \lambda_j\tau_{k+1}\alpha_{ijk} + \lambda_j\Sigma_k\eta_i + \lambda_j\Sigma_k\alpha_{ijk} + \lambda_j\eta_i\alpha_{ijk} + \tau_{k+1}\Sigma_k\eta_i + \tau_{k+1}\Sigma_k\alpha_{ijk} + \tau_{k+1}\eta_i\alpha_{ijk} + \Sigma_k\eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1}\Sigma_k + \delta_j\lambda_j\tau_{k+1}\eta_i + \delta_j\lambda_j\tau_{k+1}\alpha_{ijk} + \delta_j\lambda_j\Sigma_k\eta_i + \delta_j\lambda_j\Sigma_k\alpha_{ijk} + \delta_j\lambda_j\eta_i\alpha_{ijk} + \delta_j\tau_{k+1}\Sigma_k\eta_i + \delta_j\tau_{k+1}\Sigma_k\alpha_{ijk} + \delta_j\tau_{k+1}\eta_i\alpha_{ijk} + \delta_j\Sigma_k\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\Sigma_k\eta_i + \lambda_j\tau_{k+1}\Sigma_k\alpha_{ijk} + \lambda_j\tau_{k+1}\eta_i\alpha_{ijk} + \lambda_j\Sigma_k\eta_i\alpha_{ijk} + \tau_{k+1}\Sigma_k\eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1}\Sigma_k\eta_i + \delta_j\lambda_j\tau_{k+1}\Sigma_k\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1}\eta_i\alpha_{ijk} + \delta_j\lambda_j\Sigma_k\eta_i\alpha_{ijk} + \delta_j\tau_{k+1}\Sigma_k\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\Sigma_k\eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1}\Sigma_k\eta_i\alpha_{ijk} + E_{k+1} + S_i + \epsilon_{ijk+1}$
Top-down & Bottom-up	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \rho_{ijk} + \eta_i + \alpha_{ijk} + \delta_j\lambda_j + \delta_j\tau_{k+1} + \delta_j\rho_{ijk} + \delta_j\eta_i + \delta_j\alpha_{ijk} + \lambda_j\tau_{k+1} + \lambda_j\rho_{ijk} + \lambda_j\eta_i + \lambda_j\alpha_{ijk} + \tau_{k+1}\rho_{ijk} + \tau_{k+1}\eta_i + \tau_{k+1}\alpha_{ijk} + \rho_{ijk}\eta_i + \rho_{ijk}\alpha_{ijk} + \eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1} + \delta_j\lambda_j\rho_{ijk} + \delta_j\lambda_j\eta_i + \delta_j\lambda_j\alpha_{ijk} + \delta_j\tau_{k+1}\rho_{ijk} + \delta_j\tau_{k+1}\eta_i + \delta_j\tau_{k+1}\alpha_{ijk} + \delta_j\rho_{ijk}\eta_i + \delta_j\rho_{ijk}\alpha_{ijk} + \delta_j\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\rho_{ijk} + \lambda_j\tau_{k+1}\eta_i + \lambda_j\tau_{k+1}\alpha_{ijk} + \lambda_j\rho_{ijk}\eta_i + \lambda_j\rho_{ijk}\alpha_{ijk} + \lambda_j\eta_i\alpha_{ijk} + \tau_{k+1}\rho_{ijk}\eta_i + \tau_{k+1}\rho_{ijk}\alpha_{ijk} + \tau_{k+1}\eta_i\alpha_{ijk} + \rho_{ijk}\eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1}\rho_{ijk} + \delta_j\lambda_j\tau_{k+1}\eta_i + \delta_j\lambda_j\tau_{k+1}\alpha_{ijk} + \delta_j\lambda_j\rho_{ijk}\eta_i + \delta_j\lambda_j\rho_{ijk}\alpha_{ijk} + \delta_j\lambda_j\eta_i\alpha_{ijk} + \delta_j\tau_{k+1}\rho_{ijk}\eta_i + \delta_j\tau_{k+1}\rho_{ijk}\alpha_{ijk} + \delta_j\tau_{k+1}\eta_i\alpha_{ijk} + \delta_j\rho_{ijk}\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\rho_{ijk}\eta_i + \lambda_j\tau_{k+1}\rho_{ijk}\alpha_{ijk} + \lambda_j\tau_{k+1}\eta_i\alpha_{ijk} + \lambda_j\rho_{ijk}\eta_i\alpha_{ijk} + \tau_{k+1}\rho_{ijk}\eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1}\rho_{ijk}\eta_i + \delta_j\lambda_j\tau_{k+1}\rho_{ijk}\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1}\eta_i\alpha_{ijk} + \delta_j\lambda_j\rho_{ijk}\eta_i\alpha_{ijk} + \delta_j\tau_{k+1}\rho_{ijk}\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\rho_{ijk}\eta_i\alpha_{ijk} + \delta_j\lambda_j\tau_{k+1}\rho_{ijk}\eta_i\alpha_{ijk} + E_{k+1} + S_i + \epsilon_{ijk+1}$

Table S2.4: Mathematical structure of the full extinction models. All symbols are as in Table S2.2. TIB refers to a model based on the original Theory of Island Biogeography, without any trophic effects.

Model	Mathematical structure
Null	$X_{ijk+1} = E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
TIB	$X_{ijk+1} = \lambda_j + \tau_{k+1} + \lambda_j\tau_{k+1} + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Species-richness	$X_{ijk+1} = \lambda_j + \tau_{k+1} + \Sigma_k + \lambda_j\tau_{k+1} + \lambda_j\Sigma_k + \tau_{k+1}\Sigma_k + \lambda_j\tau_{k+1}\Sigma_j + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Top-down	$X_{ijk+1} = \lambda_j + \tau_{k+1} + \rho_{ijk} + \lambda_j\tau_{k+1} + \lambda_j\rho_{ijk} + \tau_{k+1}\rho_{ijk} + \lambda_j\tau_{k+1}\rho_{ijk} + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Top-down & Species-richness	$X_{ijk+1} = \lambda_j + \tau_{k+1} + \Sigma_k + \rho_{ijk} + \lambda_j\tau_{k+1} + \lambda_j\Sigma_j + \lambda_j\rho_{ijk} + \tau_{k+1}\Sigma_k + \tau_{k+1}\rho_{ijk} + \Sigma_k\rho_{ijk} + \lambda_j\tau_{k+1}\Sigma_k + \lambda_j\tau_{k+1}\rho_{ijk} + \lambda_j\Sigma_k\rho_{ijk} + \tau_{k+1}\Sigma_k\rho_{ijk} + \lambda_j\tau_{k+1}\Sigma_k\rho_{ijk} + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Bottom-up	$X_{ijk+1} = \lambda_j + \tau_{k+1} + \eta_i + \alpha_{ijk} + \lambda_j\tau_{k+1} + \lambda_j\eta_i + \lambda_j\alpha_{ijk} + \tau_{k+1}\eta_i + \tau_{k+1}\alpha_{ijk} + \eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\eta_i + \lambda_j\tau_{k+1}\alpha_{ijk} + \lambda_j\eta_i\alpha_{ijk} + \tau_{k+1}\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\eta_i\alpha_{ijk} + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Bottom-up & Species-richness	$X_{ijk+1} = \lambda_j + \tau_{k+1} + \Sigma_k + \eta_i + \alpha_{ijk} + \lambda_j\tau_{k+1} + \lambda_j\Sigma_k + \lambda_j\eta_i + \lambda_j\alpha_{ijk} + \tau_{k+1}\Sigma_k + \tau_{k+1}\eta_i + \tau_{k+1}\alpha_{ijk} + \Sigma_k\eta_i + \Sigma_k\alpha_{ijk} + \eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\Sigma_k + \lambda_j\tau_{k+1}\eta_i + \lambda_j\tau_{k+1}\alpha_{ijk} + \lambda_j\Sigma_k\eta_i + \lambda_j\Sigma_k\alpha_{ijk} + \lambda_j\eta_i\alpha_{ijk} + \tau_{k+1}\Sigma_k\eta_i + \tau_{k+1}\Sigma_k\alpha_{ijk} + \tau_{k+1}\eta_i\alpha_{ijk} + \Sigma_k\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\Sigma_k\eta_i + \lambda_j\tau_{k+1}\Sigma_k\alpha_{ijk} + \lambda_j\tau_{k+1}\eta_i\alpha_{ijk} + \lambda_j\Sigma_k\eta_i\alpha_{ijk} + \tau_{k+1}\Sigma_k\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\Sigma_k\eta_i\alpha_{ijk} + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Top-down & Bottom-up	$X_{ijk+1} = \lambda_j + \tau_{k+1} + \rho_{ijk} + \eta_i + \alpha_{ijk} + \lambda_j\tau_{k+1} + \lambda_j\rho_{ijk} + \lambda_j\eta_i + \lambda_j\alpha_{ijk} + \tau_{k+1}\rho_{ijk} + \tau_{k+1}\eta_i + \tau_{k+1}\alpha_{ijk} + \rho_{ijk}\eta_i + \rho_{ijk}\alpha_{ijk} + \eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\rho_{ijk} + \lambda_j\tau_{k+1}\eta_i + \lambda_j\tau_{k+1}\alpha_{ijk} + \lambda_j\rho_{ijk}\eta_i + \lambda_j\rho_{ijk}\alpha_{ijk} + \lambda_j\eta_i\alpha_{ijk} + \tau_{k+1}\rho_{ijk}\eta_i + \tau_{k+1}\rho_{ijk}\alpha_{ijk} + \tau_{k+1}\eta_i\alpha_{ijk} + \rho_{ijk}\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\rho_{ijk}\eta_i + \lambda_j\tau_{k+1}\rho_{ijk}\alpha_{ijk} + \lambda_j\tau_{k+1}\eta_i\alpha_{ijk} + \lambda_j\rho_{ijk}\eta_i\alpha_{ijk} + \tau_{k+1}\rho_{ijk}\eta_i\alpha_{ijk} + \lambda_j\tau_{k+1}\rho_{ijk}\eta_i\alpha_{ijk} + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$

S2.2: *Best-fit models*

Table S2.5: Mathematical structure of the best-fitting initial immigration models after model simplification. All symbols are as in Table S2.2. TIB refers to a model based on the original Theory of Island Biogeography, without any trophic effects.

Model	Mathematical structure
TIB	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \delta_j\lambda_j + E_{k+1} + S_i + \epsilon_{ijk+1}$
Species-richness	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \Sigma_{k+1} + \delta_j\lambda_j + \lambda_j\Sigma_{k+1} + E_{k+1} + S_i + \epsilon_{ijk+1}$
Top-down	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \rho_{ijk+1} + \delta_j\lambda_j + \delta_j\rho_{ijk+1} + \tau_{k+1}\rho_{ijk+1} + E_{k+1} + S_i + \epsilon_{ijk+1}$
Top-down & Species-richness	Equivalent to Top-down model
Bottom-up	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \alpha_{ijk+1} + \delta_j\lambda_j + \delta_j\alpha_{ijk+1} + \lambda_j\alpha_{ijk+1} + \delta_j\lambda_j\alpha_{ijk+1} + E_{k+1} + S_i + \epsilon_{ijk+1}$
Bottom-up & Species-richness	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \Sigma_{k+1} + \alpha_{ijk+1} + \delta_j\lambda_j + \lambda_j\Sigma_{k+1} + \lambda_j\alpha_{ijk+1} + E_{k+1} + S_i + \epsilon_{ijk+1}$
Top-down & Bottom-up	$C_{ijk+1} = \delta_j + \lambda_j + \tau_{k+1} + \rho_{ijk+1} + \alpha_{ijk+1} + \delta_j\lambda_j + \lambda_j\rho_{ijk+1} + \lambda_j\alpha_{ijk+1} + \tau_{k+1}\rho_{ijk+1} + E_{k+1} + \epsilon_{ijk+1}$

Table S2.6: Mathematical structure of the best-fitting repeat immigration models after model simplification. All symbols are as in Table S2.2. TIB refers to a model based on the original Theory of Island Biogeography, without any trophic effects.

Model	Mathematical structure
TIB	$C_{ijk+1} = \lambda_j + \tau_{k+1} + \lambda_j\tau_{k+1} + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Species-richness	Equivalent to TIB model
Top-down	Equivalent to TIB model
Top-down & Species-richness	Equivalent to TIB model
Bottom-up	$C_{ijk+1} = \lambda_j + \tau_{k+1} + \eta_i + \lambda_j\tau_{k+1} + \lambda_j\eta_i + \tau_{k+1}\eta_i + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Bottom-up & Species-richness	Equivalent to Bottom-up model
Top-down & Bottom-up	Equivalent to Bottom-up model

Table S2.7: Mathematical structure of the best-fitting extinction models after model simplification. All symbols are as in Table S2.2. TIB refers to a model based on the original Theory of Island Biogeography, without any trophic effects.

Model	Mathematical structure
TIB	$X_{ijk+1} = \lambda_j + \tau_{k+1} + \lambda_j \tau_{k+1} + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Species-richness	$X_{ijk+1} = \lambda_j + \tau_{k+1} + \Sigma_k + \lambda_j \tau_{k+1} + \lambda_j \Sigma_k + \tau_{k+1}\Sigma_k + \lambda_j \tau_{k+1} \Sigma_k + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Top-down	Equivalent to TIB model
Top-down & Species-richness	Equivalent to Species-richness model
Bottom-up	$X_{ijk+1} = \tau_{k+1} + \eta_i + \alpha_{ijk} + \tau_{k+1}\eta_i + \eta_i \alpha_{ijk} + W_{ijq} + E_{k+1} + \epsilon_{ijk+1}$
Bottom-up & Species-richness	$X_{ijk+1} = \lambda_j + \tau_{k+1} + \Sigma_k + \eta_i + \alpha_{ijk} + \lambda_j \Sigma_k + \tau_{k+1}\Sigma_k + \tau_{k+1}\eta_i + \tau_{k+1}\alpha_{ijk} + \Sigma_k \eta_i + E_{k+1} + W_{ijq} + \epsilon_{ijk+1}$
Top-down & Bottom-up	Equivalent to Bottom-up model

S2.3: Summary tables for best-fit models

Table S2.8: Summary tables of the best-fit Theory of Island Biogeography (TIB), Species-richness (SR), Top-down (TD), Bottom-up (BU), Bottom-up & Species-richness (BU & SR), and Top-down & Bottom-up (TD & BU) models for probability of initial immigration. The best-fit Top-down & Species-richness model was identical to the best-fit species-richness model and is not shown. Standardised effects (βs) and intercepts shown refer to the same scale as the logit-transformed data (e.g., a 1 day increase in time between censuses or a 1m increase in distance from the mainland). Models are as in Table S2.5. The intercept of the null model was -3.84 (p<0.001). Standardised effects of 0 were not included in the best-fit versions of each model. An empty cell indicates that the term was not part of the full model and hence could not appear in the best-fit version.

Fixed effect	TIB		SR		TD		Bottom-up		BU & SR		TD & BU	
	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Intercept	-3.77	<0.001	-3.78	<0.001	-3.98	<0.001	-3.87	<0.001	-3.84	<0.001	-4.02	<0.001
Distance	-55.7	<0.001	-56.5	<0.001	-57.1	<0.001	-61.7	<0.001	-55.3	<0.001	-56.3	<0.001
Diameter	0.977	0.015	0.938	0.016	-0.122	0.865	-0.041	0.938	0.425	0.353	-0.711	0.34
Timesince	11.1	<0.001	10.7	<0.001	17.8	<0.001	11.1	<0.001	10.7	<0.001	18.1	<0.001
Species			1.19	0.07					1.1	0.103		
Predators					0.251	0.081					0.239	0.104
Animal Prey							0.22	0.112	0.125	0.368	0.119	0.387
Distance:Diameter	327	<0.001	304	<0.001	322.7	<0.001	205	0.045	314	<0.001	333	<0.001
Distance:Animals							12.7	0.568	0	NA	0	NA
Diameter:Species			7.31	0.026					6.59	0.048		
Diameter:Predators					1.29	0.08					1.29	0.077
Diameter:Animals							2.27	0.002	1.13	0.027	1.32	0.009
Time:Predators					-9.01	0.108					-9.32	0.099
Distance:Diameter:Animals							285	0.05	0	NA	0	NA

Table S2.9: Summary tables of the best-fit Theory of Island Biogeography (TIB) and Bottom-up models for probability of repeat immigration. The best-fit Top-down, Species-richness, and Top-down & Species-richness models were identical to the best-fit TIB model, while the best-fit Bottom-up & Species-richness and Top-down & Bottom-up models were identical to the best-fit Bottom-up model and are not shown. Standardised effects (βs) and intercepts shown refer to the same scale as the logit-transformed data (e.g., a 1 day increase in time between censuses or a 1m increase in distance from the mainland). The best-fitting Species-richness, Top-down, and Top-down & Species-richness models were identical to the best-fitting TIB model, and the best-fit Bottom-up & Species-richness and Top-down & Bottom-up models were identical to the Bottom-up model. Models are as in Table S2.6. The intercept of the null model was -2.77 (p<0.001). Standardised effects of 0 were not included in the best-fit versions of each model. An empty cell indicates that the term was not part of the full model and hence could not appear in the best-fit version.

Fixed effect	TIB		Bottom-up	
	β	p-value	β	p-value
Intercept	-2.91	<0.001	-2.82	<0.001
Diameter	-0.671	0.486	0.504	0.637
Time	-46.8	0.137	-76.8	0.027
Basal resources			-0.164	0.431
Diameter:Time	-464	0.052	-431	0.073
Diameter:Basal			-2.52	0.025
Time:Basal			51.5	0.024

Table S2.10: Summary tables of the best-fit Theory of Island Biogeography (TIB), Species-richness, Top-down, Bottom-up, and Bottom-up & Species-richness (BU & SR) models for extinction probability. The best-fit Top-down model was identical to the best-fit TIB model, the best-fit Top-down & Species-richness model was identical to the best-fit Species-richness model, and the best-fit Top-down & Bottom-up model was identical to the best-fit Bottom-up model. None are shown here. Standardised effects (βs) and intercepts shown refer to the same scale as the logit-transformed data (e.g., a 1 day increase in time between censuses or a 1m increase in distance from the mainland). The best-fitting Top-down model was identical to the best-fitting TIB model, the best-fitting Top-down & Species-richness model was identical to the best-fitting Species-richness model, and the best-fitting Top-down & Bottom-up model was identical to the best-fitting Bottom-up model. Models are as in Table S2.7. The intercept of the null model was -0.587 (p<0.001). Standardised effects of 0 were not included in the best-fit versions of each model. An empty cell indicates that the term was not part of the full model and hence could not appear in the best-fit version.

Fixed effect	TIB		Species-richness		Bottom-up		BU & SR	
	β	p-value	β	p-value	β	p-value	β	p-value
Intercept	-0.462	<0.001	-0.59	<0.001	-0.174	0.863	1.22	0.007
Diameter	0.437	0.419	-0.836	0.276	0	NA	0.009	0.987
Time	60.0	<0.001	23.9	0.252	91.9	<0.001	117	<0.001
Species-richness			4.00	0.001			4.75	<0.001
Basal resources					-0.470	0.646	-1.87	<0.001
Animals					0.201	0.844	-1.19	0.003
Diameter:Time	140	0.008	-209	0.210	0	NA	0	NA
Diameter:Species			-2.55	0.652			-9.02	0.031
Time:Species			546	0.017			166	0.016
Time:Basal					-57.7	<0.001	-69.4	<0.001
Time:Animals							-20.4	0.146
Species:Basal							-3.70	0.036
Basal:Animals					-1.64	0.135	0	NA
Diameter:Time:Species			2.74×10^3	0.037			0	NA

S2.4: Details of models not described in the main text

Initial immigration models

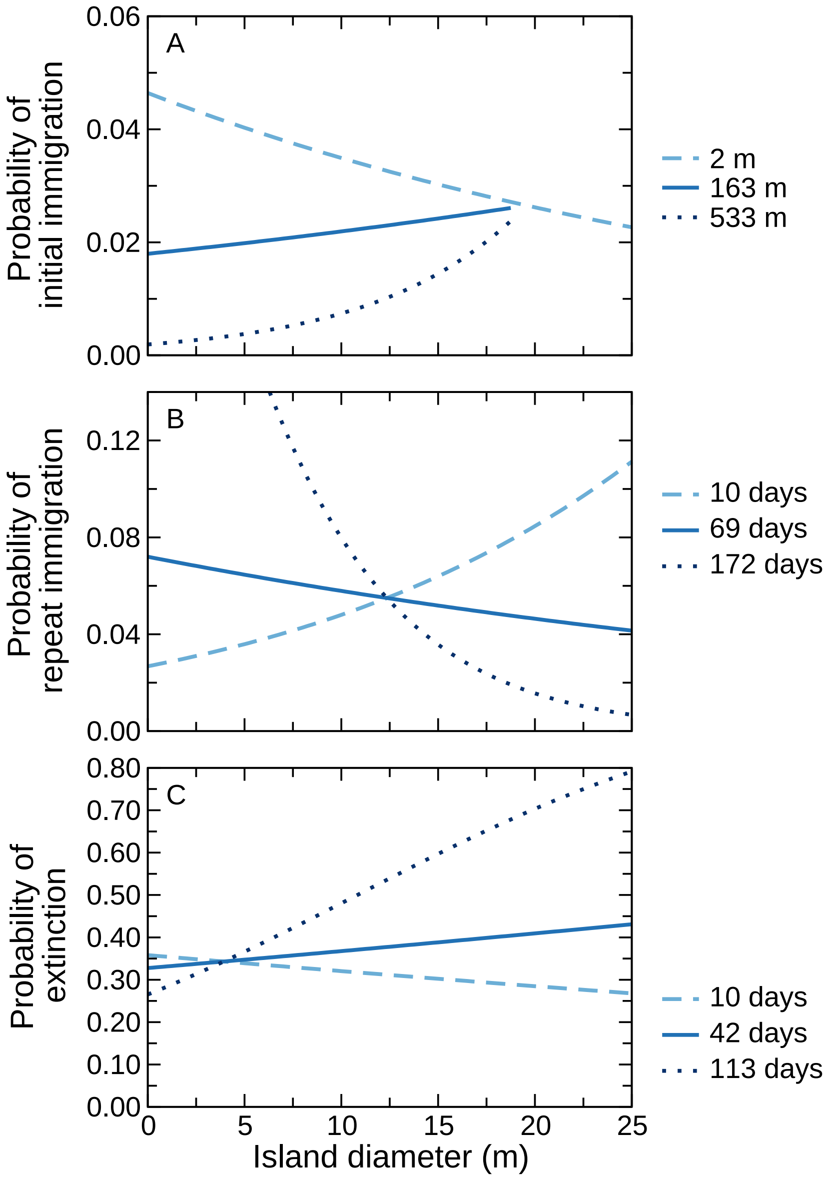

Figure S2.1: Predicted per-species probabilities of initial immigration, repeat immigration, and extinction as a function of island diameter in models based on the classic Theory of Island Biogeography. **(A)** Predicted initial immigration probability was affected by island diameter, distance from the mainland, and interval between censuses. For islands that were relatively far from the mainland, a species' immigration probability increased with island size. The opposite trend occurred for islands close to the mainland. Predictions are shown for an island close to the mainland (2m, light, dashed line), a moderate distance from the mainland (163 m, solid line), and far from the mainland (533 m, dark, dotted line). All predictions used the mean observed interval between censuses of 37 days and were based on a sample size of 18,420 opportunities for initial immigration. Predicted probability of initial immigration increased linearly with an increasing interval between censuses (not shown). **(B)** Predicted repeat immigration probability varied with island diameter and interval between censuses. For short to moderate intervals between censuses, repeat immigration probability increased with island diameter. When the interval between censuses was short, immigration probability decreased with increasing island diameter. **(C)** Predicted extinction probability increased with increasing island diameter, and this increase was steeper when the interval between censuses was long. In panels **(B)** and **(C)**, predicted per-species probabilities of immigration and extinction are shown for the minimum observed interval between censuses of 10 days (dashed line), a moderate interval of 28 days (solid line), and a large interval between censuses (76 days). Predictions were based on N=1,674 and N=1,943 opportunities for repeat immigration and extinction, respectively.

The best-fitting TIB model for initial immigration included main effects for diameter, distance from the mainland, interval between censuses, and interactions between diameter and both distance and interval between censuses (Table S2.5). This model significantly improved upon the AIC of the null model (χ^2=52.0, df=4, p<0.001; *Table 3A, main text*). As we expected, species were less likely to immigrate to more isolated islands ($\beta_{Distance}$=-55.7), although this effect was reversed on large islands ($\beta_{Distance:Diameter}$=327). More intuitively, probability of immigration also increased when the interval between censuses was long (β_{Time}=11.1; Fig. S2.1).

The best-fitting species-richness model also improved on the fit of the null model (χ^2=60.9, df=6, p<0.001). As in the TIB model, a species' probability of immigration increased with increasing island diameter and interval between censuses, and decreased with increasing distance from the mainland. All effect sizes were very similar to those in the TIB model (Table S2.8). Contrary to our expectations, species' probability of immigration also increased with increasing species richness ($\beta_{Species}$=7.31).

The best-fitting top-down model also significantly improved upon the fit of the null model (χ^2=60.7, df=7, p<0.001). As with the species-richness model, in the top-down model a species' probability of immigration decreased with increasing distance from the mainland, except on large islands (Table S2.8). Species with predators present were more likely to immigrate, especially on large islands ($\beta_{Predators}$=0.251, $\beta_{Diameter:Predators}$=1.29). However, for these species

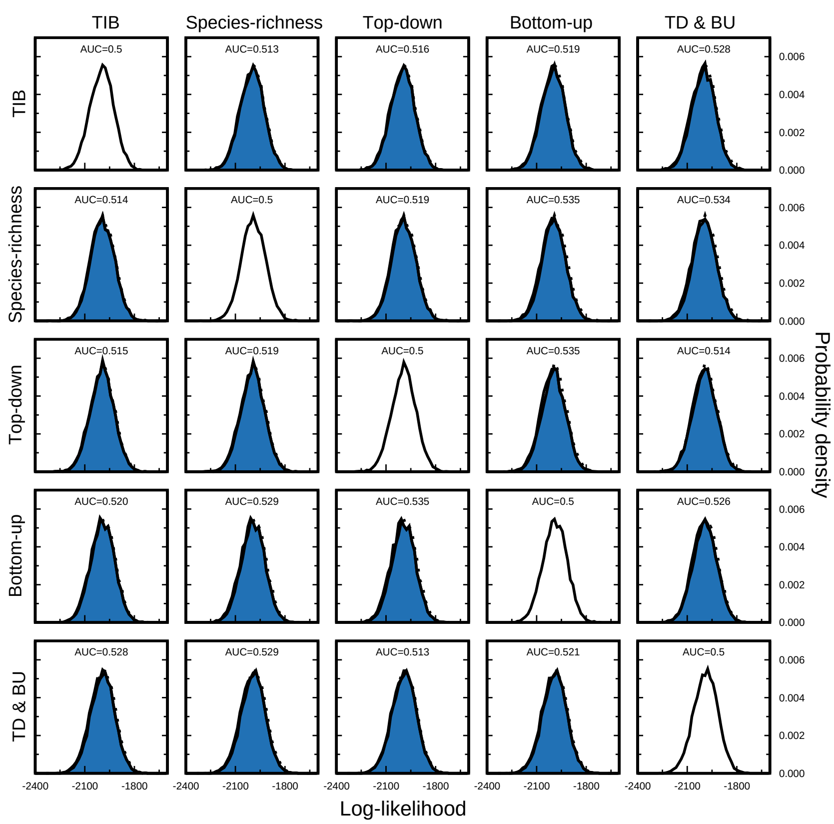

Figure S2.2: Hypothesis comparison for Theory of Island Biogeography (TIB), species-richness, top-down, bottom-up, and top-down & bottom-up (TD & BU) initial immigration models. Row names indicate the model from which test data was generated; column names indicate the model used to fit the test data. Each plot shows the histogram of log-likelihoods of obtaining test data from one model using another, based on 10,000 randomly-generated test datasets. Dotted curves indicate the success of a given model at predicting itself, as do plots on the diagonal. The grey shaded regions indicate overlap between the two models, where a given dataset was equally likely to have come from either model. All pairs of models have AUC's close to 0.5, indicating that the likelihood of observing a given dataset was approximately equal assuming either model were true.

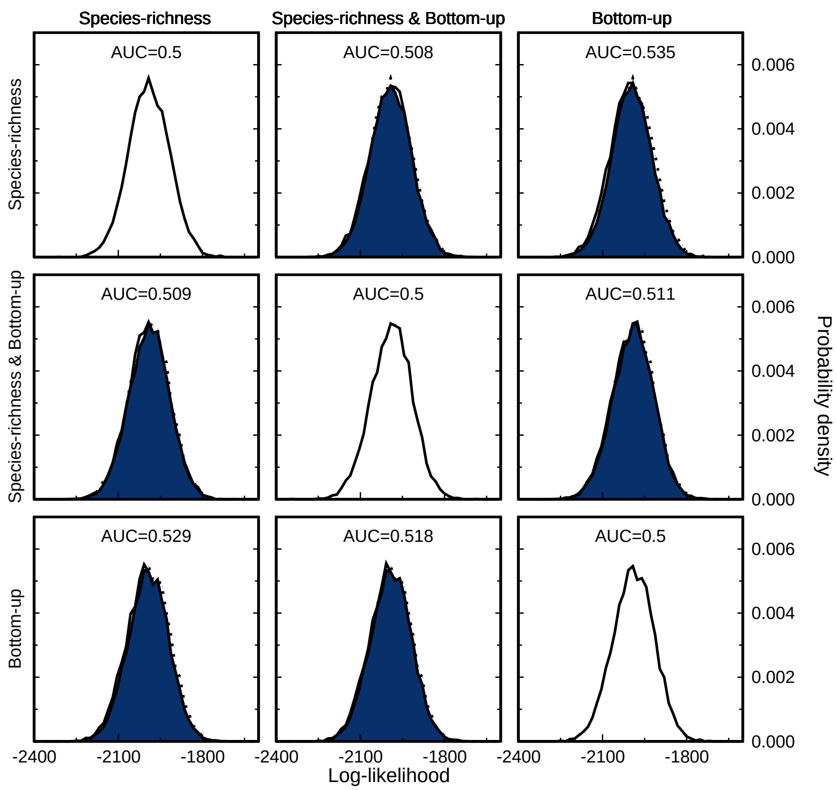

Figure S2.3: Hypothesis comparison for species-richness, species-richness & bottom-up, and bottom-up initial immigration models. Row names indicate the model from which test data was generated; column names indicate the model used to fit the test data. Each plot shows the histogram of log-likelihoods of obtaining test data from one model using another, based on 10,000 randomly-generated test datasets. Dotted curves indicate the success of a given model at predicting itself, as do plots on the diagonal. The grey shaded regions indicate overlap between the two models, where a given dataset was equally likely to have come from either model. Data generated by each model was fit well by either of the other models. This indicates that all three models capture very similar variation in the data.

likelihood of immigration increased less with increasing interval between censuses than for other species (β_{Time}=17.8, $\beta_{Time:Predators}$=-9.01).

The bottom-up model, which also significantly improved upon the null model (χ^2=63.7, df=8, p<0.001), also included similar terms for distance from the mainland, interval between censuses, and the interaction between distance and island diameter (Table S2.8). Contrary to our expectations, the bottom-up model did not include any terms for the ability to consume basal resources. However, species with animal prey present were more likely to immigrate than those without animal prey present ($\beta_{Animals}$=0.22). This effect was stronger on larger islands, islands farther from the mainland, and especially on large, isolated islands ($\beta_{Distance:Animal}$=12.7, $\beta_{Diameter:Animal}$=2.27, $\beta_{Distance:Diameter:Animal}$=285).

The best-fit top-down & species-richness model was identical to the best-fit species-richness model. The best-fit bottom-up & species-richness model, however, included terms for species richness and the presence of animal prey similar to those in the species-richness and bottom-up models in addition to similar terms to those in the TIB model (Table S2.8). Despite combining features of the species- richness and bottom-up models, the bottom-up & species-richness model did not significantly improve upon the fit of either (χ^2=5.92, df=2, p=0.052 and χ^2=0, df=0, p>0.999). Each of the species-richness, bottom-up, and bottom-up & species-richness models all fit data generated by any of the other models extremely well (Fig. S2.3).

Repeat immigration models

The best-fitting TIB model did not significantly improve upon the null model (χ^2=6.09, df=3, p=0.107). The TIB model included terms for island diameter, interval between censuses, and their interaction, but no terms relating to distance from the mainland (Table S2.6). In this model, a species' probability of re-immigration decreased with increasing island diameter ($\beta_{Diameter}$=-0.671), an effect which was strengthened when the interval between censuses was large ($\beta_{Diameter:Time}$=-464; Fig. S2.1). The species-richness and top-down models both reduced to the best-fitting TIB model, indicating that the number of species or presence of predators on an island explained little variation in the data. Unsurprisingly, the combined model including species-richness and top-down effects also reduced to the best-fitting TIB model.

Extinction models

The best-fitting TIB model for extinction was the full model (χ^2=59.8, df=3, p<0.001; Table S2.7), and this model had a lower AIC than the null model (*Table 3C, main text*). Contrary to our expectations, the data indicated that extinction probability increased on larger islands ($\beta_{Diameter}$=0.437). More intuitively, extinction probability also increased with increasing intervals between censuses (β_{Time}=60.0). The larger the island, the larger this effect ($\beta_{Diameter:Time}$=140; Fig. S2.1).

Similarly, the best-fitting species-richness model was the full model, which had a lower AIC than both the null and TIB models (*Table 3C, main text*). Unlike the TIB model, the species-richness model predicted that extinction probability would decrease on larger islands ($\beta_{Diameter}$=-0.836), and that this effect would be stronger with large census intervals ($\beta_{Diameter:Time}$=-209). As expected, probability of extinction increased with species richness ($\beta_{Species}$=4.00), although

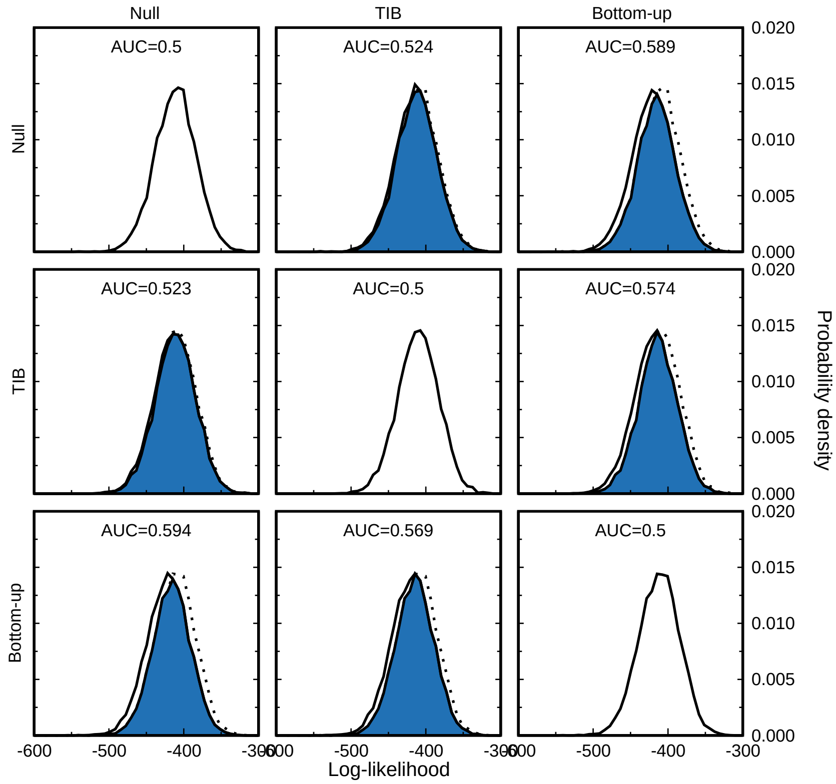

Figure S2.4: Hypothesis comparison for null, Theory of Island Biogeography (TIB), and bottom-up repeat immigration models. Note that the best-fitting species-richness and top-down models were identical to the best-fitting TIB model while the best-fitting top-down & bottom-up model was identical to the best-fitting bottom-up model. Row names indicate the model from which test data was generated; column names indicate the model used to fit the test data. Each plot shows the histogram of log-likelihoods of obtaining test data from one model using another, based on 10,000 randomly-generated test datasets. Dotted curves indicate the success of a given model at predicting itself, as do plots on the diagonal. The grey shaded regions indicate overlap between the two models, where a given dataset was equally likely to have come from either model. All pairs of models have AUC's close to 0.5, indicating that the likelihood of observing a given dataset was approximately equal assuming either model were true.

this effect was weaker on larger islands ($\beta_{Diameter:Species}$=-2.55). Because of a strong three-way interaction between diameter, species richness, and time between censuses, any of the above relationships could be reversed when both species richness and the interval between censuses were sufficiently large (or when both were small) ($\beta_{Diameter:Time:Species}$=2740). Nevertheless, overall the species-richness model generated very similar predictions to those of the TIB model (Fig. S2.5). The best-fitting top-down model was identical to the TIB model while the best-fitting top-down & species-richness model was identical to the species-richness model.

The bottom-up & species-richness model provided significant statistical improvement over both the species-richness and bottom-up models (χ^2=44.8, df=3, p<0.001 and χ^2=19.5, df=5, p=0.002, respectively). In this model, as in the bottom-up model, extinction probabilities were lower for species with animal prey available or able to consume basal resources ($\beta_{Animals}$=-1.19, β_{Basal}=-1.87). Further, the increase in probability of extinction with increasing interval between censuses was weaker for these species. Unlike the bottom-up model, the bottom-up & species-richness model also included

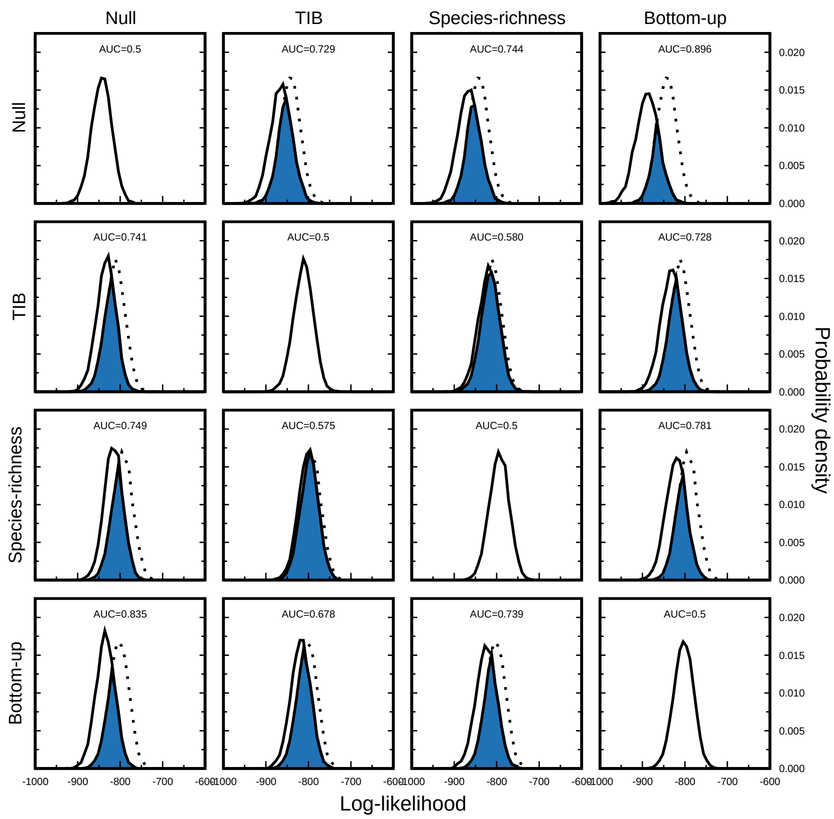

Figure S2.5: Hypothesis comparison for null, Theory of Island Biogeography (TIB), species-richness and bottom-up extinction models. The top-down model was identical to the TIB model while the top-down & bottom-up was identical to the bottom-up model. Row names indicate the model from which test data were generated; column names indicate the model used to fit the test data. Each plot shows the histogram of log-likelihoods of obtaining test data from one model using another, based on 10,000 randomly-generated test datasets. Dotted curves indicate the success of a given model at predicting itself, as do plots on the diagonal. The grey shaded regions indicate overlap between the two models, where a given dataset was equally likely to have come from either model.

positive effects of species-richness and the interaction between species-richness and census interval on probability of extinction ($\beta_{Species}$=4.75 and $\beta_{Time:Species}$=166). Despite these additional terms, the bottom-up & species-richness model captured similar variation in the data to the bottom-up model (average pairwise AUC = 0.618, Fig. S2.6). In addition, the parameters of the combined and bottom-up models were qualitatively similar (Table S2.10), suggesting that the statistical gains of the combined model may be due to over-fitting.

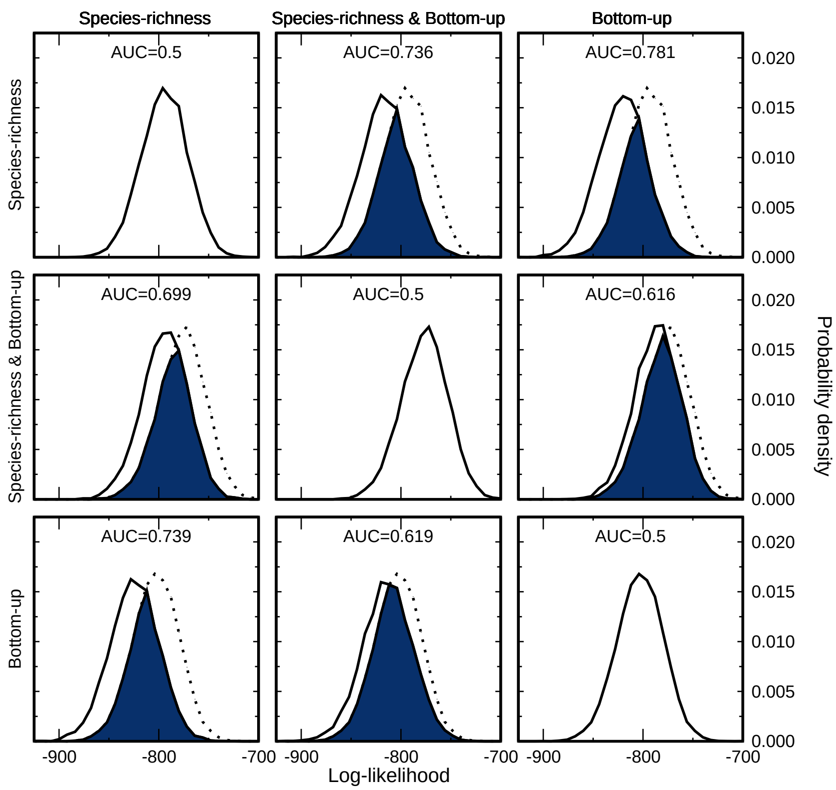

Figure S2.6: Hypothesis comparison for species-richness, species-richness & bottom-up, and bottom-up extinction models. Row names indicate the model from which test data was generated; column names indicate the model used to fit the test data. Each plot shows the histogram of log-likelihoods of obtaining test data from one model using another, based on 10,000 randomly-generated test datasets. Dotted curves indicate the success of a given model at predicting itself, as do plots on the diagonal. The grey shaded regions indicate overlap between the two models, where a given dataset was equally likely to have come from either model. Data generated by the species-richness model was poorly fit by the bottom-up and combined models, and vice versa. In contrast, pairings of the bottom-up and combined models had AUC's close to 0.5. This indicates that adding species-richness effects to the bottom-up model did not capture any variation not already explained by bottom-up effects.

S2.5: Cumulative species richness plots for islands not shown in the main text

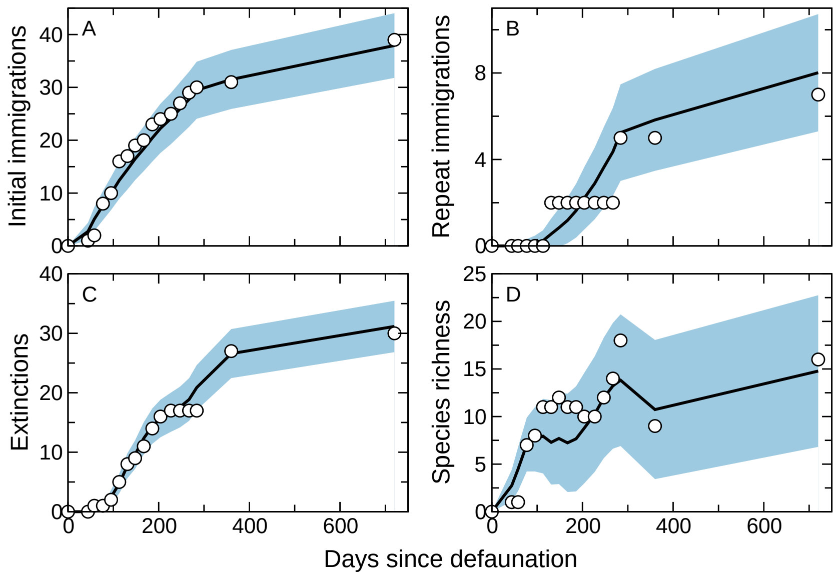

Figure S2.7: Initial immigrations, repeat immigrations, extinctions, and species richness over time for island E1, (11m in diameter, 533m from the mainland). **(A)-(D)** We show the cumulative values for the observed experiment (white circles) along with the equivalent values as predicted by the the best-fitting models for initial immigration, repeat immigration, and extinction (i.e., top-down & bottom-up, bottom-up, and bottom-up models, respectively). We obtained the model predictions for total species richness at each census by adding predicted immigrants and subtracting predicted extinctions. In all panels, the solid line indicates the mean prediction while the shaded area corresponds to one standard deviation.

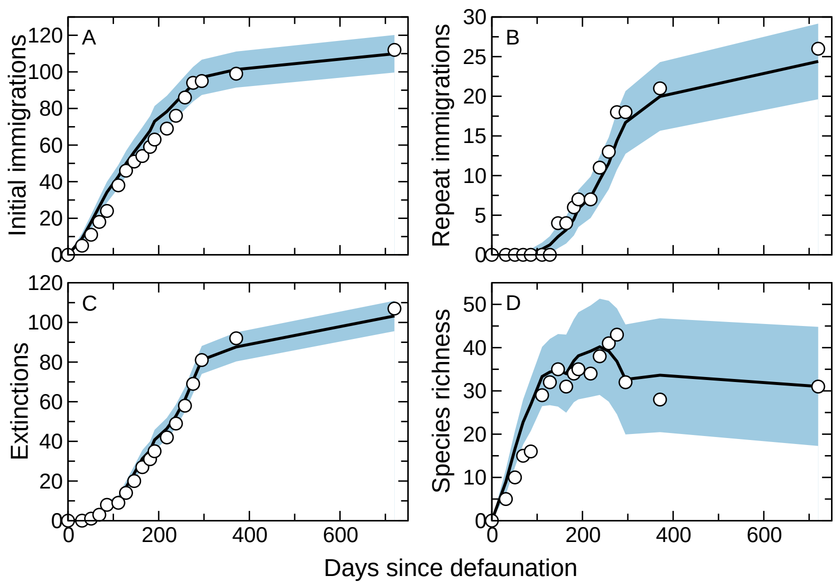

Figure S2.8: Initial immigrations, repeat immigrations, extinctions, and species richness over time for island E2 (12m in diameter, 2m from the mainland). **(A)-(D)** We show the cumulative values for the observed experiment (white circles) along with the equivalent values as predicted by the the best-fitting models for initial immigration, repeat immigration, and extinction (i.e., top-down & bottom-up, bottom-up, and bottom-up models, respectively). We obtained the model predictions for total species richness at each census by adding predicted immigrants and subtracting predicted extinctions. In all panels, the solid line indicates the mean prediction while the shaded area corresponds to one standard deviation.

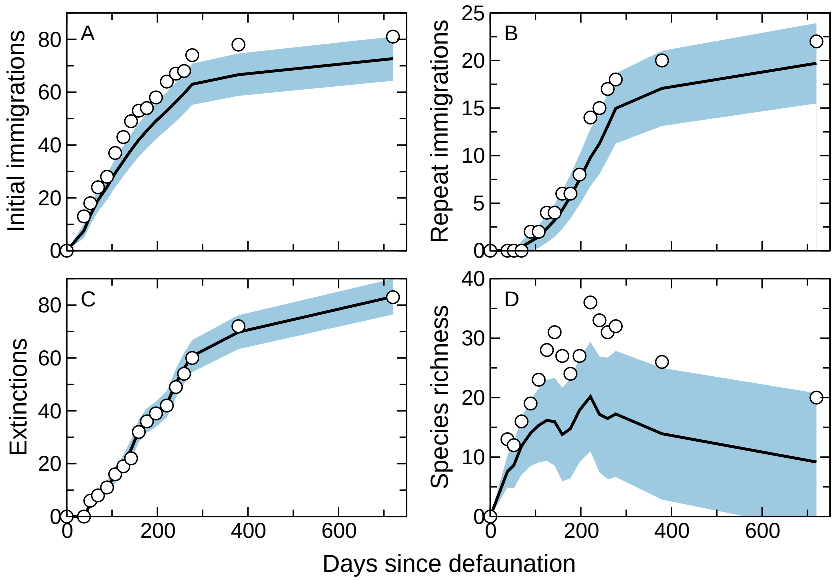

Figure S2.9: Initial immigrations, repeat immigrations, extinctions, and species richness over time for island E3 (12m in diameter, 172m from the mainland). **(A)-(D)** We show the cumulative values for the observed experiment (white circles) along with the equivalent values as predicted by the the best-fitting models for initial immigration, repeat immigration, and extinction (i.e., top-down & bottom-up, bottom-up, and bottom-up models, respectively). We obtained the model predictions for total species richness at each census by adding predicted immigrants and subtracting predicted extinctions. In all panels, the solid line indicates the mean prediction while the shaded area corresponds to one standard deviation.

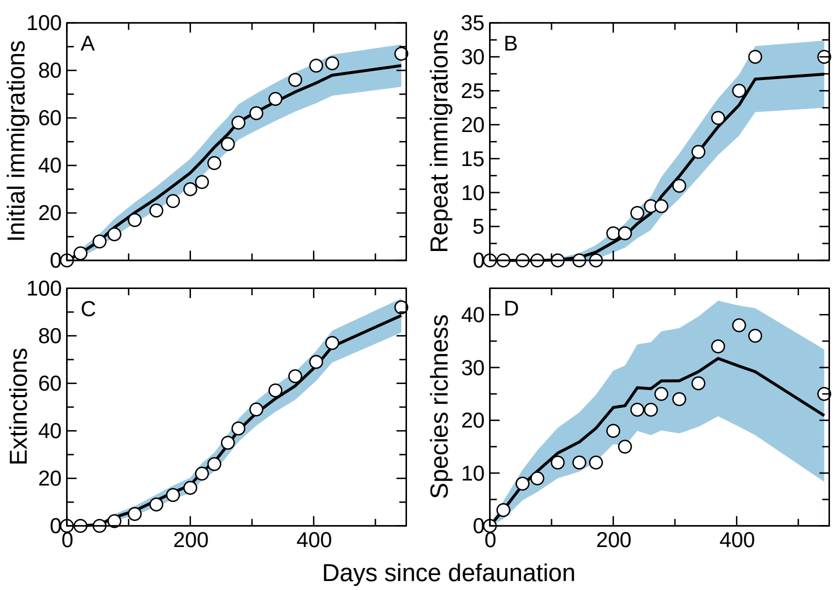

Figure S2.10: Initial immigrations, repeat immigrations, extinctions, and species richness over time for island E7 (25m in diameter, 15m from the mainland). **(A)-(D)** We show the cumulative values for the observed experiment (white circles) along with the equivalent values as predicted by the the best-fitting models for initial immigration, repeat immigration, and extinction (i.e., top-down & bottom-up, bottom-up, and bottom-up models, respectively). We obtained the model predictions for total species richness at each census by adding predicted immigrants and subtracting predicted extinctions. In all panels, the solid line indicates the mean prediction while the shaded area corresponds to one standard deviation.

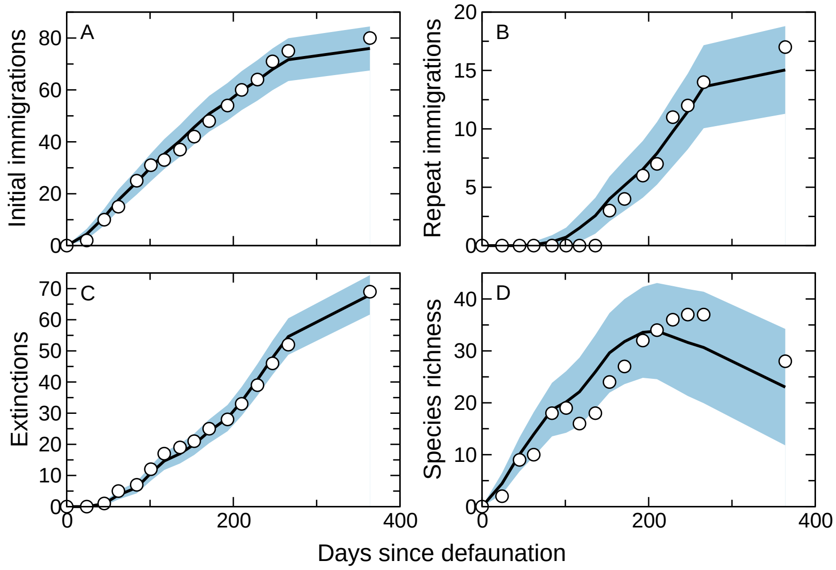

Figure S2.11: Initial immigrations, repeat immigrations, extinctions, and species richness over time for island E9 (18m in diameter, 379m from the mainland). **(A)-(D)** We show the cumulative values for the observed experiment (white circles) along with the equivalent values as predicted by the the best-fitting models for initial immigration, repeat immigration, and extinction (i.e., top-down & bottom-up, bottom-up, and bottom-up models, respectively). We obtained the model predictions for total species richness at each census by adding predicted immigrants and subtracting predicted extinctions. In all panels, the solid line indicates the mean prediction while the shaded area corresponds to one standard deviation.

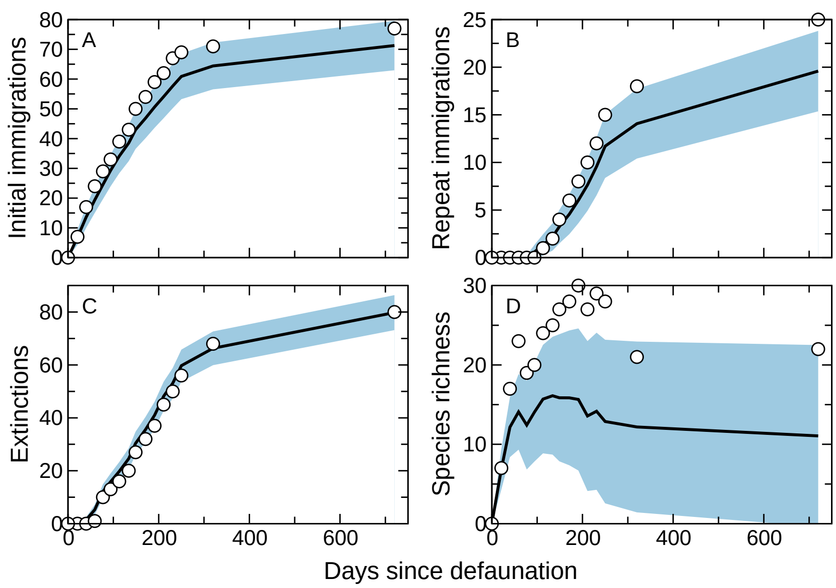

Figure S2.12: Initial immigrations, repeat immigrations, extinctions, and species richness over time for island ST2 (11m in diameter, 154m from the mainland). **(A)-(D)** We show the cumulative values for the observed experiment (white circles) along with the equivalent values as predicted by the the best-fitting models for initial immigration, repeat immigration, and extinction (i.e., top-down & bottom-up, bottom-up, and bottom-up models, respectively). We obtained the model predictions for total species richness at each census by adding predicted immigrants and subtracting predicted extinctions. In all panels, the solid line indicates the mean prediction while the shaded area corresponds to one standard deviation.

Supporting Information S3

Supporting information for Chapter 3:

Conservation of interaction partners between related plants varies widely across communities and between plant families.

Alyssa R. Cirtwill, Giulio V. Dalla Riva, Nick J. Baker,
Joshua A. Thia, Christie J. Webber, Daniel B. Stouffer

S3.1. Original sources for networks

Table S3.1: Original sources for all networks used in this analysis. PH indicates a plant-herbivore network, and PP a plant-pollinator network.

Network	Network type	Source
1	PH	(Basset and Samuelson, 1996)
2	PH	(Blüthgen et al., 2006)
3	PH	(Bodner et al., 2010)
4	PH	(Cagnolo et al., 2011)
5	PH	(Coley et al., 2006)
6	PH	(Ibanez et al., 2013)
7	PH	(Novotny et al., 2012)
8	PH	(Otte and Joern, 1976)
9	PH	(Peralta et al., 2014)
10	PH	(Sheldon and Rogers, 1978)
11	PH	(Ueckert and Hansen, 1971)
12	PP	(Arroyo et al., 1982)
13	PP	(Arroyo et al., 1982)
14	PP	(Arroyo et al., 1982)
15	PP	(Barrett and Helenurm, 1987)
16	PP	(Clements and Long, 1923)
17	PP	(Dicks et al., 2002)
18	PP	(Dicks et al., 2002)
19	PP	(Dupont et al., 2003)
20	PP	(Elberling and Olesen, 1999)
21	PP	Elberling, H. & Olesen, J. M. (unpubl.).
22	PP	(Olesen and Jordano, 2002)
23	PP	Olesen, J. M. (unpubl.).
24	PP	(Ollerton et al., 2003)
25	PP	(Hocking, 1968)
26	PP	(Petanidou, 1991)
27	PP	(Herrera, 1988)
28	PP	(Memmott, 1999)
29	PP	Olesen, J. M. (unpubl.).
30	PP	(Inouye and Pyke, 1988)

Table S3.1, continued.

Network	Network type	Source
31	PP	(Kevan, 1970)
32	PP	(Kato et al., 1990)
33	PP	(Medan et al., 2002)
34	PP	(Medan et al., 2002)
35	PP	(Mosquin and Martin, 1967)
36	PP	(Motten, 1982)
37	PP	(McMullen, 1993)
38	PP	(Primack, 1983)
39	PP	(Primack, 1983)
40	PP	(Primack, 1983)
41	PP	(Ramirez and Brito, 1992)
42	PP	(Ramirez, 1989)
43	PP	(Schemske et al., 1978)
44	PP	(Small, 1976)
45	PP	(Smith-Ramírez et al., 2005)
46	PP	(Percival, 1974)
47	PP	Olesen, J. M. (unpubl.).
48	PP	(Montero, 2005)
49	PP	(Montero, 2005)
49	PP	(Stald, 2003)
50	PP	(Ingversen, 2006)
51	PP	(Ingversen, 2006)
52	PP	(Philipp et al., 2006)
53	PP	(Montero, 2005)
54	PP	(Kato, 2000)
55	PP	(Lundgren and Olesen, 2005)
56	PP	(Bundgaard, 2003)
57	PP	(Dupont et al., 2009)
58	PP	(Dupont et al., 2009)
59	PP	(Bek, 2006)
60	PP	(Stald, 2003)
61	PP	(Vázquez, D. P., 2002)
62	PP	(Witt, 1998)
63	PP	(Yamazaki and Kato, 2003)
64	PP	(Kakutani et al., 1990)
65	PP	(Kato and Miura, 1996)
66	PP	(Kato et al., 1993)
67	PP	(Inoue et al., 1990)
68	PP	(Bartomeus et al., 2008)
69	PP	(Bezerra et al., 2009)

S3.2. Supplemental within-network results

The frequency of the no-overlap pattern increased significantly with decreasing phylogenetic distance in both pollination and herbivory networks ($\beta_{\delta+\delta\rho}$ = 11.21; P<0.001 and β_{δ} = 26.96; P=0.006). In both cases, this indicates that overlap of interaction partners decreases with increasing phylogenetic distance. This is the same trend as observed in the other patterns of overlap (see *Results, Chapter 3*).

S3.3. Supplemental within-family results

Families associated with the largest changes in overlap

The largest decreases in total overlap with increasing phylogenetic distance were associated with *Apocynaceae*, *Lacistemataceae*, *Olacaceae*, *Sapotaceae*, and *Chrysobalanaceae*. The largest increases in the no overlap pattern with increasing phyloganaetic overlap were associated with *Apocynaceae*, *Begoniaceae*, *Gleicheniaceae*, *Myricaceae*, and *Siparunaceae*. The largest increases in total overlap with increasing phylogenetic distance were associated with *Malpighiaceae*, *Plumbaginaceae*, *Surianaceae*, *Cactaceae*, and *Goodeniaceae*. The largest decreases in the no overlap pattern with increasing phylogenetic distance were associated with *Malpighiaceae*, *Surianaceae*, *Plumbaginaceae*, *Goodeniaceae*, and *Cactaceae*. These orders were similar to those for the partial overlap pattern (see *Results, Chapter 3*).

Expanded figure 14

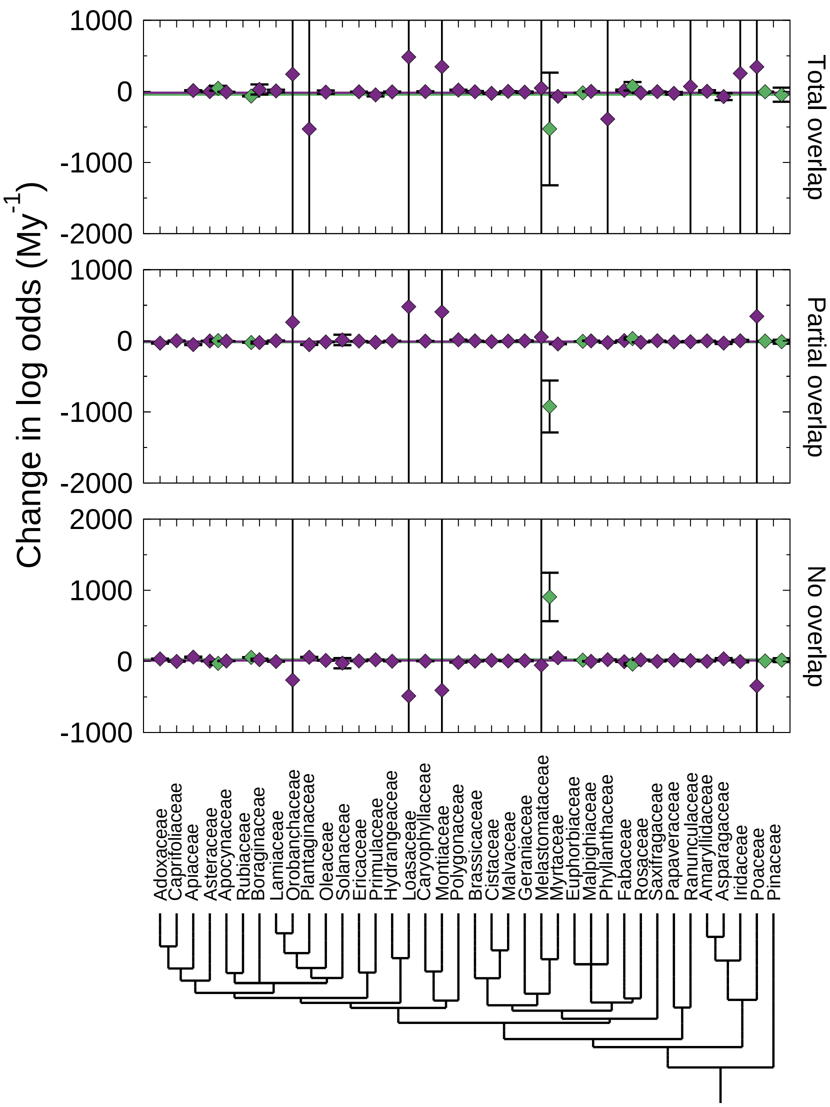

Figure S3.1: Change in log odds of observing different patterns of pairwise niche overlap per million years of divergence time between a pair of plants in 38 separate plant families. Families in pollination networks are indicated by dark purple diamonds while families in herbivory networks are indicated by pale green circles. Note that changes in log odds are analogous to the slopes of the regression lines from Eq.2-3 (*Results, Chapter 3*) in logit-transformed space and represent the change in the probability of observing a pattern of overlap per million years of divergence time. We also show the slope of the relationship between the log-odds of observing each overlap pattern and phylogenetic distance across all plant families in herbivory (pale, green horizontal line) and pollination (dark, purple horizontal line) networks. The phylogenetic tree below the plots indicates the relatedness between plant families. Error bars represent 95% confidence intervals.

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Supporting information S4

Supporting information for Chapter 4:

Are high-arctic plant-pollinator networks unravelling in a warming climate?

Alyssa R. Cirtwill, Tomas Roslin, Claus Rasmussen, Jens Mogens Olesen, & Daniel B. Stouffer

S4.1 - Simulated dates of first interaction

Methods

To test whether our results are vulnerable to small errors in the estimation of species' dates of first interaction, we repeated all analyses that included date of first interaction as a predictor using 1000 simulated dates of first interaction for each species. The simulations were designed to give reasonable dates of first interaction based on the distribution of observed interactions for each species, and for the community as a whole. We obtained separate sets of dates for each species for each year. Within each year, we also simulated dates for plants and insects independently. As we did not want to alter the number of interactions in the networks, and as our results did not vary depending on the method used to account for the tentatively-dated species (see *S4.2-3*), we used the single best-guess dates when creating simulated datasets.

For each species type (plant or insect) in each year (1996, 1997, 2010, or 2011), we first fit a linear regression of interaction dates against species identity, with no intercept. This gave us the mean values of the normal distributions that best described the observed interactions for each species. In order to account for the varying amounts of information we had about different species, we weighted the regression using the number of observed interactions for each species. Thus, the confidence intervals of the fitted means were narrower for species with many observed interactions and wider for species with few observed interactions.

To obtain simulated dates of first interactions, we simulated 1000 sets of interaction dates using the linear regression described above, and then took the earliest date for each species as its simulated date of first interaction. Note that simulating interactions in this way generated datasets of the same size and structure as the observed dataset, such that species with only one observed interaction also had only one simulated interaction. We then used these sets of simulated earliest interactions to repeat our tests for Hypotheses 5, 6, and 7 (i.e., that species active at different times of the year will have different roles, that their roles will change in different ways between decades, and that the magnitude of change in species' roles will depend on the magnitude and direction of change in their dates of first interaction). We present the results of these repeated analyses below.

Hypothesis 5

Species with different dates of interaction had different roles in both the observed data (see main text) and in the majority of simulated datasets. Specifically, plants with different dates of first interaction had significantly different roles in 602/1000 simulated datasets for the yearly networks and 765/1000 datasets for the monthly networks. Pollinators, on the other hand, had significantly different roles in 655/1000 simulated datasets for the yearly networks and 659/1000 datasets for the monthly networks. In general, however, the results for the observed data were significantly more extreme than the simulated datasets. For plants, this was true in the yearly networks, while for the monthly networks the result from the observed dataset were similar to those from the simulated datasets (p=0.004 for the yearly networks and p=0.354 for the monthly networks; Fig. S4.1). For insects, on the other hand, the F-statistic from the observed dataset were more extreme than those obtained from the simulated datasets in both monthly and yearly datasets (p<0.001 for both monthly and yearly webs). This indicates that our results for pollinators were more susceptible to observation error in dates of first interaction than were our results for the plants. However, as the majority of our simulation results remained significant we can still be confident that different dates of first interaction are indeed associated with different roles. This is also the case for plants' roles in yearly webs, although we can be more confident in this case because the values for the observed and simulated datasets were more similar.

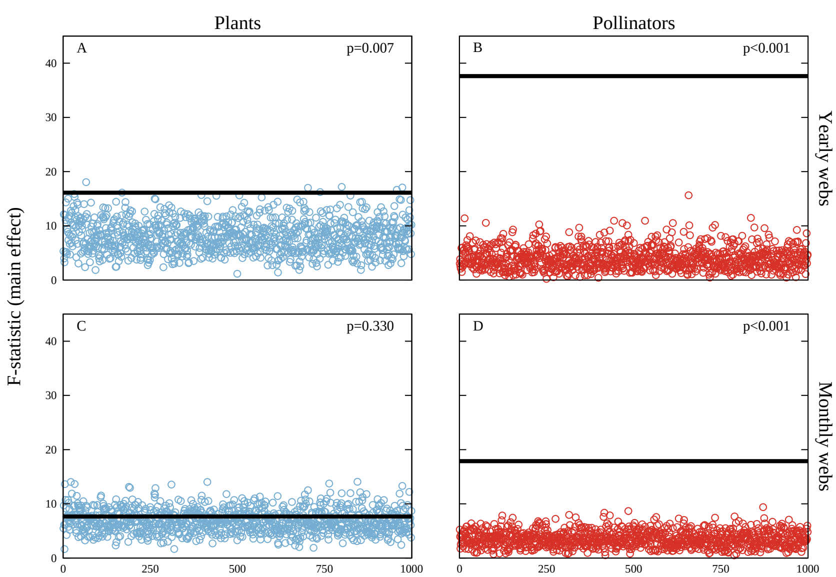

Figure S4.1: Values of the F-statistics for the main effect of date of first interaction in a PERMANOVA test of species' roles against date of first interaction, decade, and their interaction (Hypothesis 5). In each panel we show the F-statistics for 1000 simulated dates of first interaction (circles) as well as the value of the F-statistic for the observed dataset (horizontal line). We also give the probability that the F-statistic from the observed dataset was significantly larger than the F-statistics from the simulated datasets.

In addition to testing whether species with different dates of first interaction had different structural roles, we repeated our CAP analysis testing whether this difference could be explained by changes in network structure. As with the PERMANOVA described above, the relationship between species' roles and their dates of first interaction remained significant after accounting for network structure in most of the simulated datasets (984/1000 for plants' roles in yearly networks, 996/1000 for plants' roles in monthly networks, 573/1000 for pollinators' roles in yearly networks, and 819/1000 for pollinators' role in monthly networks). Our results for plants' roles were similar in the observed and simulated datasets for both the yearly and monthly networks (p=0.075 and p=0.546, respectively; Fig. S4.2). As with the PERMANOVA results, this suggests that our results for plants' roles are relatively robust to noise in our estimates of first date of interaction. The F-statistics we observed for insects' roles, however, were significantly greater than those we obtained from the simulated datasets (p<0.001 for both network types). This suggests that our results for insects' roles are much more sensitive to potential errors in estimates of species' dates of first interaction. Nevertheless, as the majority of simulated datasets also gave significant results, we remain confident in our results.

Hypothesis 6

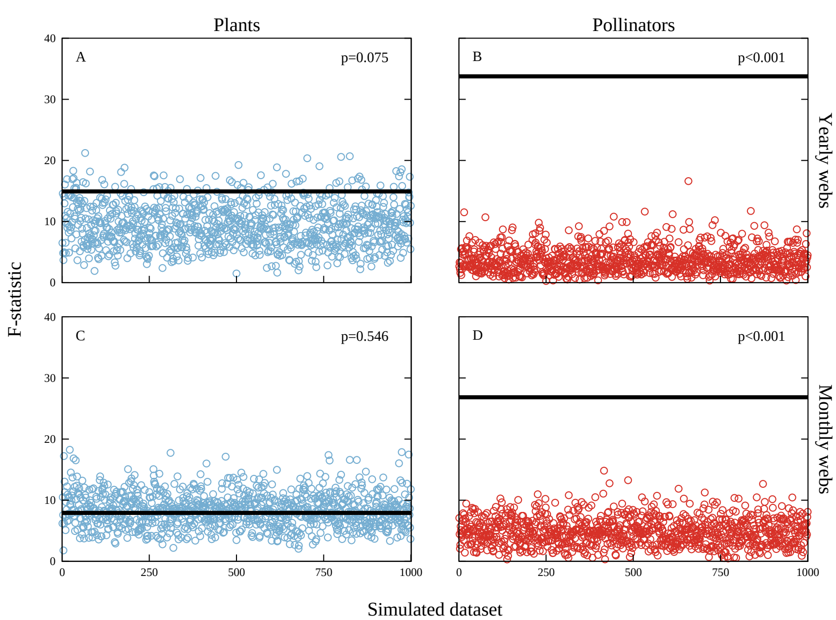

Figure S4.2: Value of the F-statistic for a CAP analysis of species' roles against date of first interaction, constrained by network structure (Hypothesis 5). In each panel we show the F-statistics for 1000 datasets with simulated dates of first interaction (circles) as well as the F-statistic for the observed dataset (horizontal line). In each panel, we also give the probability that the F-statistic from the observed dataset was more extreme than the values from the simulated datasets.

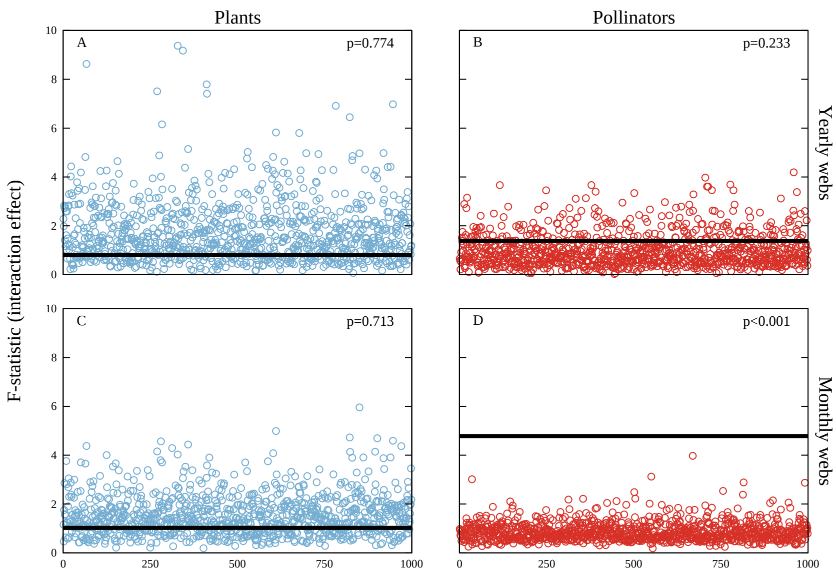

Figure S4.3: Values of the F-statistics for the interaction term in a PERMANOVA test of species' roles against date of first interaction, decade, and their interaction (Hypothesis 6). In each panel we show the F-statistics for 1000 simulated dates of first interaction (circles) as well as the value of the F-statistic for the observed dataset (horizontal line). We also give the probability that the F-statistic from the observed dataset was significantly larger than the F-statistics from the simulated datasets.

The PERMANOVA we used to test Hypothesis 5 was also used to test Hypothesis 6, that relationships between species' roles and their dates of first interaction would change between decades. Consistent with our observed result that this relationship did not change between decades for plants, this relationship was significant in only 317/1000 simulated datasets for yearly webs and 101/1000 simulated datasets for the monthly webs, and the F-statistics we obtained from the simulated datasets were not significantly different from those we found in the observed datasets in both cases (p=0.790 for the yearly webs and p=0.724 for the monthly webs; Fig. S4.3). Likewise, our results for insects' roles in yearly webs were similar for the observed and simulated datasets (p=0.220), with only 31/1000 simulated datasets showing a significant change in the relationship between species' roles and their dates of first interaction between decades. The results for simulated datasets in the monthly networks were similar, with only 20/1000 datasets showing a significant change in the relationship. This contrasts strongly with the significant result in the observed dataset. Moreover, the F-statistic we obtained from the observed data for insects' roles in monthly networks was significantly larger than the results we obtained from the simulated datasets (p<0.001). This indicates that this result may be more susceptible to errors in estimation of species' dates of first interaction.

Hypothesis 7

Finally, we compared the correlations between the magnitude of change in species' roles and the magnitude of change in dates of first interaction in the observed dataset with the correlations in the simulated datasets. As in the main text, we analysed species with advancing and retreating phenologies separately. For plants' roles in yearly webs, 98/1000 simulated datasets had significant results for species with advancing phenologies and 78/1000 had significant results for species with retreating phenologies. This is consistent with the non-significant results for the observed dataset, and indeed the observed correlations were not significantly different from those in the simulated datasets (p=0.515 for species becoming active earlier in the year and p=0.633 for species becoming active later; Fig. S4.3). This was also the case for plants' roles in monthly networks, with few simulated datasets yielding significant results (494/1000 for plants becoming active earlier and 231/1000 for plants becoming active later) and the observed correlations not significantly different from those obtained using simulated datasets (p=0.475 and p=0.549, respectively). Likewise, most of the simulated datasets yielded non-significant results for insects' roles in yearly webs (84/1000 for those active earlier and 22/1000 for those active later) and the observed correlations were similar to those from the simulated datasets (p=0.526 and p=0.278, respectively). This was also true for insects' roles in monthly networks for species becoming active earlier (67/1000 simulated datasets with significant results, p=0.191 for the observed correlation being different from those in the simulated datasets) and those becoming active later (107/1000, p=0.45).To reiterate, in all cases the correlations in our observed dataset were not significantly different from the correlations in our simulated datasets.

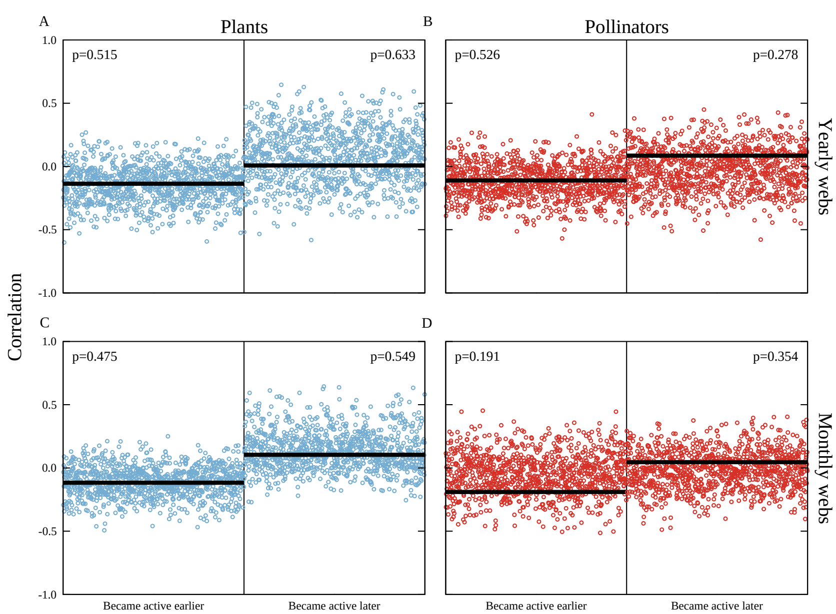

Figure S4.4: R^2 values for correlations between the change in species' roles and change in their dates of first interaction between decades (Hypothesis 7). As we expected that species with advancing and retreating phenologies might show different trends, we analysed each group separately. In each panel, we show the values for 1000 datasets using simulated dates of first interaction (circles) as well as the value from the observed dataset (horizontal lines). We also show the probability that the R^2 value from the observed dataset is more extreme than the values from the simulated datasets.

This indicates that all of these results are quite robust to mis-estimation of species' dates of first interaction.

S4.2 - Tentatively-dated observations: methods

There were 94 interactions in our dataset which could not be ascribed to a definite date. Of these, 41 were observed in 1996 and the remaining 53 were observed in 1997. The interactions involve five insect species visiting 15 plant species. *Boloria chariclea* visited all 15 plants, *Colias hecla* visited four plant species, *Limnophyes brachytomus* visited three, and *Paraphaenocladius impensus* and *Syngrapha parilis* each visited one. In addition to visiting the most plant species, *Boloria chariclea* was observed far more often than any of the other insects (79 of the 94 tentatively-dated observations).

Each interaction is associated with a range of possible dates where the plant had been observed flowering and the insect had been observed at the site. Within this range, we used the earliest date that was not associated with a definitively-labelled interaction as the best-guess date for the interaction. This date was used to include the interaction in the monthly networks described in the main text. Because of the uncertainty regarding these dates, we repeated our analyses using two other methods of assigning these interactions. First, we excluded these interactions from the monthly networks entirely. As each interaction was definitively associated with a particular year, however, we included the interactions in the yearly networks. This method underestimated the number of interactions in the 1996 and 1997 monthly networks but presented no risk of assigning an interaction incorrectly. Second, we included the interaction in all networks describing any part of the range of potential dates. This included the yearly networks, as in the other methods, and any relevant monthly networks. This method over-estimates the number of interactions in the 1996 and 1997 monthly networks, but does not exclude any of the pollination interactions that occurred. These three methods of assembling the monthly networks cover a range of conservatism and all have different attendant biases. As described below, all results were qualitatively identical regardless of the method use.

S4.3 - Tentatively-dated observations: results

Change in network structure

When tentatively-dated observations were not included in the monthly webs, change in network structure between decades was very similar to the change in network structure when tentatively-dated observations were only included on their most likely date. That is, the structure of monthly networks did not change between decades ($F_{1,10}$=2.13, p=0.091 for a PERMANOVA of monthly network structure against decade) *except* when controlling for differences between months ($F_{1,10}$=2.24, p=0.042 for a PERMANOVA of monthly network structure against decade, stratified by month). When tentatively-dated observations were included for all dates within the probable range, however, the structure of monthly networks differed between decades regardless of whether differences between months were taken into account ($F_{1,10}$=4.03, p=0.002 for a PERMANOVA of monthly network structure against decade; $F_{1,10}$=4.27, p=0.002 for a similar PERMANOVA, stratified by month). Despite this minor difference, all three methods of accounting for tentatively-dated observations agree that, if the differences between networks describing June, July, and August in different years are taken into account, network structure undoubtedly changed between the 1990's and the 2010's.

Change in species' roles

Changes in plants' and pollinators' roles in the monthly networks were similar regardless of the way in which the tentatively-dated interactions were included. Plants' roles changed between decades regardless of whether these interactions were included only in the yearly networks or for the full range of possible dates ($F_{1,227}$=2.28, p=0.017 and $F_{1,247}$=5.78, p<0.001, respectively, for a PERMANOVA of monthly roles against decade, stratified by species). This was also the case for pollinators' roles ($F_{1,458}$=13.5, p<0.001 and $F_{1,455}$=7.96, p<0.001, respectively). In all cases, these results were qualitatively identical to those presented in the main text.

The extent of change in species' roles varied between months regardless of how the tentatively-dated interactions were treated. That is, the interaction term in a PERMANOVA of species' roles against decade, month, and their interaction was significant whether the tentatively-dated observations were included only in the yearly networks or for the full range of possible dates ($F_{1,223}$=2.13, p=0.011

and $F_{1,243}$=4.65, p<0.001, respectively for plants and $F_{2,451}$=2.78, p=0.003 and $F_{2,454}$=4.85, p<0.001, respectively, for pollinators).

Effect of date of first interaction on species' roles

Our results relating species' roles to their dates of first interaction were also robust to different ways of including the tentatively-dated observations. Plants' roles initially did not appear to be related to their dates of first interaction when tentatively-dated observations were included in only the yearly networks ($F_{1,225}$=7.36, p=0.126 in a PERMANOVA of PERMANOVA of plants' roles against decade, date of first interaction, and the interaction between them). After controlling for network structure, however, plants' roles were related to their dates of first interaction, as in the *Main Text* ($F_{1,216}$=7.63, p<0.001 for a CAP of plants' roles against their date of first interaction, conditioned by network structure). When the tentatively-dated observations were included across the full range of possible dates, plants' roles varied with their dates of first interaction whether or not network structure was taken into account ($F_{1,245}$=11.1, p=0.016 for a PERMANOVA similar to that described above, and $F_{1,236}$=11.7, p<0.001 for a CAP as described above). The relationship between plants' roles and their dates of first interaction did not vary between decades regardless of how tentatively-dated interactions were included, again as in the *Main Text* ($F_{1,225}$=1.08, p=0.344 when these interactions were included in the yearly webs only and $F_{1,245}$=0.761, p=0.660 when they were included across the range of possible dates).

Pollinators' roles were likewise associated with their dates of first interaction regardless of whether the tentatively-dated interactions were included in the yearly webs only or in all possible dates ($F_{1,453}$=15.9, p=0.001 and $F_{1,456}$=20.5, p<0.001, respectively, for the main effect of date in PERMANOVAs of pollinators' monthly roles against decade, date of first interaction, and their interaction). This relationship remained significant when accounting for network structure ($F_{1,447}$=19.3, p<0.001 and $F_{1,444}$=14.791, p<0.001, respectively, in the CAPs described above). Unlike plants' roles, but consistent with the results we present in the *Main Text*, the relationship between pollinators' roles and their dates of first interaction changed between decades whether we included the tentatively-dated observations in the yearly webs only or for the full range of potential dates ($F_{1,444}$=14.8, p<0.001 and $F_{1,447}$=27.4, p<0.001, respectively, for the interaction term in the PERMANOVAs described above). As with our other results, these are identical to the results presented in the main text where tentatively-dated interactions were included only on their most probable date.

Magnitude of change in roles and change in dates of first interaction

The magnitude of change in plants' roles was not related to the magnitude of change in their dates of first interaction for species which became active earlier in the year (R^2=0.094, p=0.440 when tentatively-dated observations were included in the yearly webs only and R^2=0.014, p=0.620 when these observation were included for the full range of potential dates). For plants which became active later in the year, on the other hand, change in roles was related to change in dates of first interaction (R^2=0.107, p=0.017 and R^2=0.084, p0.034, respectively).

For pollinators, these patterns were reversed. Change in roles was related to change in dates of first interaction for species which became active earlier in 2010-2011 than in 1996-1997 (R^2=0.028, p=0.012; and R^2=0.028, p=0.020, respectively). For species which became active later in 2010-2011, this relationship was not significant (R^2<0.001, p=0.292; and R^2=0.016, p=0.310, respectively). Once again, these results are all qualitatively identical to those in the main text. This indicates that including using the best-guess dates for the tentatively-dated observations did not affect our results.

7553 ## *S4.4 - Supplemental figures*

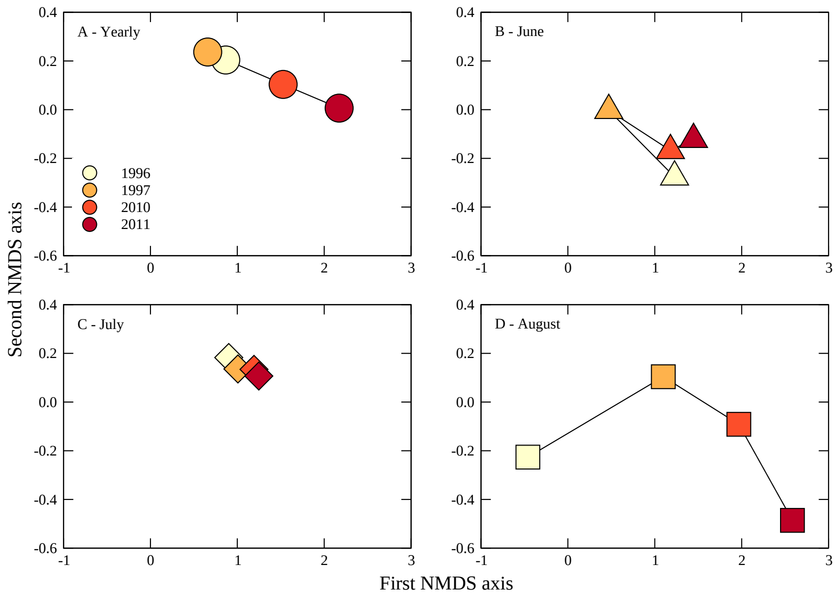

Figure S4.5: The structure of plant-pollinator networks at Zackenberg, Greenland changed between years. A) Yearly networks generally increased along the first NMDS axis and decreased slightly along the second NMDS axis. The structures of the 1996 and 1997 webs were very similar, with larger changes from 1997 onwards. The changes in the structure of monthly networks was more variable. B-D) In June, the 1996, 2010, and 2011 networks were fairly similar while the 1997 web was lower along the first NMDS axis; the July networks were very similar in all four years; and the August networks increased along the first NMDS axis in every year and showed a hump-shaped trend along the second NMDS axis. Moving from negative to positive values along the first axis represented a shift from high frequencies of motifs representing tightly-knit groups to high frequencies of more loosely-connected motifs. Moving from negative to positive values of the second NMDS axis corresponds to an increase in larger (five or six species) motifs and a decrease in smaller motifs.

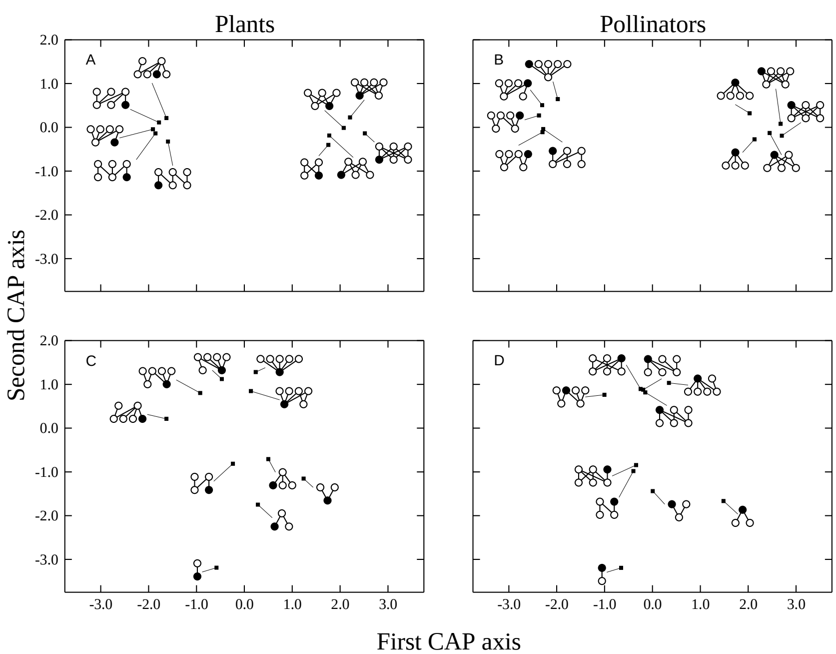

Figure S4.6: Here we show the five motifs most strongly associated with the two axes of a constrained analysis of principal coordinates (CAP) of species' roles conditioned by the overall network structure. As plants and their pollinators never occupy the same positions within motifs, we analysed the two groups separately. In both cases, however, the axes had similar interpretations. A-B) Moving from negative to positive values along the first axis represented a shift from high frequencies of positions that tend to represent specialists to high frequencies of positions that tend to represent generalists. C-D) Moving from negative to positive values along the second axis, meanwhile, represented a shift from high frequencies of positions in small motifs to high frequencies of positions in large motifs. Positions that were strongly associated with an axis are indicated in black. Small dots indicate the exact location of each position with respect to the two axes.

Supporting Information S5

Supporting information for Chapter 5:

Concomitant predation on parasites is highly variable but constrains the ways in which parasites contribute to food-web structure

Alyssa R. Cirtwill & Daniel B. Stouffer

S5.1. Additional References and Description of Food Webs

Table S5.1: Locations and original sources for food-web datasets. The Ythan web used is version 3 from Huxham et al. (1996). Following Huxham et al. (1996), species 100 in this web was removed as it is an animal with no recorded resources in the food web. This also resulted in the removal of one link $100 \rightarrow 85$ where species 100 appeared as a resource.

Site	Source	Location
Bahia	Hechinger et al. (2011)	Bahia Falsa, Baja California Mexico
Carpinteria	Hechinger et al. (2011)	Carpinteria Salt Marsh, California USA
Estero	Hechinger et al. (2011)	Estero de Punta Banda, Baja California Mexico
Fjord	Thieltges et al. (2011a)	Flensburg Fjord, Baltic Sea Germany/Denmark
Otago	Mouritsen et al. (2011)	Otago Harbour New Zealand
Sylt	Thieltges et al. (2011b)	Sylt Tidal Basin, North Sea Germany/Denmark
Ythan	Huxham et al. (1996)	Ythan Estuary, Scotland UK

Trophic groups of free-living species were defined based on the free-living webs. Top predators (T) were defined as species with prey but no predators, basal resources (B) as species with predators but no prey, and intermediate consumers (I) were all remaining species (that is species with both predators and prey). Cannibalistic species were considered to be intermediate consumers, as some individuals serve as prey to their conspecifics even if they are not prey to other species (Williams and Martinez, 2000). Parasites were defined by the authors of the original food webs, and included species ranging from apicomplexan and ciliate protozoans to nematode, trematode, and cestode worms to parasitic copepods (Dunne et al., 2013; Huxham et al., 1996; Hechinger et al., 2011; Mouritsen et al., 2011; Thieltges et al., 2011b,a). Any species with both parasitic and free-living life stages was considered a parasite.

Table S5.2: Representation of each type of species across the different food webs. Type "free-living" refers to webs with free-living species only while type "par & con" refers to "parasite" and "concomitant" webs which include parasites and free-living species. S refers to the total species richness in each web. $\%_F$, $\%_T$, $\%_I$, $\%_B$, and $\%_P$ refer to the proportion of species that are free-living, top predators, intermediate consumers, basal resources, and parasites, respectively.

Site	Type	S	$\%_F$	$\%_T$	$\%_I$	$\%_B$	$\%_P$
Bahia	free-living	119	100	7	79	14	0
Bahia	par & con	171	70	5	55	10	30
Carpinteria	free-living	107	100	5	84	11	0
Carpinteria	par & con	165	65	3	55	7	35
Estero	free-living	138	100	7	83	10	0
Estero	par & con	214	64	4	54	6	36
Flensburg	free-living	77	100	12	80	8	0
Flensburg	par & con	123	62	7	50	5	38
Otago	free-living	123	100	26	71	3	0
Otago	par & con	142	87	23	61	3	13
Sylt	free-living	126	100	21	74	5	0
Sylt	par & con	161	78	17	58	3	22
Ythan	free-living	91	100	34	62	4	0
Ythan	par & con	133	68	23	42	3	32

Table S5.3: Frequency of different types of links across the different food webs. L refers to the total number of links in each web while $F \rightarrow F$, $P \rightarrow F$, $P \rightarrow P$, $F \xrightarrow{t} P$, and $F \xrightarrow{c} P$ to the number of links describing predation among free-living species, parasitism, predation between parasites, target predation of free-living species on parasites, and concomitant predation on parasites, respectively. Note that neither $F \xrightarrow{t} P$ nor $P \rightarrow P$ links were observed in the Ythan web.

Site	Type	L	$F \rightarrow F$	$F \rightarrow P$	$P \rightarrow P$	$P \xrightarrow{t} F$	$P \xrightarrow{c} F$
Bahia	free-living	1075	1075	0	0	0	0
Bahia	parasite	2232	1075	807	165	185	0
Bahia	concomitant	3765	1075	807	165	185	1533
Carpinteria	free-living	963	963	0	0	0	0
Carpinteria	parasite	2180	963	755	166	296	0
Carpinteria	concomitant	3762	963	755	166	296	1582
Estero	free-living	1647	1647	0	0	0	0
Estero	parasite	3324	1647	835	169	673	0
Estero	concomitant	5805	1647	835	169	673	2481
Fjord	free-living	577	577	0	0	0	0
Fjord	parasite	966	577	271	40	78	0
Fjord	concomitant	1428	577	271	40	78	462
Otago	free-living	1200	1200	0	0	0	0
Otago	parasite	1481	1200	173	19	89	0
Otago	concomitant	1852	1200	173	19	89	371
Sylt	free-living	1047	1047	0	0	0	0
Sylt	parasite	1944	1047	552	69	276	0
Sylt	concomitant	3033	1047	552	69	276	1089
Ythan	free-living	416	416	0	0	0	0
Ythan	parasite	593	416	177	0	0	0
Ythan	concomitant	1268	416	177	0	0	675

S5.2. Supplemental methods: quantifying species' and links' roles

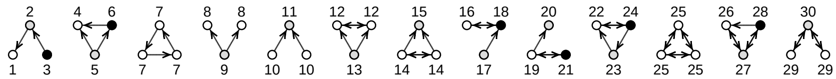

Figure S5.1: Three-species motifs with unique positions numbered.

Interactions between species are a direct consequence of the motif structure of a food web. Motifs are the set of 13 three-species subwebs describing all possible interaction patterns of three species (Milo et al., 2002; Stouffer et al., 2007, Fig. S5.1). Each motif contains one or more unique positions, indicating a unique way in which a species' interactions are organised in that motif (e.g., the top predator, intermediate consumer, and resource in a three- species food chain) (Stouffer et al., 2012). In the 13 three-species motifs, there are 30 such positions (Kashtan et al., 2004; Stouffer et al., 2012). Similarly, there are 24 unique link types connecting species (Fig. S5.2). By counting the frequency c_{ij}^{w} with which each species i in community s in web type w (i.e., free-living, parasite, or concomitant) occurs in each position j, we obtained a vector $\overrightarrow{f_{si}^{w}}$ describing the overall role of that species within its food web,

$$\overrightarrow{f_{si}^{w}} = \{c_{i1}, c_{i2}, ..., c_{i29}, c_{i30}\}_{s}^{w} . \tag{9}$$

The same process was used to determine the roles of links between species, giving a vector

$$\overrightarrow{f_{sl}^{w}} = \{c_{l1}, c_{l2}, ..., c_{l23}, c_{l24}\}_{s}^{w} . \tag{10}$$

that describes the role $\overrightarrow{f_{sl}^{w}}$ for each link l in community s in web type w.

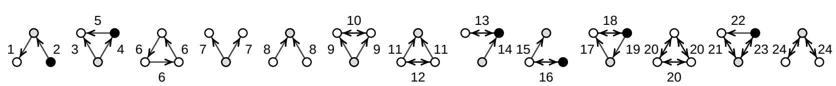

Figure S5.2: Three-species motifs with unique links numbered.

S5.3. Supplemental methods: role dispersion & diversity

As described in Chapter 5, we quantified the distribution of species' and links' roles by their role dispersion and role diversity (Fig. *20, Chapter 5*). In order to quantify role diversity, we first needed to identify subsets of species (or links) that have statistically-similar motif-based roles; that is, clusters of species (or links) that appear in the same motif positions more often than one would expect by chance. To perform a clustering of this nature, we followed a recently-proposed method that is an extension of community detection algorithms for complex networks to the case of detecting groups of nodes in bipartite networks with weighted edges (Sales-Pardo et al., 2007; Stouffer et al., 2012). Here, the bipartite network consists of each species (or link) in our dataset on one side and the different motif positions on the other. Each edge in this network is weighted by the frequency c_{sij}^{w} with which the species or link i in community s in web type w occupies position j. The clustering algorithm consists of maximising an objective function M (referred to as "modularity") that is high when nodes in the same cluster tend to occupy the same positions with similar frequencies and low otherwise (Stouffer et al., 2012).

We used a stochastic and heuristic optimisation method known as simulated annealing (Kirkpatrick et al., 1982) to cluster nodes (species or links) while maximising modularity (Sales-Pardo et al., 2007; Girvan and J., 2002). Since this procedure is not always guaranteed to find a global optimum, and since we are most interested in the expected variety of clusters per group as a proxy for role diversity, we performed this modularity maximisation 100 separate times for roles of species and links in each community. As with dispersion, we included the roles of free-living species from the "free-living" web as well as the roles of parasites from both the "parasite" and "concomitant" webs. We then calculated the weighted average number of clusters containing each type of species (or link) across the 100 modularity-maximised clusterings following

$$\hat{N}_j = \sum_i p_i N_{ij} \quad , \quad p_i = e^{M_i} / \sum_k e^{M_k} \, , \tag{11}$$

where M_i is the modularity of a given clustering i, $\sum_k e^{M_k}$ is the sum of modularities over all k clusterings, and p_i is the relative probability of obtaining a clustering i weighted by its modularity; N_{ij} is the number of clusters containing species type j in clustering i, and $\hat{N}_j$ is the weighted average of the number of clusters containing species (or link) type *j* (Sales-Pardo et al., 2007). We assume that each cluster represents a unique structural role, therefore this average number of clusters provides an estimate of the role diversity for each type of species and links.

S5.4. Supplemental results: median roles

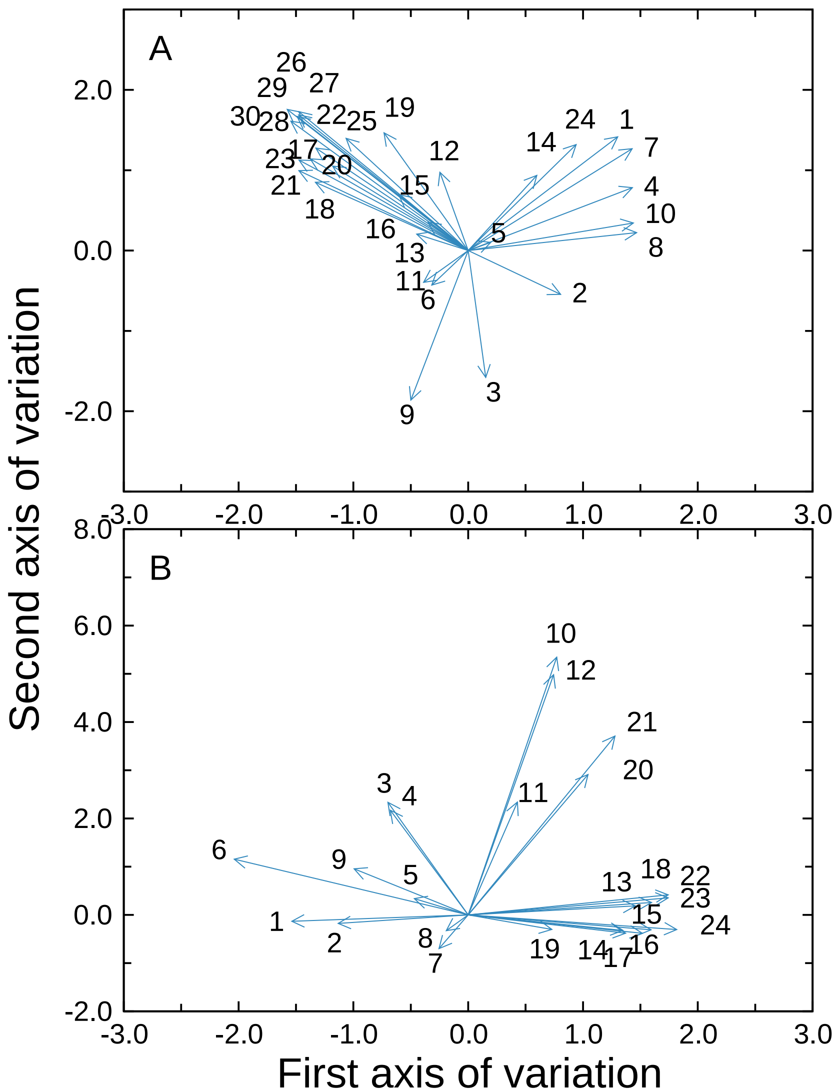

Figure S5.3: The major axes of variation for median roles demonstrated key differences in the roles of different types of species and links. **(A)** The first major axis of variation for species roles corresponded to a split between positions in motifs containing only one-way interactions and positions in motifs containing at least one two-way interaction. This axis separates the roles of parasites including concomitant predation from other types of roles (Fig. *21A, Chapter 5*). The second major axis was largely defined by positions representing the base of a three-species food chain (3) and a species with two predators which do not eat each other. These positions are most common in the roles of basal resources. **(B)** The first major axis of variation for link roles also corresponds to a split between positions in motifs that contain only one-way interactions and those in motifs containing at least one two-way interaction. Positions associated with two-way interactions were more frequent in the roles of concomitant predation links than in other role types (Fig. *21B, Chapter 5*). The second axis is largely determined by two positions representing mutual predation between species with a common prey or common predator. These positions are most common in the roles of links describing predation between parasites.

When comparing across different types of species, we found that trophic group was a significant predictor of median roles, as hypothesised ($F_{4,1432} = 218.15$, $p = 0.001$; Fig. *21A, Chapter 5*). The P roles were between those of I and T free-living species, and they slightly overlapped with each. The P_c roles, in contrast, were distinct from all other role types. They were separated from T, I, and P roles along the first correspondence analysis axis (which accounted for 64.9% of total variance in species roles) and separated from B roles along the second correspondence analysis axis (which explained 13.0% of total variance).

The first axis corresponds mainly to a split between positions in motifs containing only one-way interactions and positions in motifs with at least one two-way interaction (Fig. S5.3A). T, I, and P roles are associated with a greater frequency of one-way motifs, while P_c roles are associated with a greater frequency of two-way motifs. The second axis was largely defined by the frequencies of positions 3 and 9 (Fig. S5.2). Position 3 represents the base of a three-species food chain, while position 9 represents a species which is preyed upon by two other species (apparent competitors). These positions are more frequent in B roles and less frequent in other types of roles.

When comparing different types of links, we found that link type significantly predicted median roles ($F_{4,20908} = 1018.75$, $p < 0.001$; Fig. *21B, Chapter 5*). There was a great deal of overlap between the median roles of F$\rightarrow$P and F$\rightarrow$F links while the median roles of P$\rightarrow$P links were highly variable across communities. In general, the roles of P$\xrightarrow{c}$F and P$\rightarrow$P links showed more variation along the first principal-component axis (which accounted for 60.7% of total variance in link roles) while the roles of F$\rightarrow$F links, F$\rightarrow$P links, and P$\xrightarrow{t}$F links showed more variation along the second principal-component axis (which accounted for 15.2% of total variance).

As with species roles, the first correspondence axis corresponds to a split between one-way interactions and two-way interactions (Fig. S5.3B). Two-way interaction positions were more frequent in the roles of concomitant predation links and less frequent in other groups. The second axis corresponds to mainly to link positions 10 and 12, which represent species with a common prey that consume each other and species which consume each other and have a common predator, respectively (Fig. S5.3). These link positions are most common in links describing predation among parasites.

S5.5. Supplemental results: species roles

Dispersion

We determined the overall relationship between species-richness and role dispersion using the model

$$\sigma_{gs} = \beta_1 B_g + \beta_2 I + g + \beta_3 T_g + \beta_4 P_g + \beta_5 P_{\mathrm{cg}} + \beta_6 N_{gs} + \beta_6 P_g N_{gs}\,. \quad (12)$$

where σ_{gs} is the dispersion of group g (B, I, T, P, or $\mathrm{P_c}$) in community s (e.g., Ythan), B_g, I_g, T_g, P_g, and P_{cg} are dummy variables that equal 1 if g is the corresponding group type (i.e., B_g=1 if g represents the roles of basal resources), N_{gs} is the number of species N in group g at community s, and $P_g N_{gs}$ represents the number of species N in group g at community s if g represents the roles of parasites without concomitant predation links.

We then removed the non-significant overall effect of species richness (Table S5.4), leaving the model,

$$\sigma_{gs} = \beta_1 B_g + \beta_2 I_g + \beta_3 T_g + \beta_4 P_g + \beta_5 P_{\mathrm{cg}} + \beta_6 P_g N_{gs}\,, \quad (13)$$

which was used to compare the dispersions of B, I, T, and $\mathrm{P_c}$ roles as well as the slope of P role dispersion over species richness.

Table S5.4: Standardised effects, t-values, and p-values for all terms included in models 1 and 2, as well as the F-statistic, degrees of freedom, and p-value of each model overall

	Model 1			Model 2		
Parameter	Effect	t-value	p-value	Effect	t-value	p-value
B	0.251	11.903	<0.001	0.261	12.703	<0.001
I	0.255	3.961	<0.001	0.352	17.133	<0.001
T	0.213	9.050	<0.001	0.233	11.344	<0.001
P	0.189	3.157	0.004	0.189	3.081	0.005
P_{c}	0.268	6.825	<0.001	0.320	15.611	<0.001
N_{gs}	0.001	1.563	0.129		NA	
$P_g N_{gs}$	0.002	1.128	0.269	0.003	2.195	0.036
F-statistic		160.6			178.1	
Degrees of freedom		7, 28			6, 29	
Overall p-value		<2.2e-16			<2.2e-16	

Table S5.5: Standardised effects, z-values, and p-values for all terms included in models 3 and 4, as well as the AIC and degrees of freedom of each model overall

	Model 3			Model 4		
Parameter	Effect	t-value	p-value	Effect	t-value	p-value
Intercept		NA		0.189	0.802	0.422
B	0.291	0.901	0.368		NA	
I	0.566	0.892	0.372		NA	
T	-0.128	-0.320	0.749		NA	
P	0.617	1.407	0.159		NA	
P_c	1.558	4.081	<0.001	1.151	5.632	<0.001
N	0.007	1.000	0.317	0.012	2.968	0.003
AIC		122.56			108.83	
Degrees of freedom		26			29	

Diversity

We tested the effect of species richness on role diversity using the model,

$$\delta_{gs} = \beta_1 B_g + \beta_2 I_g + \beta_3 T_g + \beta_4 P_g + \beta_5 P_{\mathrm{cg}} + \beta_6 N_{gs}\,, \qquad (14)$$

where δ_{gs} is the role diversity of trophic group g in community s and all other symbols are as in the dispersion models above. Only $\mathrm{P_c}$ roles had a diversity significantly different from zero and there was no significant effect of species richness. This model was also used in the Tukey's HSD test of mean diversities across groups, as the reduced model used to establish the mean diversity of $\mathrm{P_c}$ roles,

$$\delta_{gs} = \beta_0 + \beta_1 P_{\mathrm{cg}} + \beta_2 N_{gs}\,, \qquad (15)$$

did not include intercepts for other role types (Table S5.5).

S5.6. Link roles

Dispersion

We examined the effect of link richness on the dispersion of link roles using the model,

$$\sigma_{ls} = \beta_1 F \rightarrow F_l + \beta_2 F \rightarrow P_l + \beta_3 P \xrightarrow{t} F_l + \beta_4 P \xrightarrow{c} F + \beta_5 P \rightarrow P_l + \beta_6 N_{ls} + \beta_7 P \rightarrow P_l N_{ls}\,, \quad (16)$$

where σ_{ls} is the dispersion of the roles of link type l in community s, $F \rightarrow F_l$, $F \rightarrow P_l$, $P \xrightarrow{t} F_l$, $P \xrightarrow{c} F_l$, and $P \rightarrow P_l$ are dummy variables that are equal to 1 if link type l is the relevant type (i.e., $F \rightarrow F_l$=1 for $F \rightarrow F$ links) and 0 otherwise, N_{ls} is the number of links of type l in community s, and $P \rightarrow P_l N_{ls}$ is an additional effect of link richness specific to $P \rightarrow P$ roles, only the model above which includes the interaction between link richness and $P \rightarrow P$ roles showed any significant effect of link richness on link role dispersion. This model was used to conclude that link richness does not affect the dispersion of $F \rightarrow F_l$, $F \rightarrow P_l$, $P \xrightarrow{t} F_l$, and $P \xrightarrow{c} F_l$ roles.

We then used the reduced model,

$$\sigma_{ls} = \beta_1 F \rightarrow F_l + \beta_2 F \rightarrow P_l + \beta_3 P \xrightarrow{t} F_l + \beta_4 P \xrightarrow{c} F_l + \beta_5 P \rightarrow P_l + \beta_7 P \rightarrow P_l N_{ls}\,, \quad (17)$$

which includes an effect of link richness for $P \rightarrow P$ roles only, to calculate the confidence intervals in Fig. *23*, *Chapter 5*). The best parameter estimates returned by the two models were very similar (Table S5.6).

Diversity

Finally, we determined that there was no effect of link richness on link role diversity using the model

$$N_{ls} = \beta_1 F \rightarrow F_l + \beta_2 F \rightarrow P_l + \beta_3 P \xrightarrow{t} F_l + \beta_4 P \xrightarrow{c} F_l + \beta_5 P \rightarrow P_l + \beta_7 N_{ls}\,, \quad (18)$$

where N_{ls} is the role diversity for link type l in community s and all other symbols are as above. We then used the model

Table S5.6: Standardised effects, t-values, and p-values for all terms included in models 5 and 6, as well as the F-statistic, degrees of freedom, and p-value of each model overall.

	Model 5			Model 6		
Parameter	Effect	t-value	p-value	Effect	t-value	p-value
$F \rightarrow F_l$	0.345	12.066	<0.001	0.359	19.342	<0.001
$F \rightarrow P_l$	0.295	13.524	<0.001	0.302	16.282	<0.001
$P \xrightarrow{t} F_l$	0.264	12.504	<0.001	0.267	13.338	<0.001
$P \xrightarrow{c} F_l$	0.450	14.179	<0.001	0.466	25.095	<0.001
$P \rightarrow P_l$	0.262	6.709	<0.001	0.262	6.783	<0.001
N_{ls}	<0.001	0.640	0.528		NA	
$P \rightarrow P_l N_{ls}$	0.001	4.095	<0.001	0.001	4.195	<0.001
F-statistic		260.3			310.4	
Degrees of freedom		7, 26			6, 27	
Overall p-value		<2.2e-16			<2.2e-16	

$$\delta_{ls} = \beta_1 F \rightarrow F_l + \beta_2 F \rightarrow P_l + \beta_3 P \xrightarrow{t} F_l + \beta_4 P \xrightarrow{c} F_l + \beta_5 P \rightarrow P_l \,, \quad (19)$$

to generate confidence intervals in Fig. S5.4. Although the estimated diversities for each link type differed between models (Table S5.7), the standard errors on these estimates were large, such that different types of links did not have significantly different role diversities.

Table S5.7: Standardised effects, z-values, and p-values for all terms included in models 7 and 8, as well as the AIC and degrees of freedom of each model overall.

	Model 7			Model 8		
Parameter	Effect	t-value	p-value	Effect	t-value	p-value
$F \rightarrow F_l$	12.147	2.676	0.013	8.616	2.886	0.007
$F \rightarrow P_l$	9.022	3.465	0.015	7.201	2.412	0.023
$P \xrightarrow{t} F_l$	9.483	3.350	0.009	8.533	2.646	0.013
$P \xrightarrow{c} F_l$	14.730	2.929	0.007	10.553	3.535	0.001
$P \rightarrow P_l$	7.507	2.316	0.028	7.133	2.212	0.035
N_{ls}	-0.004	-1.032	0.311		NA	
AIC		237.35			236.63	
Degrees of freedom		27			28	

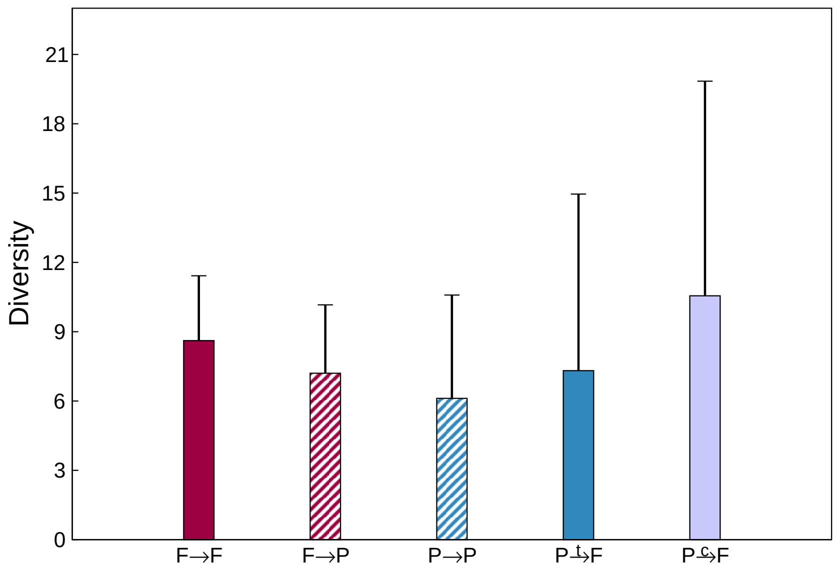

Figure S5.4: Diversity of unique roles was not related to the number of links in a community for any link type. Diversity of unique roles did not differ across link types.

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Supporting Information S6

Supporting information for Chapter 6:

Taking the scenic route: trophic transmission of parasites and the properties of links along which they travel.

Alyssa R. Cirtwill, Clement Lagrue, Robert Poulin, & Daniel B. Stouffer

S6.1: Detailed methods for data collection

Study lakes and sampling sites

Detailed field data on food web composition and structure, including parasites, was obtained from four lake ecosystems. Based on existing knowledge and accessibility, Lake Hayes, Lake Tuakitoto, Lake Waihola, and Tomahawk Lagoon (South Island, New Zealand) were selected to provide a variety of lake types (size, depth, altitude; Table S6.1) and freshwater communities (coastal versus alpine, oligotrophic versus eutrophic, tidal or not, etc.). Within each lake, 4 sampling sites were selected along the littoral zone. Site selection was partly restricted by accessibility and sampling permit specification (New Zealand Department of Conservation permit OT-34204-RES and Fish and Game New Zealand permit to capture fish for research purposes), but was ultimately made to represent all habitat types (substrate, macrophytes, riparian vegetation, etc.) present within each lake. Sampling sites consisted of 225m^2 square areas (15m $\times$ 15m) with one side of the square following the lake shore line (Figure S6.1). Distances between sampling sites varied within and among lakes according to lake size and shape as well as sampling site distribution (Table S6.1; Figure S6.1). The four lakes were sampled in early spring, mid-summer, and late autumn (austral seasons: September 2012, January and May 2013). In each lake and in each season (4 lakes $\times$ 3 seasons = 12 full sets of samples), fish, benthic and demersal invertebrates, plankton, periphyton, and macrophytes were sampled in each sampling site to determine their local species composition, density and/or biomass as well as that of their parasites, and potential temporal and spatial variability within and among lakes. In all cases, we averaged values across the four sites within a lake and sampling period prior to any analysis.

Table S6.1: Geographical locations and characteristics of the four study lakes (South Island of New Zealand), and distance between sampling sites (straight lines).

Lake	GPS coordinates	Surface area (km^2)	Depth (m)		Altitude (m)	Dist. between sites (m)		
			Mean	Max		Min	Mean	Max
Hayes	44°58′59.4"S 168°48′19.8"E	2.76	3.1	33	329	314	1190	2250
Tuakitoto	46°13′42.5"S 169°49′29.2"E	1.32	1.0	3	15	417	794	1590
Waihola	46°01′14.1"S 170°05′05.8"E	6.35	1.3	2	4	1330	1620	2020
Tomahawk Lagoon	45°54′06.0"S 170°33′02.2"E	0.10	1.0	1	15	124	253	438

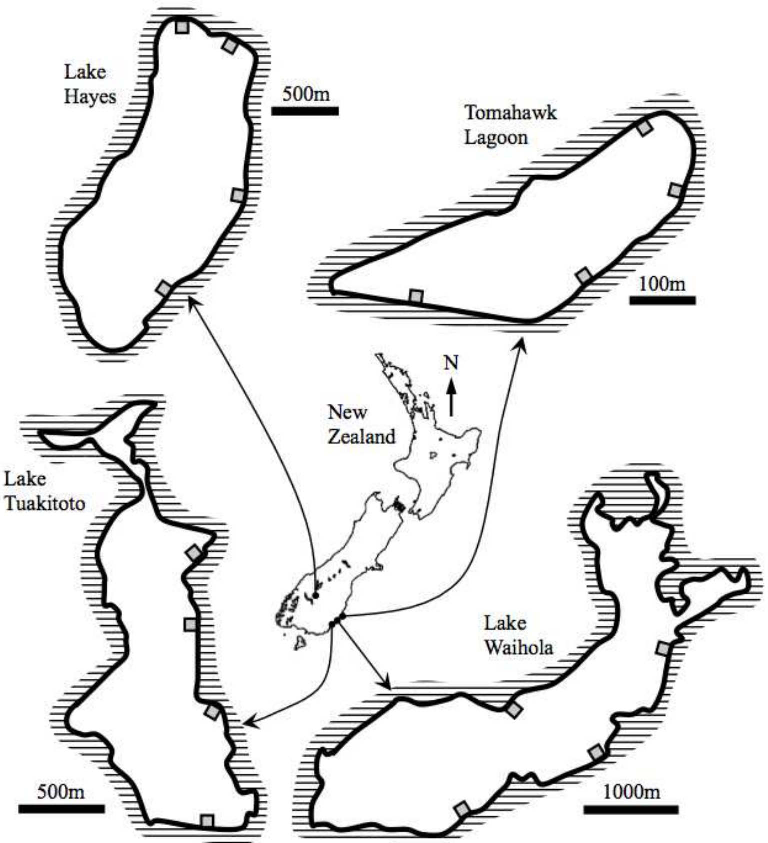

Figure S6.1: Location, size (see scale bars) and shape of the four study lakes on the South Island of New Zealand. The position of the 4 sampling sites per lake is indicated by shaded squares (not drawn to scale).

Field sampling

Fish

Fish were sampled once per season at each sampling site in each lake (1 sample $\times$ 4 sites $\times$ 3 seasons = 12 replicates per lake). We used a combination of fish-catching gear types following a standardised protocol so that samples represented accurately fish diversity and density (Hayes, 1989). First, two fyke nets and ten minnow traps were set in the evening. Fyke nets were positioned perpendicularly to the shore at either edge of the sampling site (i.e., 15m apart) to stop and capture fish swimming in and out of the focal 225m^2 area. Fyke nets consist of a cylinder of netting (2m length, 15mm mesh size) wrapped around a series of hoops to create a trap. Fish enter through the mouth of the trap and are retained by a series of funnel-shaped constrictions. One leader (or wing) is attached to the mouth and used to direct fish into the fyke net. The leader (3m length, 50cm height, 15mm mesh size) has a float-line at the top and lead-line at the bottom to keep it upright in the water and in close contact with the substrate. To prevent fish from swimming around it, the end of the leader was securely anchored to the lake shore. Along with the two fyke nets, 10 minnow traps were set overnight in each sampling site. Traps were set diagonally across the sampling area at regular intervals (i.e., ≈1.7m apart). Minnow traps are small fish traps that typically consist of two funnel-shaped entrances (25mm entrance diameter) at either end of a mesh box (40 × 25 × 25cm, 2mm mesh size). Fyke nets and minnow traps were set during the night, when fish are more active, as they are passive sampling methods relying on fish to willingly encounter and enter traps (Hubert, 1996). The next day, all trapped fish were recovered from the nets and a subsample of fish from each species was set aside for later dissection. Remaining individuals were identified to species, counted and measured to the nearest mm (fork length). These fish were then released at least a hundred meters away from the sampling site.

Fish sampling was then complemented using two 15m long multi-mesh gillnets. Gillnets were benthic weighted sets with top floats, 1.5m high and comprised 3 panels of 25, 38 and 56mm meshes, each 5m long. Nets were set 15m apart similarly to fyke nets, perpendicularly to the shore line and anchored to the lake shore on the edge of the 225m^2 sampled area with the finer mesh panel closer to shore on one side and further from shore on the other. Gillnets covered the whole water column in all cases and were checked every 15 min for an hour. Fish caught in the nets were removed immediately to avoid excessive accumulation and potential visual deterrence to incoming fish (Lagrue et al., 2011). Fish caught in fyke nets and gillnets were either entering or exiting the sampling site and thus considered as site “users/occupants". All fish were identified, counted, and measured. Again, a subsample was kept for later dissection and the remaining fish released away from the sampling site.

Finally, fish sampling was completed using a standard, fine-mesh purse seine net. As an active sampling method, seine netting captures small and/or sedentary (i.e., resident) fish that are not captured by passive gear like fyke nets or gillnets (Thorogood, 1986). The seine net was 20m long and 1.5m high (5mm mesh size), thus covering the whole water column, and dragged by two people across the whole sampling area, catching virtually all small, sedentary fish remaining in the 225m^2 area. A final subsample of fish was kept for dissections and all other fish captured in the seine net were identified, counted, measured, and immediately released. All fish set aside for later dissection were killed immediately following University of Otago

Animal Ethics Committee guidelines (permit ET 10/12) to inhibit the digestion process and stored on ice to preserve internal tissues, stomach contents, and parasites for future identification, counts, and other measures.

Plankton

Four plankton samples were taken per site and per season in each lake (4 samples × 4 sites × 3 seasons = 48 replicates per lake). Sampling was done at night when planktonic organisms migrate up from the shelter of the substrate to the top water layers of the lake (Iwasa, 1982; Haney, 1988; Rhode et al., 2001). Samples were taken using plankton net tows. The net used was a conical device (25cm mouth diameter) made of fine nylon mesh (90μm mesh size) pulled through the water for a set distance. Since we sampled the littoral zone of shallow lakes, water depth was always less than a meter. We thus used a three meter horizontal pull repeated four times within each sampling area (i.e., four samples per site). Samples were distributed haphazardly across the 225m^2 area. Animals captured at the bottom of the net were rinsed into a storage jar and fixed in 70% ethanol for later identification and count. The amount of water from which zooplankton are removed was estimated as length of tow (3m) times mouth diameter of the net (25cm). Plankton density and biomass could thus be later determined using the sample count, volume of water filtered, and water depth at the sampling site.

Demersal and benthic invertebrates

Six demersal and six benthic invertebrate samples were taken per site and per season in each lake (6 samples × 4 sites × 3 seasons = 72 replicates per lake for each sample type). Benthic sampling was done using a standard Surber sampler net with a 0.1m^2 horizontal metal frame (0.33 × 0.3m) fitted with a 250μm mesh collecting net (Surber, 1937; Fenchel, 2011). Samples were taken by embedding the Surber's metal frame into the lake bottom. Substrate and macrophytes enclosed within the frame were manually scooped up into the net to a depth of 5cm so that animals living on or within (hyporheic habitat) the substrate were captured into the net. Demersal invertebrates living on or near the substrate but either too fast or too rare to be captured in Surber nets were sampled using a rectangular dip net (i.e., a 30cm wide and 22cm high frame fitted with a 250μm mesh net and attached to a long pole). Each demersal sample consisted of a fast, two meter long sweep of the net along the lake bottom without dredging the substrate. Again, the 12 samples (6 benthic and 6 demersal) were distributed haphazardly across the 225m^2 sampling area so that none overlapped. Substrate, wood debris, and macrophytes contained in the net (Surber or dip net) were placed into a bucket of water and stirred, shaken, and/or scrubbed to dislodge attached invertebrates, and then transferred into another bucket. Animals and substrate remaining in the first bucket were transferred onto a sieve (250μm mesh size) so fine sediment could be rinsed off. Samples were then stored individually in jars filled with 70% ethanol for later sorting, identification, count, and measurement of invertebrates. Benthic and demersal invertebrate density and biomass were then determined using sample counts and sampling surface area.

Periphyton

Periphyton growing on hard substrate (rocks, gravels) was brushed off rocks with a toothbrush and rinsed with lake water into a container. We used a 3.9cm diameter PVC pipe as a template to standardise sampling surface (11.9cm^2; Hughes et al., 2012). Periphyton from soft sediment bottom (sand or mud) was sampled from the top 5mm layer of sediments. The top half of a Petri dish (9cm in diameter, 63.6cm^2 sampling surface) was pushed into the lake bottom sediment and a small spatula was slipped under, sealing the sample inside the Petri dish. Then the sample was lifted and rinsed with lake water into a container. Five samples of periphyton, distributed haphazardly across the 225m^2 area, were taken per sampling site. The number of periphyton samples from soft and hard substrate parts of each sampling site was representative of the relative proportion of each substrate type within each sampling area. Samples were preserved in Lugol's solution and stored in the dark for later identification and count (Wood et al., 2012).

Macrophytes

Macrophytes recovered in benthic invertebrate samples were used to examine macrophyte diversity and abundance within sampling sites. During benthic sampling, macrophytes transferred into Surber nets with substrate and invertebrates were recovered, rinsed to dislodge invertebrates and wash off all sediment, and bagged into zip-lock bags. Macrophyte samples were frozen for later sorting, identification and biomass assessment.

Birds

Birds could not be sampled for dissections (permission was not granted by the New Zealand Department of Conservation). However, species composition and relative species abundances of the bird communities foraging at each sampling site of each lake and during each season were assessed by visual counts carried out from shore with binoculars. Once per site and per season, birds present around each sampling area were identified to species (Heather and Robertson, 1996). Birds were observed over a one hour period and every bird present or passing through a 200m radius zone centred on the sampling site was counted. Given the small size of Tomahawk Lagoon, all birds present on the lake were identified and counted. Note that bird counts were done during the day and did not account for highly secretive and/or nocturnal bird species like the Australasian bittern (*Botaurus poiciloptilus*) or marsh crake (*Porzana pusilla*). However, these birds are rare and represent a negligible fraction of the bird populations in our study lakes.

Laboratory analyses

Fish

In the laboratory, fish were identified to species, measured to the nearest mm (fork length), weighed to the nearest 0.01g and then dissected. Their gastrointestinal tract, from esophagus to anus, and all internal organs (heart, liver, gall bladder, gonads, swim bladder, etc.) were removed and preserved in 70% ethanol for later diet and parasite analyses. Fish bodies were frozen individually.

All fish bodies were later examined for parasites. The head, gills, eyes, brain, and spine of each fish were examined under a dissecting microscope using fine forceps to pull apart fish tissues to obtain an accurate overall parasite count for each fish. Soft tissues (muscle and skin) were removed from the spine, crushed between two glass plates, and examined by transparency under a dissecting microscope to identify and count parasites. Internal organs and gastrointestinal tract were first rinsed in water to wash off the ethanol. The digestive tract was then separated from other organs. Liver, swim bladder, gall bladder, gonads, and other organs and tissues from the body cavity (fat, mesentery, kidneys, heart, etc.) were all screened for parasites. Finally, the digestive tract was dissected and stomach contents were removed and examined. Prey items were counted and identified to genus or species when possible to assess diet composition and the dietary importance of each prey taxon. Esophagus, stomach, pyloric ceca (when present), intestine, and rectum were then examined for gastrointestinal parasites. All parasites were identified, counted, and a subsample of 20 individuals per genus/species (or all individuals when less than 20 were found in a fish) were measured to the nearest 0.01mm (diameter for spherical parasites; length, width, and thickness for flattened ellipsoids; length and width for cylinder-shaped parasites).

Plankton

Plankton samples were examined under a dissecting microscope. All individuals were counted, identified to genus, and a subsample of 20 individuals per genus per sample (or all individuals when less than 20 were found in a sample) was measured to the nearest 0.01mm (body length) to assess potential within genus variations in body size across sites, seasons, and/or lakes. Planktonic crustaceans were examined for parasites by crushing subsamples of individuals from each genus between two glass plates, but no metazoan parasite could be detected in any sample.

Demersal and benthic invertebrates

Demersal and benthic samples were sorted under a dissecting microscope. All invertebrates were separated from debris and sediment, identified to genus or species when possible (using identification keys; see Winterbourn et al., 1989; Moore, 1997; Chapman et al., 2011), and counted. Again, a subsample of 20 individuals per taxon (genus or species) and per sample (or all individuals when less than 20 were found in a sample) were measured to the nearest 0.01mm (body length) to assess potential within-taxon variations in body size across sites, seasons, and/or lakes. Invertebrates were then dissected under a dissecting microscope using fine forceps and examined for parasites. For abundant invertebrate taxa (chironomid larvae, gastropods, amphipods, etc.), subsamples of 20 to 80 individuals per sample were dissected. All parasites were identified, counted, and a subsample of 20 individual parasites per genus/species (or all individuals when less than 20 were found in a sample) were measured to the nearest 0.01mm (diameter for spherical parasites; length, width, and thickness for flattened ellipsoids; length and width for cylinder shaped parasites). Stomach contents of carnivorous invertebrates (odonate larvae, leeches, Trichoptera larvae, etc.) were also examined.

Prey items were counted and identified to genus or species when possible to assess diet composition and the dietary importance of particular prey taxa.

Periphyton

Periphyton samples were topped up with distilled water to standardise sample volume to 50ml and stored in the dark until analysis. Samples were then homogenised and, using a compound microscope and a Palmer-Maloney counting chamber, algae, diatoms, and cyanobacteria cells were identified and counted. An aliquot of the homogenised sample was first transferred into the counting chamber and cells were allowed to settle at the bottom. Cells were then counted and identified following standard protocols for quantitative periphyton analysis (Biggs and Kilroy, 2000). Because of their small size, periphyton cells were not measured. Mean body sizes of the different taxa recorded were obtained from the literature and used to calculate body volumes for each taxon and for later biomass estimation (Biggs and Kilroy, 2000).

Macrophytes

Macrophytes from each sample were sorted by species and identified (Clayton and Edwards, 2006). Plants were patted dry to eliminate excess moisture and weighed to determine the fresh weight of each species (all individuals combined) within each sample.

Body mass

Body mass was calculated/measured differently for different types of organisms. Parasites were too small to be individually weighed and body measurements indicated that they varied little in size within each life stage of each taxonomic species. We thus calculated body volume for the subsamples of parasite individuals measured during host dissection based on the most appropriate formula for each species' shape (e.g. adult nematodes and acanthocephalans, trematode rediae and sporocysts = cylinder, adult trematodes = flattened ellipsoid, encysted juvenile trematodes [metacercariae] = spheres). Body volume was then calculated for each life stage of each species and their volume was converted to mass assuming their density equalled that of water. We could thus calculate a mean ($\pm$ SE) individual body mass for each life stage of each parasite species. In the case of trematodes in their snail first intermediate host, since rediae or sporocysts are the product of clonal multiplication, all rediae or sporocysts have the same genotype (with infrequent exceptions) and are issued from the same larva hatched from a single egg. Individual parasite body mass was thus considered as the sum of all rediae/sporocysts present in a snail host. Although rediae and sporocysts size (length and width) and volume (cylinder) were measured or calculated for each redia/sporocyst for convenience, individual parasite body mass for that life stage was reported as the total body mass of all rediae/sporocysts present in a snail host.

Most free-living invertebrates were large enough to be weighed individually (isopods, chironomids, odonates, large Trichoptera larvae, adult hemiptera, molluscs, leeches, etc.). Invertebrates varied little in size within taxonomic species or genus and by weighing a subsample of individuals for each taxon (to the nearest 0.01mg) we could calculate the mean body mass of an individual for all invertebrate taxa. For small free-living invertebrates, which varied little in size intraspecifically (amphipods, small Trichoptera larvae, oligochaetes, planktonic crustaceans, etc.), we pooled 5, 10, or 20 conspecific individuals (depending on individual body size) from random subsamples, weighed them as a group, and from the total mass calculated the average body mass of one individual.

For fish, each individual was weighed individually and fish body mass could be directly inferred from the data. Consequently fish body mass data for a given species varied across lakes and seasons, while the body mass of smaller organisms was treated as constant for each genus/species (or life stage of parasites within a taxonomic genus/species).

Similarly to parasites, periphyton cells were too small to be weighed. Taxon-specific sizes and shapes were obtained from the literature and used to calculate body volume (Biggs and Kilroy, 2000). Body volume was then converted to body mass assuming their density equalled that of water.

Density

Density of organisms (number of individuals per m^2 and its variance) was calculated for all taxa except macrophytes for which only biomass (mg per m^2) was estimated. For fish, we obtained a single estimate of abundance (number of fish per species) per sampling site per season. Since we used a combination of passive and active gear types and virtually captured all fish individuals present in (sedentary individuals) or passing through (user/occupant) each sampling area, we considered the number of fish captured as representative of the fish community present at and/or using the site. Fish density was thus calculated as the total number of fish captured divided by the surface of the entire sampling area (225m^2). One value of fish density was thus obtained per sampling site per season per lake and for each species present.

Densities of benthic and demersal invertebrates were simply calculated as the number of individuals of each taxon captured in a sample divided by the surface of the lake bottom sampled, regardless of water depth since these organisms live in, on and/or close to the substrate. Sample surface was 0.1m^2 for benthic and 0.6m^2 (0.3m net width $\times$ 2m sweep of the net) for demersal invertebrates. Invertebrate densities were calculated for all samples and could then be used to estimate mean densities per site, season and/or lakes.

Plankton density in each sample was first expressed as the number of individuals per m^3 of water filtered by dividing the number of individuals captured in a sample by the volume of the sample (0.15m^3; 0.25m net diameter and 3m net tow). Density per m^3 was then converted to density per m^2 by projection of the number of individuals per plankton taxon contained in 1m^3 of lake water onto the flat surface necessary to contain that 1m^3 of water according to water depth at each sampling site.

Parasite populations are usually quantified as individuals per host rather than per surface area. Here, we calculated parasite densities (individuals per m^2) to provide a common metric for all free-living and parasite taxa. Also, because distinct life stages of parasites with complex life cycles exploit completely different host species, we estimated parasite densities separately for each life stage of these parasites (trematodes, nematodes, acanthocephalans, etc.). Parasite abundance (mean number of parasites per individual host) was first calculated for each parasite taxon in each host species from dissection data. Parasite abundance was then multiplied by host density (number of hosts per m^2) to obtain parasite density. Parasite densities were also estimated in all individual samples. In the case of trematode parasites in their snail host, we did not count each individual redia or sporocyst as separate individual parasites, since these are the product of clonal multiplication. All rediae or sporocysts are issued from the same larva hatched from a single egg and were considered as a single individual. Density of these life stages was thus estimated as the number of infected snail hosts per m^2.

Density of periphyton was calculated from the number of cells counted in the volume of the subsample contained in a Palmer-Maloney counting chamber (0.05ml). By multiplying the number of periphyton cells found in the subsample by 1000 we obtained an estimation of the number of cells in a whole sample. That number was then divided by sampling surface (11.9cm^2 for hard substrate and 63.6cm^2 for soft sediments) to obtain periphyton density (cells per m^2) in each sample. Mean density per site, season and/or lake could then be estimated.

Density of birds was estimated per species from the number of individuals identified during bird counting. Density (number of individuals per m^2) was thus calculated as the number of birds counted per species divided by the area sampled. Area sampled corresponded to the whole lake for Tomahawk Lagoon or circular sector centred on each sampling site and delimited by two 150m shoreline radii and an arc within which birds were counted.

Biomass

Biomass of organisms (mg fresh weight per m^2) was calculated for all taxa. For fish, only one biomass estimate could be calculated per site in each season (4 biomass estimates per season in each lake) because only one density estimate was obtained per site. First we calculated a mean body mass for each fish species in each sampling site. Mean body mass of each species was then multiplied by the species density (number of individuals per m^2) in the same sampling site, giving the biomass of each species in each sampling site for all seasons and lakes.

For invertebrates and parasites, biomass was simply the product of the mean individual body mass of each taxon by the density (number of individuals per m^2) of that particular taxon in each sample. We thus obtained biomass estimates for all individual samples.

Biomass of macrophytes was calculated as the mass of each species (mg of fresh weight per sample) recovered in Surber nets during benthic samples divided by the surface sampled (0.1m^2 with Surber nets). Since 6 replicates were taken in each site, a mean macrophyte biomass per site could be calculated.

Biomass of birds was calculated for each species as the product of the density (number of individuals per m^2) of each species observed at each sampling site by the mean individual body mass obtained from the literature.

Weighted trophic links

Because we recorded diet of predatory taxa both qualitatively and quantitatively, we could calculate weighted trophic links. While the diets of primary consumers were estimated from the literature and the actual food sources available in each sampling site, stomach contents recorded during dissections of predator taxa were used to calculate the proportion of each prey taxon in the diet of predators, both numerically and in terms of biomass/energy transfer. First, we calculated the proportional contribution of each resource taxon, in terms of biomass, to the total diet of a consumer taxon, and assigned a fraction (between 0 and 1) to each resource-consumer link such that the sum of all trophic links toward any consumer species equalled 1. This was done for all consumers.

The diet of grazers and detritivores could not be quantified from stomach contents. Instead, we assumed that the diet of grazers consisted of a mixture of periphyton taxa proportional to their local abundance at the site and season of sampling. The diet of detritivores was assumed to consist entirely of detritus (not measured in the present study).

Many of the top predators in the 4 lake food webs considered here are birds. Because we were not allowed to sample birds, we used published information on their diet (O'Donnell, 1982; Sagar, P.M., Schwarz, A.-M., Howard-Williams, 1995; Wakelin, 2004) to establish the relative composition of their diet in terms of the main groups of fish or invertebrates or macrophytes. We assumed the diet of the birds at out study site matched that of the same bird species studied elsewhere, and used (where necessary) the species available locally to reconstruct the most likely diet of each bird species.

The 'diet' of each parasite taxon consists of the range of host species they use. For host-specific parasites, i.e., those occurring in only one host species at a given stage of their life cycle, the diet consists only of that host (a single trophic link of value 1 going to the parasite). For parasite species or life stages using more than one host species, we calculated the proportional contribution of each host taxon, in terms of the proportion of the parasite population harboured by each host, to the total diet of the parasite. Each link from a particular host was then assigned a fraction (between 0 and 1) such that the sum of all trophic links toward any parasite equalled 1.

Finally, many parasites are consumed by non-host predators that capture and eat their current host, a phenomenon known as concomitant predation on parasites. This creates trophic links in which these parasites become resources for the non-host predators. From stomach content analysis of all predator taxa, we estimated the contribution of concomitant predation on parasites to each predator's diet. Furthermore, we determined whether parasites consumed by non-host predators were digested and thus assimilated to the predator's diet or simply lost in the faeces without being digested; trematode metacercariae protected by thick cysts are often passed through the faeces intact and should not be included in the predator's diet. For each parasite life stage of each species, the mean number of parasites per prey item was multiplied by the mean number of individual prey consumed by unsuitable hosts for that parasite. For parasites actually digested by the predator, after converting this number of parasites eaten into biomass, these new links were added to the more traditional prey-predator links going to a consumer, and as above assigned a fraction (always very small) representing their contribution to the total diet of the consumer.

Potential host taxa for parasite life stages

Table S6.2: Potential host taxa for the parasite life stages observed in this dataset. For each life stage, we identify the host taxa for both the focal life stage and the next life stage in the parasite life cycle. If the next life stage is free-living or the current life stage is the adult (final) stage in the parasite's life cycle, there are no future hosts (indicated by a '-'). In our null model which accounted for parasites' host specificity, only those links where the prey was a potential current host and the predator was a potential future host were included as possible "transmission" links; links where the prey was a potential current host but the predator was not a potential future host were considered possible "loss" links; and all other links were categorised as "unused" (see *Material and Methods, Chapter 6* for details).

Parasite	Life stage	Host for focal stage	Host for next stage
Acanthocephalus galaxii	Cystacanth	Amphipod	Fish
Acanthocephalus galaxii	Adult	Fish	-
Anisakidae sp.	Larva	Unknown	Fish
Apatemon sp.	Metacercaria	Fish	Bird
Apatemon sp.	Sporocyst	Gastropod	-
Aporocotylid sp. I	Sporocyst	Gastropod	-
Coitocaecum parvum	Metacercaria	Amphipod or Mysid	Fish
Coitocaecum parvum	Sporocyst	Gastropod	-
Coitocaecum parvum	Adult	Fish	-
Deretrema sp.	Adult	Fish	-
Eustrongylides sp.	Larva	Fish	Bird
Gymnocephalous sp. I	Redia	Gastropod	-
Hedruris spinigera	Larva	Amphipod	Fish
Hedruris spinigera	Adult	Fish	-
Hydracarina sp.	Larva	Insects (aquatic)	-
Lepocreadiidae sp.	Metacercaria	Leech	Bird
Maritrema poulini	Metacercaria	Amphipod or Isopod	Bird
Maritrema poulini	Sporocyst	Gastropod	-
Microphalloidea sp.	Metacercaria	Trichoptera	Bird
Microphallus livelyi	Metacercaria	Gastropod	Bird
Microphallus sp.	Metacercaria	Amphipod or Isopod	Bird
Neoechinorhynchus sp.	Adult	Fish	-
Notocotylus sp.	Metacercaria	Mollusc	Bird
Notocotylus sp.	Redia	Gastropod	-
Plagiorchioid sp.	Sporocyst	Gastropod	-
Pronocephaloid sp. I	Metacercaria	Mollusc	Bird
Pronocephaloid sp. I	Redia	Mollusc	-
Pronocephaloid sp. IV	Metacercaria	Mollusc	Bird
Pronocephaloid sp. IV	Redia	Mollusc	-
Stegodexamene anguillae	Metacercaria	Fish	Fish
Stegodexamene anguillae	Redia	Mollusc	-
Stegodexamene anguillae	Adult	Fish	-
Telogaster opisthorchis	Metacercaria	Fish	Fish
Telogaster opisthorchis	Redia	Mollusc	-
Telogaster opisthorchis	Adult	Fish	-
Tylodelphys sp.	Metacercaria	Fish	Bird
Virgulate sp. I	Sporocyst	Mollusc	-
Unidentified "*Apatemon sp.*"	Metacercaria	Odonate	Bird
Unidentified cestode *sp.*	Larva	Fish	Bird
Unidentified nematode *sp.*	Adult	Fish	-
Unidentified trematode *sp.*	Metacercaria	Mollusc	Bird
Unidentified trematode *sp. A*	Adult	Fish	-
Unidentified trematode *sp. B*	Adult	Fish	-

S6.2: Supplemental methods and results for links' structural properties

Methods

In addition to calculating each link's centrality, we also defined their structural roles to get a richer picture of the ways in which species are embedded in their networks. These roles describe the link's position in the network in terms of "motifs"— unique patterns of 3 interacting species that can be understood as the building blocks of networks (Milo et al., 2002; Kashtan et al., 2004; Stouffer et al., 2007). Each motif has different implications for the flow of energy and biomass through the network (Stouffer et al., 2007; Stouffer and Bascompte, 2010). For example, the populations of three species in a direct competition motif (two predators with one prey) will affect each other differently from those of the three species in an apparent competition motif (two prey with one predator). Moreover, each unique position in each motif has different implications (Cirtwill and Stouffer, 2015). For example, in the omnivory motif the top predator consumes both an intermediate consumer and a basal species that is also eaten by the intermediate consumer, and each of these links will almost certainly provide the top predator with different amounts of biomass and energy, and the top predator will in turn affect the intermediate and basal species differently. By tracking the frequency with which a link appears in each position in each motif, we thereby obtain a rich picture of the way each link is embedded in the network.

To calculate a link's structural role, therefore, we counted the frequency with which the link appears in each of the 24 unique positions in the 3-species motifs. We were interested in comparing the shapes of links' roles rather than their sizes (i.e., the number of times the link appeared across all motifs). To ensure that different role sizes did not influence our analyses, we normalised each role vector by dividing by the total number of positions in which the link appears.

After obtaining these normalised role vectors for each link, we tested whether links with different outcomes had different typical roles. We first visualised the median roles for each outcome using a canonical correspondence analysis conducted using the function cca from the package vegan (Oksanen et al., 2014) in R (R Core Team, 2014). The median roles for each outcome as determined by this analysis describe the outcomes' "typical" roles. This visualisation is equivalent to the Tukey's HSD tests performed for the univariate properties above. We then statistically compared these typical roles with a non-parametric permutational multivariate analysis of variance (PERMANOVA Anderson, 2001) by using the adonis function from the package vegan (Oksanen et al., 2014) in R (R Core Team, 2014).

Like our modified ANOVA in *Chapter 6*, the PERMANOVA compares between-group differences to within-group differences following a pseudo-F statistic (Anderson, 2001). As when testing for correlations between links' roles and other structural or dynamic properties, we defined differences between links' roles using Bray-Curtis dissimilarity, calculated using relative frequencies of positions within each role (see above). Once again, we did not assume a particular distribution of the data and computed p-values from null distributions based on permutations of the data (Anderson, 2001). As in our modified ANOVA tests, we used both the unrestrictive and taxonomically-informed null models.

Results

Links with different outcomes were associated with different structural roles, whether we used the unrestrictive or the taxonomically-informed null model ($F_{2,42019}=126.5$, $p<0.001$ in both cases). Transmission links, on average, had more positive values on both axes than loss links, and loss links in turn had more positive values on both axes than unused links (Fig. S6.5A).

To put these results into context, positive values of the first RDA axis were most strongly associated with frequent participation in the direct competition motif, where one prey has two predators, followed by the lower link in a three-species food chain (Fig. S6.5B). Negative values of this axis were strongly associated with frequent participation in the apparent competition motif, where one predator has two prey. Positive values of the second RDA axis were associated with frequent participation in both direct and apparent competition, while negative values were associated with both links in a three-species food chain. These motifs were more strongly associated with the RDA axes than any others by at least an order of magnitude. Transmission links therefore tended to appear more frequently in the bottom of food chains and in direct competition links.

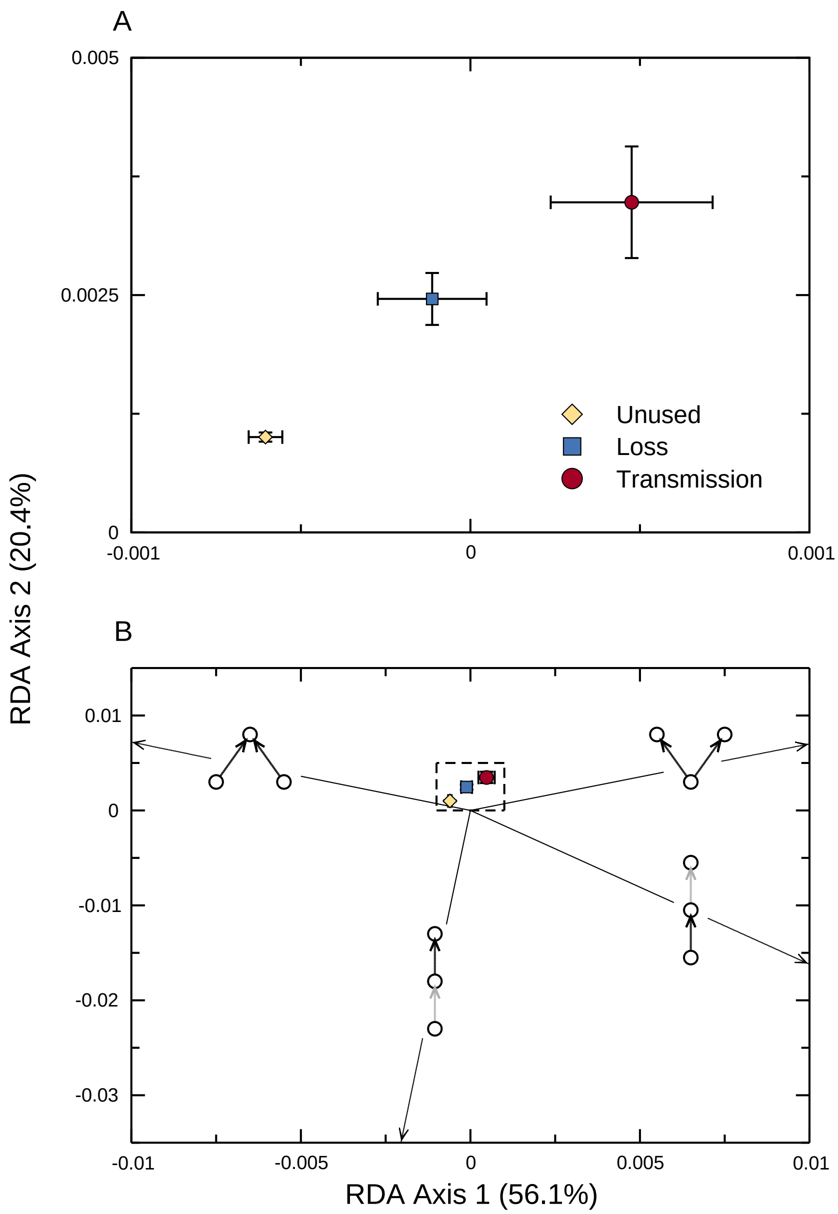

Figure S6.5: Feeding links between free-living species with different outcomes for parasites also had different structural roles. **A)** For each outcome, we show the median role across all parasite life stages (±2SE) with respect to the first two axes of a redundancy analysis that, together, explain 76.5% of variation in links' structural roles. **B)** We also show the four structural role positions that were most strongly associated with the two RDA axes. The dashed box indicates the location of panel A, while the lines indicate the strict relationship between the frequency of each position (highlighted in black) and the two RDA axes. Note that all four lines extend far beyond the borders of panel B. The four positions were: the single unique position in the direct competition motif (top right), the single unique position in the apparent competition motif (top left), the upper link in the three-species food chain (bottom centre, black link), and the lower link in the three-species food chain (bottom right, black link).

S6.3: Results of model selection

Table S6.3: AIC scores for CCAs of outcomes of links for parasites against different combinations of dynamic and structural properties. In the table below, an 'X' indicate that a property was included in the model. The models have been ranked from lowest to highest AIC. A line separates the best two models from those with AIC's significantly greater (ΔAIC>2) than the most parsimonious model.

	Properties					
Model	Contribution to predator's diet	Prey abundance	Prey biomass	Biomass transfer	Centrality	AIC score
1	X		X	X	X	18151.36
2	X	X	X	X	X	18152.12
3	X		X		X	18154.81
4	X	X	X		X	18155.54
5			X	X	X	18179.01
6		X	X	X	X	18180.02
7			X		X	18184.66
8		X	X		X	18185.60
9	X		X	X		18562.44
10	X	X	X	X		18563.40
11	X		X			18566.94
12	X	X	X			18567.89
13			X	X		18595.06
14		X	X	X		18596.25
15			X			18600.21
16		X	X			18601.35
17	X	X		X	X	18894.91
18	X	X			X	18900.35
19		X		X	X	18989.52
20		X			X	18995.31
21	X			X	X	19192.71
22	X				X	19198.19
23				X	X	19311.62
24					X	19318.33
25	X	X		X		19451.30
26	X	X				19459.78
27		X		X		19565.75
28		X				19570.78
29	X			X		19803.15
30	X					19811.87
31				X		19947.92
32						19953.55

S6.4: Testing for correlations between link properties

Methods

To control for the possibility that relationships between outcomes of feeding links and dynamic properties might be similar because of hidden relationships between the properties, we first tested for correlations between them. We did this using the R (R Core Team, 2014) function cor.test from the stats package (R Core Team, 2014). When testing for correlation between links' contributions to predators' diets and the amount of biomass they transfer, we included all links (n=2160). When testing for correlations between prey biomass or prey abundance and any other property, however, we restricted our sample to those links where the local prey biomass (n=1627) or abundance (n=1464) could be estimated.

We also tested for correlations between links' structural roles and the other predictors. To do this, we performed a series of non-parametric t-tests for multivariate independence, using the function dcor.ttest in the R (R Core Team, 2014) package energy (Rizzo and Szekely, 2014). Once again, we included only those links where biomass or abundance had been estimated when testing for correlations involving prey biomass or abundance. This function tests for correlations between the inter-point distances in two datasets. In our case, these were the sets of structural roles for each link and the set of links' contributions to predators' diets (or any other univariate predictor we considered). We defined differences between links' roles using Bray-Curtis dissimilarity (Anderson, 2001; Baker et al., 2015; Cirtwill and Stouffer, 2015) since it measures differences between roles based only on positions in which at least one of the links appears. That is, this dissimilarity is not affected by "double zeros" such that links which appear in few positions are not considered more similar to each other due to the large number of shared zeros frequencies. We also wished to avoid a situation in which two links involved in different numbers of positions would be interpreted as having different roles even if they occurred with the same frequencies across all positions; we therefore calculated the dissimilarities based on positions' relative frequencies (that is, the number of times a link appears in a position divided by the number of times it appeared in any position). As all of the other properties we tested were univariate and Bray-Curtis dissimilarity could not be used, we calculated Euclidean distances between links for these properties.

Results

As we expected, there were significant correlations among many of the dynamic properties we investigated. The contribution of a link to the predator's diet was significantly and positively correlated with the local abundance of the prey species (R^2=0.073, p=0.005), the local biomass of the prey species (R^2=0.198, p<0.001), and the amount of biomass transferred along the link (R^2=0.238, p<0.001). However, not all of these properties were correlated amongst themselves. In particular, prey abundance and prey biomass were not significantly correlated with the amount of biomass transferred along a link (R^2=0.016, p=0.537 and R^2=0.024, p=0.326, respectively). Prey abundance and biomass were strongly correlated with each other (R^2=0.521, p<0.001). It is worth noting that, even though many of these properties were significantly correlated, the correlations tended to be both weak and potentially non-linear (Fig. S6.2). We therefore present the results for each property separately.

Centrality was significantly and positively correlated with the contribution of a link to the predator's diet, the abundance of the prey, and the amount of biomass transferred along a link (R^2=0.088, p<0.001; R^2=0.071 , p=0.007; and R^2=0.133, p<0.001, respectively; Fig. S6.3). Centrality was not, however, correlated with prey biomass (R^2=0.030, p=0.227). Links' structural roles, meanwhile, were strongly correlated with each of the link's contribution to the predator's diet, the biomass of the prey, the abundance of the prey, and the amount of biomass transferred along a link (t_{2158}=685, p<0.001; t_{1625}=57.9, p<0.001; t_{1462}=69.5, p<0.001; and t_{2158}=69.5, p<0.001, respectively). Finally, centrality and link's structural roles were also significantly correlated t_{2158}=35.4, p<0.001). Once again, however, the correlations between centrality and dynamic properties were weak and non-linear, while the linearity of correlations between structural roles and other properties is difficult to assess. We therefore present all results independently but note the potential for confounding effects between properties.

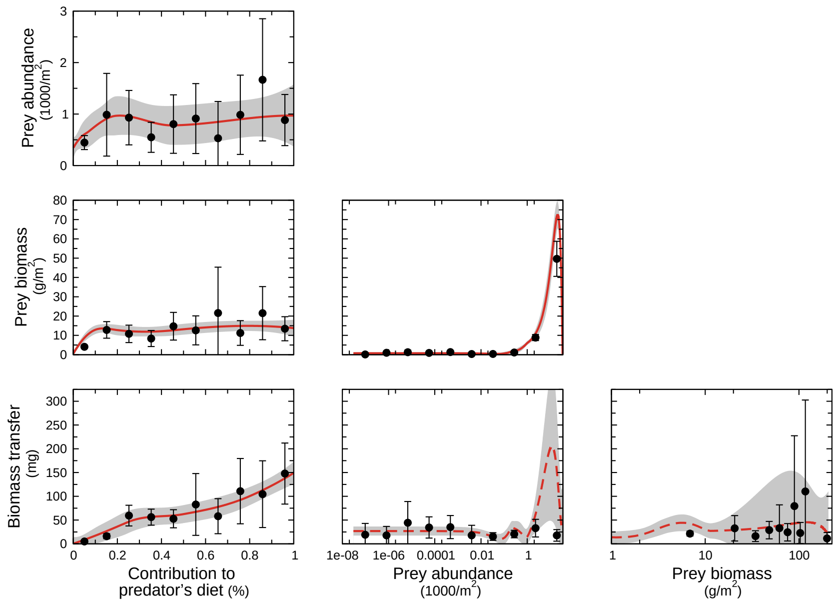

Figure S6.2: The contribution of a feeding link to the predator's diet was positively correlated with the local abundance and biomass of the prey (p<0.001 in both cases) and with the amount of biomass transferred along the link (p<0.001). Likewise, prey abundance was correlated with prey biomass (p<0.001). The amount of biomass transferred along a link, however, was not correlated with the abundance or biomass of the prey (p=0.537 and p=0.326, respectively). For each pair of properties, we show the best-fit loess regression (red line) with a 95% confidence interval (shaded area) together with the means ($\pm$2 SE) of the observed property for 10 bins (sizes of bins vary depending on the regression). Note that even when the correlations between two dynamic properties were significant, the correlations were weak and appeared non-linear. We therefore present the results for all four dynamic properties.

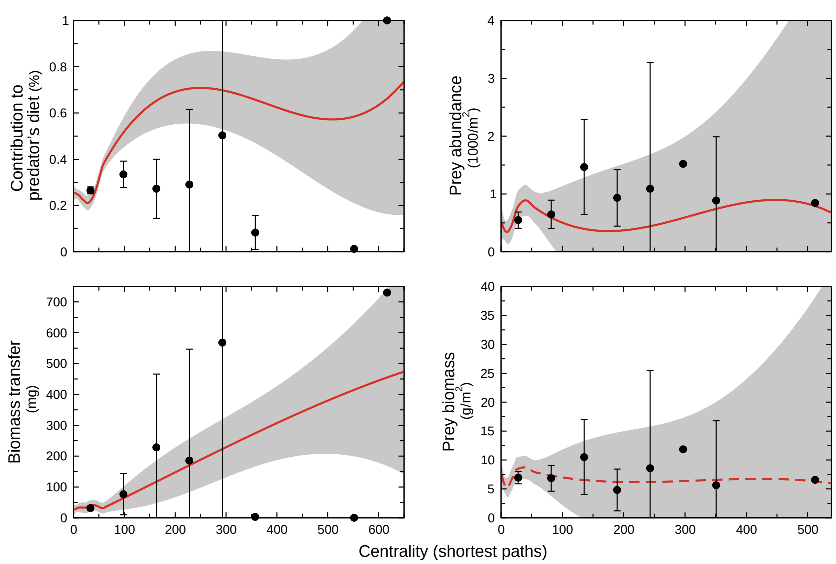

Figure S6.3: A link's centrality was significantly correlated with its contribution to the predator's diet (p<0.001), the abundance of the prey (p=0.007), and the amount of biomass transferred along the link (p<0.001). Centrality was not, however, correlated with the biomass of the prey (p=0.227). For each pair of properties, we show the best-fit loess regression (red line) with a 95% confidence interval (shaded area) together with the means ($\pm$2 SE) of the observed property for 10 bins (sizes and numbers of bins vary depending on the regression). Note that even when the correlations between two properties were significant, the correlations were weak and appeared non-linear. As with the dynamic properties, we therefore present the results for centrality separately.

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Supporting Information SA

Supporting information for Appendix:

Are parasite richness and abundance linked to prey species richness and individual feeding preferences in fish hosts?

Alyssa R. Cirtwill, Daniel B. Stouffer, Robert Poulin, & Clement Lagrue

S7.1. Supplemental results: biomass of trophically transmitted parasites

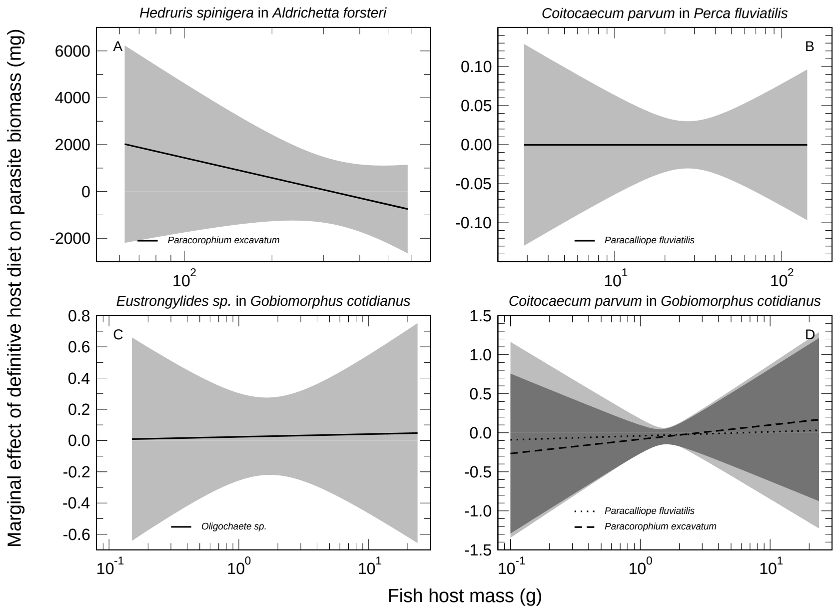

Figure SA.1: Marginal effects of the proportion of intermediate host prey in the diet of fish hosts on the total biomass of trophically-transmitted parasites in individual hosts in the four parasite-fish host taxon combinations for which models could be fitted; (A) *Hedruris spinigera* in *Aldrichetta forsteri*, (B) *Coitocaecum parvum* in *Perca fluviatilis*, (C) *Eustrongylides* sp. in *Gobiomorphus cotidianus* and (D) *C. parvum* in *G. cotidianus*. Intermediate host prey taxa are also identified within each panel. Marginal effects are obtained by summing the effect of proportion of intermediate host with the effect of the interaction between fish host mass and proportion of intermediate hosts across the observed range of fish host masses. We show mean marginal effects (black lines) with 95% confidence intervals (grey). See Fig. *A1, Appendix* for details about the interpretation of marginal effects.

In general, relationships between parasite biomass and proportions of intermediate hosts in the diet of fish hosts were similar to the relationships with parasite abundances described above (Fig. S7.1). The main distinction was that, unlike the abundance of *H. spinigera* in *A. forsteri*, the biomass of the parasite did not vary significantly with the proportion of *P. excavatum* in the fish's diet, and there was no significant interaction with the size of *A. forsteri* (β_2 = 758, P = 0.607; β_3 = -560, P = 0.456). As such, host diet did not affect the biomass of *H. spinigera* for *A. forsteri* of any size (Fig. S7.1A; Table S7.2). Also unlike abundance, the biomass of *Eustrongylides* sp. in *G. cotidianus* increased with the proportion of intermediate hosts in the fish's diet (β_2 = 2.96, $P<0.001$). However, there was no significant interaction with fish host size (β_3 = 0.009, P = 0.874) and the high degree of variance associated with this interaction meant that, overall, the biomass of *Eustrongylides* sp. did not vary with the diet of *G. cotidianus* (Fig. S7.1C). More similarly, neither the biomass of *C. parvum* in *P. fluviatilis* nor the biomass of *C. parvum* in *G. cotidianus* varied with the proportion of intermediate hosts in the fishes' diets (β_2 = -2.80x10^{-4}, P = 0.960 and β_2 = -0.029, P = 0.557; β_3 = -0.048, P = 0.434, respectively). Further, there were no significant interactions between proportions of intermediate hosts and fish host size (β_3 = 2.09x10^{-6}, P = 0.999 and β_4 = 0.038, P = 0.906; β_5 = 0.134, P = 0.676). Therefore there was no overall effect of the proportion of either intermediate host on *C. parvum* biomass (Fig. S7.1B, D; Table S7.2).

Table SA.1: Geographical locations and characteristics of the four lakes sampled for *G. cotidianus* (South Island of New Zealand).

Lake	GPS coordinates	Surface area (km^2)	Depth (m) Mean - Max	Altitude (m)	Trophic status	Tidal
Hayes	44○58′59.4"S 168○48′19.8"E	2.76	3.1 - 33	329	Mesotrophic	No
Tuakitoto	46○13′42.5"S 169○49′29.2"E	1.32	0.95 - 3	5	Mesotrophic	Yes
Waihola	46○01′14.1"S 170○05′05.8"E	6.35	1.3 - 2.2	4	Eutrophic	Yes
Tomahawk Lagoon	4○54′06.0"S 170○33′02.2"E	0.096	1.0 - 1.2	15	Eutrophic	No

Table SA.2: Estimated fixed effects in equation 3 (with P-values in parentheses). β_1 indicates the effect of fish host mass on the biomass of the parasite, β_2 and β_3 the effects of the proportions of two intermediate hosts in the diet of the fish host, and β_4 and β_5 the effects of the interaction between proportion of intermediate host and fish host mass. NA indicates that only one intermediate host was found in the gut contents of the fish host. Estimates are based on averages over the full equation 2 and all possible reduced models, weighted by AIC.

Fish host	Parasite	β_1	β_2	β_3	β_4	β_5
Aldrichetta	*Hedruris*	14.0	758	NA	-560	NA
forsteri	*spinigera*	(0.224)	(0.607)		(0.456)	
Perca	*Coitocaecum*	0.003	-2.80x10^{-4}	NA	2.09x10^{-6}	NA
fluviatilis	*parvum*	(0.823)	(0.960)		(0.999)	
Gobiomorphus	*Eustrongylides*	2.96	0.036	NA	0.009	NA
cotidianus	sp.	(<0.001)	(0.775)		(0.874)	
Gobiomorphus	*Coitocaecum*	0.004	-0.029	-0.048	0.038	0.134
cotidianus	*parvum*	(0.948)	(0.557)	(0.434)	(0.906)	(0.676)

Table SA.3: Estimated fixed effects in equation 2 (with P-values in parentheses) where proportions of intermediate hosts were determined using masses of intermediate hosts. β_1 indicates the effect of fish host mass on the abundance of the parasite, β_2 and β_3 the effects of the proportions of two intermediate hosts in the diet of the fish host, and β_4 and β_5 the effects of the interaction between proportion of intermediate host and fish host mass. NA indicates that only one intermediate host was found in the gut contents of the fish host. Estimates are based on averages over the full equation 2 and all possible reduced models, weighted by AIC.

Fish host	Parasite	β_1	β_2	β_3	β_4	β_5
Aldrichetta	*Hedruris*	0.208	44.6	NA	0.991	NA
forsteri	*spinigera*	(<0.001)	(0.002)		(0.898)	
Perca	*Coitocaecum*	0.113	0.007	NA	0.024	NA
fluviatilis	*parvum*	(0.839)	(0.991)		(0.964)	
Gobiomorphus	*Eustrongylides*	0.438	0.287	NA	0.028	NA
cotidianus	sp.	(<0.001)	(0.075)		(0.725)	
Gobiomorphus	*parvum*	0.131	-0.847	1.07	6.10	-4.41
cotidianus	*parvum*	(0.191)	(0.358)	(0.229)	(0.250)	(0.405)

Table SA.4: Estimated fixed effects in equation 3 (with P-values in parentheses) where proportions were determined based on the masses of each intermediate host. β_1 indicates the effect of fish host mass on the biomass of the parasite, β_2 and β_3 the effects of the proportions of two intermediate hosts in the diet of the fish host, and β_4 and β_5 the effects of the interaction between proportion of intermediate host and fish host mass. NA indicates that only one intermediate host was found in the gut contents of the fish host. Estimates are based on averages over the full equation 2 and all possible reduced models, weighted by AIC.

Fish host	Parasite	β_1	β_2	β_3	β_4	β_5
Aldrichetta	*Hedruris*	10.4	2.88×10^{3}	NA	3.45×10^{2}	NA
forsteri	*spinigera*	(0.415)	(0.632)		(0.287)	
Perca	*Coitocaecum*	0.003	-2.62×10^{-4}	NA	1.79×10^{-6}	NA
fluviatilis	*parvum*	(0.823)	(0.962)		(0.998)	
Gobiomorphus	*Eustrongylides*	3.27	5.19	NA	1.49	NA
cotidianus	sp.	(<0.001)	(<0.001)		(0.138)	
Gobiomorphus	*parvum*	0.042	-0.273	0.442	4.83	-4.38
cotidianus	*parvum*	(0.571)	(0.694)	(0.516)	(0.320)	(0.365)

Table SA.5: Estimated fixed effects in equation 2 (with P-values in parentheses) using absolute counts of intermediate hosts consumed rather than proportions. β_1 indicates the effect of fish host mass on the abundance of the parasite, β_2 and β_3 the effects of the counts of two intermediate hosts in the diet of the fish host, and β_4 and β_5 the effects of the interaction between number of intermediate host individuals consumed and fish host mass. NA indicates that only one intermediate host was found in the gut contents of the fish host. Estimates are based on averages over the full equation 2 and all possible reduced models, weighted by AIC.

Fish host	Parasite	β_1	β_2	β_3	β_4	β_5
Aldrichetta	*Hedruris*	0.130	1.18	NA	0.549	NA
forsteri	*spinigera*	(<0.001)	(<0.001)		(0.002)	
Perca	*Coitocaecum*	0.095	5.76	NA	-2.77	NA
fluviatilis	*parvum*	(0.922)	(0.885)		(0.993)	
Gobiomorphus	*Eustrongylides*	0.411	0.007	NA	-0.009	NA
cotidianus	sp.	(<0.001)	(0.842)		(0.889)	
Gobiomorphus	*Coitocaecum*	0.891	0.842	-0.156	4.59	-0.423
cotidianus	*parvum*	(<0.001)	(0.187)	(<0.001)	(0.013)	(0.015)

Table SA.6: Estimated fixed effects in equation 3 (with P-values in parentheses) using absolute counts of intermediate hosts consumed rather than proportions. β_1 indicates the effect of fish host mass on the abundance of the parasite, β_2 and β_3 the effects of the counts of two intermediate hosts in the diet of the fish host, and β_4 and β_5 the effects of the interaction between number of intermediate host individuals consumed and fish host mass. NA indicates that only one intermediate host was found in the gut contents of the fish host. Estimates are based on averages over the full equation 2 and all possible reduced models, weighted by AIC.

Fish host	Parasite	β_1	β_2	β_3	β_4	β_5
Aldrichetta	*Hedruris*	8.43	78.9	NA	48.5	NA
forsteri	*spinigera*	(0.212)	(0.076)		(0.222)	
Perca	*Coitocaecum*	0.003	0.025	NA	-9.42×10^{-4}	NA
fluviatilis	*parvum*	(0.824)	(0.933)		(0.999)	
Gobiomorphus	*Eustrongylides*	2.91	0.106	NA	-0.225	NA
cotidianus	sp.	(<0.001)	(0.809)		(0.823)	
Gobiomorphus	*Coitocaecum*	0.081	0.017	-9.14×10^{-4}	0.003	2.16×10^{-4}
cotidianus	*parvum*	(0.388)	(0.903)	(0.911)	(0.974)	(0.974)

Acknowledgements

I would like to thank my primary supervisor, Daniel Stouffer, for always pushing me to do the best possible science. Your energy and attention to detail are an inspiration. Thanks also to my secondary supervisor Dominique Gravel for providing key feedback that has shaped the course of this thesis.

Many thanks to Nick Baker, Katie Bowron, Camille Coux, Carol Frost, Simon Litchwark, Lupe Peralta, and Kate Wootton for making me feel at home when I arrived in New Zealand. Special thanks to Nick for all of the advice and assistance during my first attempts at programming.

Thanks also to the members of the Stouffer and Tylianakis labs, past and present, who provided feedback on drafts, support when times were tough, and smiles in the good times. I feel very lucky to have been part of such a great group of people.

Special thanks to my co-authors Clement Lagrue, Jens Olesen, Robert Poulin, Tomas Roslin, and Claus Rasmussen for sharing their data and their empirical expertise. This thesis is much stronger because of you. I would also like to thank Nick Baker, Malyon Bimler, Fernando Cagua, Camille Coux, Matthias Dehling, Marilia Gaiarsa, Giulio Dalla Riva, Daniel Stouffer, Josh Thia, and Christie Webber for their contributions to papers.

Funding for this thesis was provided by the National Science and Engineering Research Council of Canada and the University of Canterbury. Additional funds supporting conference attendance was provided by a Marsden Fund grant (to Daniel Stouffer), administered by the Royal Society of New Zealand. I also appreciate the use of computation services provided by the BlueFern University of Canterbury Super Computer and Abacus biology cluster.

I would like to thank everyone who has given me feedback on drafts and talks, supported me at conferences, and shared their research with me. These conversations are a huge part of what makes science worthwhile.

I would like to thank Karen Adair, Melissa Broussard, Carla Gomez Creutzberg, Matt Hutchinson, Bernat Bramon Mora, and Johanna Voinopol-Sassu for their advice, support and friendship.

Last but certainly not least, thanks to Dan Cirtwill, Max Cirtwill, and Susanne Collins for encouraging me to chase my dreams and to James Litchwark for making sure I always had something to smile about.