Patterns of potential cross-species transmission in planktonic multihost-multiparasite communities

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September 7, 2023

Abstract

Most parasite species infect multiple host species, and reciprocally, most hosts are infected by multiple parasites. This leads to complex webs of interactions that influence disease within the community, making it challenging to understand and predict disease spread within the community and epidemics. Here, we used network approaches to analyze a multi-year time series dataset that includes eight zooplankton host species (in the Daphnia and Ceriodaphnia genera) and seven microparasite species to examine patterns of cross-species transmission. These analyses suggest that parasite species varied in their ability to infect multiple host species and in which host species they most commonly infected. Three parasites (the bacteria Pasteuria ramosa and Spirobacillus cienkowskii and the oomycete Blastulidium paedophthorum) showed signatures of relatively high cross species transmission, while the others seemed more restricted. Even for the three common multihost parasites, our approach also revealed differences in patterns of potential cross species transmission. For P. ramosa, two host species, Daphnia dentifera and D. retrocurva, seem particularly likely to transmit across species; in contrast, for S. cienkowskii, no host species stands out as particularly important for cross species transmission. Additionally, these patterns matched those describing epidemic size, suggesting that infected host density may drive cross-species transmission. These results are based on observations of patterns of infection in natural communities, and therefore we cannot draw definitive conclusions about interspecific transmission in lakes. However, some of the patterns are supported by additional lines of evidence, and others point to interesting avenues for future research. Together, these findings provide additional evidence that network approaches can provide valuable insights into patterns of transmission in complex multihost-multiparasite communities in nature.

Introduction

Parasites are ubiquitous in nature, and also embedded in complex assemblages of interrelated species. As a result, most host species interact with more than one parasite species and many parasite species interact with multiple potential hosts (Petney and Andrews 1998; Pedersen and Fenton 2007; Rynkiewicz et al. 2015). While we know that the complex web of interactions between species within communities can influence patterns of disease (Johnson et al. 2015a), there are substantial challenges to disentangling the patterns of transmission in these complex communities – leaving an important gap in our understanding of the basic functioning of ecological communities and how changes in species in diversity might influence infectious diseases in wildlife and humans (Cable et al. 2017; Stewart Merrill et al. 2022).

To date, most studies focusing on the complexity of natural communities have focused on the impact of changes in the number of host species on patterns of disease (Johnson et al. 2015b). In particular, studies of the dilution effect have sought to understand how changes in host community diversity might influence the level of infection, especially in one focal host (Keesing et al. 2010; Civitello et al. 2015). Central to the

potential for dilution is that host species differ in their ability to host a parasite – to become infected, for parasite replication within a host, and/or in the ability to pass infections on to additional hosts (LoGiudice et al. 2003; Stewart Merrill et al. 2022). However, studies of the dilution effect still tend to focus on one parasite in a community, and we still do not understand whether certain hosts are likely to be important for transmission (or dilution) more broadly across parasites, though there is evidence that certain aspects of a host's life history might lead to certain hosts generally being highly competent (Martin et al. 2016).

At the same time, we wish to know whether it is likely that the path one parasite takes through an ecological community might be followed by another parasite. Cross-species transmission plays a crucial role in public health, as most human infectious diseases originate from zoonotic sources (Woolhouse and Gowtage-Sequeria 2005; Lloyd-Smith et al. 2009). If one parasite spills over via one particular set of host species, will another parasite most likely follow that same path, or do different parasites tend to have different networks of transmission?

Unfortunately, studying these realistic, complex communities and quantifying their ecological interactions using traditional approaches is challenging due to the sheer number of species involved and the complexity of their interactions (Runghen et al. 2021). Moreover, even in cases where it would be ethical to experimentally manipulate transmission, it often is not logistically feasible (e.g., due to challenges cultivating host species in controlled settings). These challenges are exemplified by a well-studied host-parasite system,*Daphnia* and their parasites. While this system is unusually well characterized and studied, being used as a model system for questions of disease ecology and evolution (Ebert 2005; McLean and Duffy 2020; Wale and Duffy 2021; Ebert 2022), we still lack a general understanding of basic questions such as whether particular host species are generally more prominent parts of parasite transmission, or whether different parasites tend to be transmitted via different networks of hosts.

In the present study, we took advantage of the large dataset capabilities of network approaches to (1) generate hypotheses about which hosts and parasites might warrant further study in experiments or models (Pilosof et al. 2014), and (2) produce useful metrics approximating the relative "importance" of a species and the number of connections among species (Gómez et al. 2013; Dallas et al. 2017). This allowed us to address three main questions regarding this study system. First, which parasite species in our study system show the most cross-species transmission and infect the widest breadth of host species? Second, are individual host species associated with cross-species transmission of each parasite species or are parasites more commonly host generalists, infecting many species? And third, are patterns of potential cross species transmission similar across parasite species within a community? The insights garnered from these analyses point towards topics for future studies aimed at understanding complex multihost-multiparasite communities in nature.

Methods

Study system

We studied seven host species of *Daphnia* (*D. dentifera* ,*D. retrocurva* , *D. dubia* , *D. parvula* , *D. pulicaria* , *D. ambigua* , and *D. mendotae*) and one closely related species of *Ceriodaphnia* (*C. dubia;* hereafter all hosts are collectively referred to as "*Daphnia* " for simplicity). These species occur at varying densities in our study lakes. *Daphnia* are small planktonic crustaceans which live in freshwater lakes, feeding on phytoplankton and serving as prey to small fish and invertebrate predators (Tessier and Woodruff 2002). Many parasite species infect *Daphnia* , and here we focused on seven of the most commonly occurring species in our study sites. Our parasite analysis included: one fungus: *Metschnikowia bicuspidata* ; two microsporidians: *Larsonnia obtusa* and *Gurleya vavrai* ; two bacteria: *Pasteuria ramosa* and *Spirobacillus cienkowskii* ; and two oomycetes: *Blastulidium paedophthorum* and an unknown oomycete (which grows hyphae throughout the host's body cavity, referred to as 'Spider' in the figures). Hosts were diagnosed based on characteristic symptoms of infection (Green 1974; Ebert 2005; Duffy et al. 2015). We excluded two common gut parasites of *Daphnia* (the ichthyosporean *Caullerya mesnili* and the microsporidian*Ordospora pajunii*), because they were misclassified as the same species for part of the sampling period.

We studied host and parasite communities in 15 lakes in Southeast Michigan, US over three years (2014-

2016). We sampled lakes roughly every two weeks from mid-July to mid-November each year (usually 9 total sampling events), a time period when most parasite epidemics in these lakes occur (Gowler et al. 2021). In addition to our normal sampling efforts, we sampled four of the study sites every three days during 2016 (this additional effort was due to another concurrently running project (Rogalski et al. 2021)). During each sampling event, we collected three replicate whole-water-column vertical tows from the bottom of the lake up through the surface with a 153 μm Wisconsin plankton net, pooling samples from three different locations in the deep basin of each lake for each replicate. For one replicate sample, we visually diagnosed parasite infections in live hosts under a dissection microscope at 10x magnification (or under a compound microscope at 20 to 40x magnification for early-stage infections). As *Daphnia* are mostly transparent, many parasite infections are visibly detectable with this method. We randomly subsampled the collected hosts, surveying at least 200 individuals of each host species for possible parasite infections or surveying all individuals of a given species when fewer than 200 of that host species were present. Overall, we analyzed 272,351 *Daphnia*individuals for infections during this study. We preserved the other two replicate samples in 90% ethanol; at a later date, we randomly subsampled and counted one replicate to estimate the density of each host species. Density was calculated as the number of hosts throughout the water column for a given surface area of the lake (number of hosts per m^2).

Quantifying potential cross-species transmission

To generate a plausible measure of potential cross-species transmission, we chose a method that accounts for temporal and spatial overlap of a single parasite species in multiple host species (i.e., to what degree did the same parasite species infect more than one host species on the same day in the same lake?). Our metric, which we term "epidemic overlap", measures the degree to which epidemics of a single parasite species overlapped in multiple host species within the same site over the same time period. Looking at a single parasite species in a single lake (hereafter: site) in a single year of data at a time, we compared parasite prevalence and infected host density of the parasite species in each pairwise combination of hosts (e.g., shaded region in Fig. 1, comparing *S. cienkowskii* infected host density in *D. pulicaria* and *D. retrocurva* in Bruin Lake during 2014). Only including days when two hosts were infected with the same parasite, we took the geometric mean of infected host density for each pair of host species through time and calculated the area under the overlapping epidemic curve. We used the geometric mean because, unlike the arithmetic mean, it yields a zero for any dates where prevalence in either of the hosts was zero. In this way, epidemic overlap served as a proxy for the potential amount of cross-species transmission. In the overlapping region, infections in one host could plausibly result from transmission stages of parasites produced by another host species. In other words, epidemic overlap quantifies the amount of synchrony of epidemics of the same parasite species in different host species.

Estimating host breadth and cross-species transmission by parasite species

We considered three network-level factors for each parasite species: (1) the number of different host species a parasite infected in a community (host breadth), (2) the number of predicted transmission pairs between different host species (cross-species transmission connections), and (3) a combination of the two (graph density). Calculating each of these required that we first construct networks of the host species infected by a particular parasite species (examples in Fig. 2). To do this, for each parasite species, we constructed undirected networks (i.e., edges/connections do not have a direction) of host species in each site where each node represented the population of a host species in a given community. With only the host species that were present in that site as the nodes, we added edges between host species if they had overlapping epidemics of that same parasite species. For the networks used to calculate host breadth, we added node edges to itself (loops) for each host that was infected by a given parasite (these loops are equivalent to filling a diagonal cell in the adjacency matrix). We then calculated host breadth as the proportion of all hosts in a site (observed across all time points) that were infected with a given parasite species. The networks used to estimate cross-species transmission did not contain loops (that is, node edges from a host species back to itself), because we were only interested in interconnections between species. We then estimated cross-species transmission using the proportion of realized connections between host species out of all possible connections

in the network. Finally, because both of these metrics are important for different reasons, we combined the two and calculated graph density as the proportion of realized edges out of all potential edges that could occur in a given network, including loops where a host connects to itself if it was infected. Additionally, since sites varied in their host community composition, we only included a host species in a given network if that species was found in that site; sites where the parasite was not found were dropped from the analysis.

Estimating host influence on cross-species transmission

Our goal was to see if some host species were more likely to be involved in cross-species transmission of various parasite species. For each parasite species and site, we constructed undirected networks of hosts with edge connections weighted by the epidemic overlap value for each host combination, while removing host species with no connections from the network. Because some parasites produce long-lived spores capable of delaying transmission (and for others we lack the necessary natural history information), we summed the epidemic overlap values across years for each site. Since we were purely interested in potential cross-species transmission, we did not include loops as we did for the networks described above (i.e., no connections of a host species to itself). Additionally, we dropped very small networks (two or fewer host species), because our measures of host influence were not applicable.

As a proxy for the influence of each host species on cross-species transmission of different parasites, we calculated the eigenvector centrality for the host nodes in each network. Eigenvector centrality was useful because it considers the entire network and emphasizes second order connections; a given node is more central if it is connected to other nodes that themselves are more central (Bonacich 2007). If one imagines a random walk across a network, the stationary distribution of time spent at each node is proportional to the eigenvector centrality. Eigenvector centrality scores range from 0 to 1, with 1 being most central, and were calculated on weighted networks. We chose eigenvector centrality because it emphasizes connections throughout the network, which could be important in a community disease context where there might be chains of transmission across different host species (Gómez et al. 2013).

Statistical analysis

We used the Kruskal–Wallis test to see (1) if there were differences in host breadth, cross-species transmission connections, and graph density across parasite species; (2) if there were differences in eigenvector centrality across host species; and (3) if there were differences in epidemic size of different parasites across host species. For the centrality and epidemic size data, we looked at each parasite species separately. For all comparisons, if the Kruskal–Wallis test suggested a significant main effect, we then used the Conover–Iman test with Bonferroni corrections for pairwise comparisons across parasite or host species.

We used R version 4.2.3 for all of the data processing and statistical analyses. We used the "frame2webs" function in the bipartite package (Dormann et al. 2008) to construct host adjacency matrices and the igraph package (Csardi and Nepusz 2006) to calculate network metrics such as graph density and centrality.

Results

Host-breadth and cross-species transmission connections in different parasite species networks

All parasite-specific measures—host breadth, cross-species transmission connections, and graph density—suggest parasites in these communities vary in their ability to infect and transmit between different host species (Fig. 3). Each of these three measures showed significant differences among parasite species (Kruskal–Wallis test, $p < 0.001$ for all: see Figure 3 legend for individual tests), and the median values and pairwise comparisons were largely the same across metrics. *B. paedophthorum, S. cienkowskii,* and *P. ramosa* were always infective to the most host species, with our analysis indicating that they infect approximately the same number of host species. Of these three parasite species, *B. paedophthorum* , in particular, had consistently higher values compared to the four lower scoring parasite species (i.e., *M. bicuspidata* , *L. obtusa* ,*G. vavrai* , and 'Spider'), suggesting it is more of a multihost parasite than the others.

Comparing host breadth (Fig. 3A) and cross-species transmission connections (Fig. 3B) provides information

that combined graph density (Fig. 3C) does not. For instance, *B. paedophthorum* is the only parasite species that was found in every host species at one of the sites (and, at one site with 4 hosts, all host species were infected; host breadth = 1). Also, while *G. vavrai* was found to infect multiple host species, those epidemics never overlapped in time and space (Fig. 3B, values = 0). However, overall, parasite species that had higher host breadth also tended to have more overlapping epidemics across host species.

Influence of each host species on cross-species transmission

Some host species were more central in the networks of overlapping epidemics (Figure 4), suggesting they might be more important for the cross-species transmission of particular parasites. The eigenvector centrality values depended on the host species and parasite species. Only three parasite species, *P. ramosa, B. paedophthorum,* and*S. cienkowskii* , were sufficiently common to create epidemic overlap networks large enough to calculate centrality values. Of those parasites, cross-species transmission networks of *P. ramosa*showed some hosts (*D. dentifera* and *D. retrocurva*) were more central in the network (Fig. 4A, Kruskal–Wallis test, p = 0.01), suggesting they may be more important for driving cross-species transmission. For *B. paedophthorum* , host species also varied in their centrality (Fig. 4B, Kruskal–Wallis test, p = 0.01). However, for*S. cienkowskii* , host species had indistinguishable centralities (Fig. 4C, Kruskal–Wallis test, p = 0.46). Thus, although each parasite (*P. ramosa, B. paedophthorum, and S. cienkowskii*) commonly infected multiple host species, the cross-species transmission dynamics might be different because host centrality patterns differ across parasite species; more specifically, in some cases (*S. cienkowskii*) the hosts all seem equally important, while in others (e.g., *P. ramosa*) only some hosts seem important for cross-species transmission.

D. dentifera and *D. retrocurva* consistently had the highest centrality values (Figure 4), suggesting they are more important for cross-species transmission of certain parasite species. On the other hand, the host species that were least central tended to vary depending on parasite species (note letters in Fig. 4, and see Table S1 for a full list of significant pairwise comparisons). *D. parvula* , *D. pulicaria* , and *Ceriodaphnia* were all less important for transmitting at least one parasite species. Besides *D. dentifera*and *D. retrocurva* , no other host species had significantly higher centrality than another. Thus, these two host species might be of particular interest for future work on cross-species transmission in these communities.

Broadly speaking, the three common multihost parasites—*P. ramosa, B. paedophthorum,* and *S. cienkowskii* — appear to have different patterns of cross-species transmission. For *P. ramosa* , two host species (*D. dentifera* and *D. retrocurva*) were likely most important for cross-species transmission because they had higher centrality values on average (Figure 4A). For *B. paedophthorum* only one host species (*D. dentifera*) had higher centrality values on average, while two different species (compared to*P. ramosa*) had lower centrality values (Figure 4B). However, with*S. cienkowskii* , there were no differences across host species, suggesting that host species identity is not an important driver of patterns of cross-species transmission.

Epidemic size of different parasites in a single host species

Epidemic size—measured as the area under the infected host density curve during a single epidemic—varied by host species for *P. ramosa* (Fig. 5A, $\chi^2 = 24.1$, $p < 0.001$, df = 5) and *B. paedophthorum* (Fig. 5B, $\chi^2 = 27.1$, $p < 0.001$, df = 5). For *P. ramosa* , epidemics in *D. dentifera* and *D. retrocurva* were significantly larger than those in *D. dubia* and *Ceriodaphnia* (Conover–Iman, *D.retrocurva-Ceriodaphnia* p = 0.004, *D. dentifera -Ceriodaphnia* $p < 0.001$; Fig. 5B). For*B. paedophthorum* , epidemics in *D. dentifera* and *D. retrocurva* were significantly larger than those in *D. pulicaria*(Conover–Iman, $p < 0.001$; Fig. 5B), and epidemics in *D. dentifera* were significantly larger than those in *D. parvula*(Conover–Iman, p = 0.02; Fig. 5B). Taken together with the centrality results, these results suggest host species differ in the degree to which they spread parasites to other host species.

Discussion

Using detailed multihost, multiparasite time series data and network analysis approaches, we estimated potential cross-species transmission in *Daphnia* communities. We found evidence that parasites in these lakes differ in their ability to infect and transmit between host species; three parasites showed high host

breadth and strong potential for cross-species transmission (based on high overlap of epidemics in different host species in a given lake on a given date). Focusing on these three common multihost parasites, two hosts, *D. retrocurva*and the well-studied *D. dentifera* , are most implicated in cross-species transmission. This was particularly true for the bacterium*P. ramosa* . In contrast, for another common multihost parasite, the bacterium *S. cienkowskii* , cross-species transmission seemed less reliant on particular species.

Basing our estimates of plausible cross-species transmission on synchrony of single pathogen epidemics, we found that the identity of both the pathogen and host species were important to our networks. Pathogens varied in their specificity of host species and in their apparent ability to transmit among host species. With one notable exception, pathogen species with greater host breadth (implying lesser specificity) displayed greater potential for cross-species transmission. The exception is *G. vavrai;* this parasite was found to infect multiple host species, but epidemics were separated in time leaving no potential for cross-species transmission. Notably, it has been suggested that an intermediate, non-*Daphnia* host, is required for the*G. vavrai* lifecycle (Refardt et al. 2002), which would preclude cross-species transmission among *Daphnia* in our communities for this pathogen. This is also the case for the other pathogen for which we found no potential cross-species transmission, *L. obtusa* (Refardt et al. 2002). These results thus provide support for our method of cross species transmission detection.

For our three most common pathogens, our estimates of network centrality indicate that host identity plays a strong role in potential for cross species transmission, with some host species having a disproportionate influence on community transmission. However, the degree to which host identity influenced cross species transmission – and the particular host(s) that most strongly impacted it – varied by pathogen. For two of our pathogens, a subgroup of hosts were more central within the network (supporting greater cross species transmission), while for the third,*S. cienkowskii,* no host displayed greater cross species transmission potential. Thus, this approach allows us to identify pathogen species for which some hosts might have an outsized impact on disease dynamics in these communities, and also gives us insight into multihost-multiparasite communities as a whole. The potential patterns of interspecific transmission for *P. ramosa* can be more readily studied in lab experiments, as compared to those for *S. cienkowskii* ; while experimental infections with *S. cienkowskii*are possible, they are rare and experiments are challenging. Thus, instead, molecular analyses of infections in these species seem more promising. A study of *P. ramosa* in these lake populations genotyped the parasite primarily from *D. retrocurva* and *D. dentifera* (based on the availability of infected hosts in the field), but also included several infected *D. parvula* and one*Ceriodaphnia dubia* (Shaw 2019; Shaw et al. in review). In this analysis, the genotypes found in *D. parvula* were always also found in *D. retrocurva* , but the same genotype was not found infecting both *D. dentifera* and *D. retrocurva* . Thus, the molecular results partially support the analyses here that indicate that*P. ramosa* moves between host species, but also suggests there are barriers to doing so that are not captured by these analyses. Molecular analyses of *S. cienkowskii* -infected hosts from natural populations would be interesting; based on the network analyses, we predict less genetic structuring by host species for *S. cienkowskii* .

Parasites differ in their ability to infect and transmit among different host species, though we cannot yet say why some parasite species commonly infect more host species in the same site; both pathogen life history traits and taxonomic group have been suggested as potential mechanisms (Pedersen et al. 2005). Our two bacterial parasites, *S. cienkowskii* and *P. ramosa* , generally had higher host breadth and potential cross-species transmission compared to other parasites; however, the oomycete parasites, *B. paedophthorum* and 'Spider', significantly differed in both host breadth and potential cross species transmission. Moreover, more recent work on a microsporidian gut parasite that was not included in this study, *O. pajunii* , has found substantial transmission across host species (Dziuba et al. 2023). Overall, these results make it difficult to conclude whether taxonomic group strongly influences the likelihood of cross species transmission. We know that these parasites differ in key aspects of their transmission, with some (e.g., *B. paedophthorum*) being continually shed and others (e.g., *P. ramosa, S. cienkowskii* , and*M. bicuspidata*) being obligate killers. Moreover, within a parasite species, particular aspects of phenotype or genotype can influence transmission. In *P. ramosa* , only certain genotypes can attach to the esophagus of the host, a key first step in infection

(Duneau et al. 2011; Ebert et al. 2015); when this occurs across species, infections often fail at a later (but currently unknown) stage in the infection process (Luijckx et al. 2014). In contrast, with *M. bicuspidata* , interspecific transmission seems to be asymmetric and to depend on parasite genotype and spore size (Shaw et al. 2021; Sun et al. 2023). Future studies that aim to uncover the traits that promote – or prevent – cross species transmission will help us better understand and predict infections in complex natural communities.

Host species differed in the pathogens they hosted and in their relative potential importance for cross species transmission (Fig 4). Phylogeny can influence patterns of cross-species transmission (Streicker et al. 2010; Parker et al. 2015; Mollentze and Streicker 2020). In particular, hosts might be more susceptible to parasites transmitted from more closely related host species. Our *P. ramosa* network suggests that *Ceriodaphnia* are significantly less likely to be involved in cross species transmission than *Daphnia* species, supporting a phylogenetic influence for this pathogen. This is consistent with the limited experimental evidence we have, which suggests that cross species transmission between *C. dubia* and *D. dentifera* is possible, but rare (Auld et al. 2017). It is also consistent with genetic evidence collected in 2015 (overlapping with this study), which found that the *P. ramosa* strain in *C. dubia* was an outgroup to the strains in *Daphnia* (Shaw 2019; Shaw et al. in review)). In contrast, our results for *B. paedophthorum* do not suggest challenges traversing greater phylogenetic distances, as *C. dubia* did not differ from the *Daphnia* hosts displaying the greatest transmission potential. This is also consistent with experimental studies, which were readily able to transmit this parasite across host species (Duffy et al. 2015). As *P. ramosa* is known to have a matching allele interaction with hosts (Bento et al. 2017), it is probable that pathogens with this infection strategy are more likely to have phylogenetically regulated cross-species transmission.

Within our communities, we saw variation in epidemic size within our pathogens. Focusing on the three pathogens for which we were able to create networks, we found that two (*P. ramosa* and *B. paedophthorum*) displayed significant differences in infected host density among host species (Fig 5). The patterns in infected host density closely replicated the patterns for centrality among hosts (Fig 4, Fig 5). Our remaining pathogen, *S. cienkowski* , displayed no differences in epidemic size by host and also no differences in host centrality among hosts. Previous work has demonstrated a positive (though nonlinear) causal relationship of infected host density to within species transmission (Rachowicz and Briggs 2007; Roberts and Hughes 2015). As centrality, here, represents potential cross-species transmission, our results suggest that infected host density (or epidemic size) within host species may also influence cross-species transmission. Thus, these results extend known within species transmission mechanisms to the multi host species scale.

We are beginning to disentangle the ecological and epidemiological drivers of cross species transmission (Lloyd-Smith et al. 2009; Plowright et al. 2015; Plowright et al. 2017). However, we still lack the ability to accurately predict novel transmission events and host shifts, or the degree to which cross species transmission maintains epidemics within communities (Mollentze and Streicker 2020). By applying network analysis techniques to a rich dataset from a well-studied planktonic host-parasite system, we were able to uncover host species that seem particularly important for maintaining levels of infection in a community, as well as differences among parasite species in the degree to which they infect multiple host species. Because of its correlative nature, this work cannot definitively say whether certain host or parasite species are more involved in cross-species transmission, but it can inform hypotheses about transmission patterns within this community. For example, it suggests that parasites differ in the degree to which host phylogeny constrains their ability to spillover between hosts (e.g., *P. ramosa* vs. *S. cienkowskii*). In doing so, it points to additional studies that will help us better understand this system (e.g., studies characterizing the genetic diversity of *P. ramosa* vs. *S. cienkowskii* in different host species during epidemic outbreaks). More broadly, it provides additional support for the value of network approaches for providing insights into patterns of transmission in nature and for generating hypotheses regarding transmission in complex multihost-multiparasite communities.

Acknowledgements

We are extremely grateful to Rebecca Bilich for her assistance with field sampling. This work was supported by NSF grants DEB-1305836 and 1748729 to MAD and by the Moore Foundation (GBMF9202; DOI:*https://doi.org/10.37807/GBMF9202*). The authors declare no conflicts of interest.

Authors' contributions

CDG conceived the initial idea for this study and developed it in collaboration with MCE and MAD. CDG, MAR, CLS, and KKH collected data. CDG analyzed the data with support from MCE; KMM and MAD updated the code. CDG and KMM wrote the manuscript with substantial editing by MAD. All authors were involved in editing the manuscript and gave approval for publication.

Data availability statement

The data and code used for this study are available on GitHub (https://github.com/kmmcintire/networkscst) for review purposes. Data and code for this study will be posted to Dryad upon acceptance of this manuscript.

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Figures

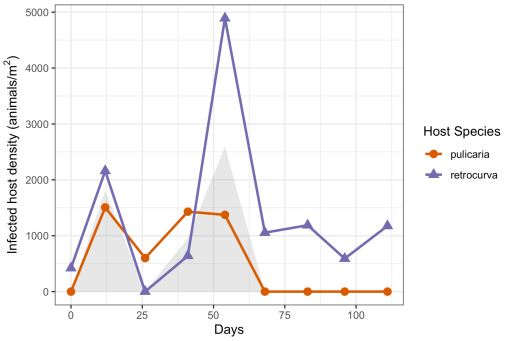

Figure 1: An example of the epidemic overlap calculation. Overlapping epidemics of *S. cienkowskii* in two different host species at Bruin Lake during 2014. Points denote sampling events and the estimated density of *D. pulicaria* (orange circles) and *D. retrocurva* (purple triangles) infected with *S. cienkowskii* through time. The shaded regions are the area calculated as epidemic overlap (*i.e.*, integrated area of the geometric mean between the two infected host densities at time points when both hosts were infected).

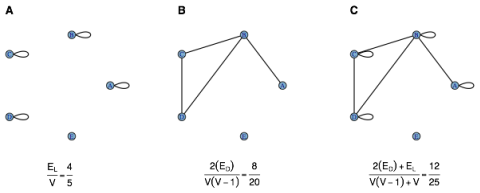

Figure 2: An example network of five host species and a single parasite species, with visualizations and calculations for (A) host breadth, (B) cross-species transmission connections, and (C) a combination of both: graph density. Each node/vertex represents a host species in the community. All hosts that were present in the community were included in the analysis, even if they were not infected by the parasite in question (*e.g.*, host "E"). For host breadth (A), each host vertex was connected by a "loop" if it was infected with the parasite in question, and host breadth was calculated as the proportion of hosts that had an edge loop (E_L) out of total vertices (V). On the other hand, in (B) host vertices were connected only by potential cross-species transmission links (meaning there was geographic and temporal overlap of infection in the species, ignoring loops), and we calculated the proportion of realized direct edge connections (E_D) out of all potential links that could occur in the network. Finally in (C), we combined the two metrics—both including loops and direct edge connections for cross-species transmission—and calculated graph density as the proportion of all realized links out of all available ones.

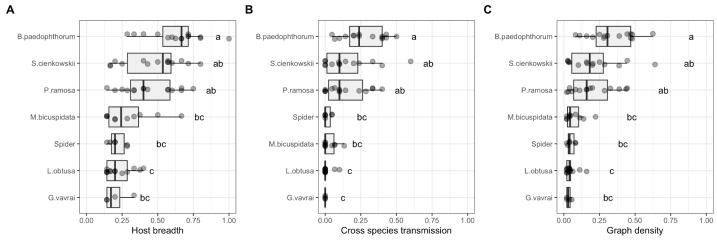

Figure 3: Network level estimates of (A) host breadth, (B) potential cross-species transmission connections, and (C) graph density all indicate that parasites in these communities vary in their ability to infect and transmit between different host species. Networks were created using the host species present in each site as nodes and epidemic overlap for connections between hosts. Connections were summed across years, so each point in the figure shows data for a single site/lake. There was a significant difference in host breadth (Kruskal–Wallis test, $\chi^2 = 35.2$, $p < 0.001$, $df = 6$), proportion of potential cross-species transmissions (Kruskal–Wallis test, $\chi^2 = 38.3$, $p < 0.001$, $df = 6$), and graph density across parasite species (Kruskal–Wallis test, $\chi^2 = 36.2$, $p < 0.001$, $df = 6$). Letters show pairwise comparisons that were significantly different based on the Conover–Iman test with Bonferroni corrections. Note that the y-axes differ slightly between the three panels, as they are ordered based on the ranking for each particular metric.

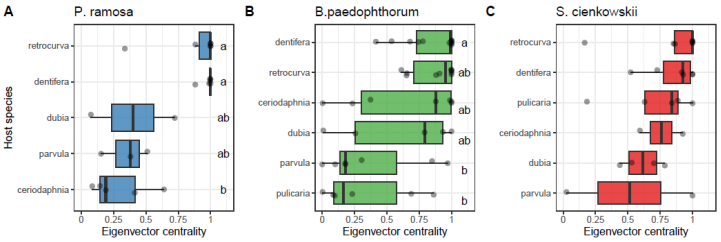

Figure 4: Eigenvector centrality values for host species in networks of *P. ramosa* (A), *B. paedophthorum* (B), and *S. cienkowskii* (C) for overlapping epidemics by infected host density, showing differences by parasite species and host species. Each point represents the centrality values for a host in a given site for a given parasite species. Centrality values varied significantly by host species for *P. ramosa* infected host density (A, Kruskal–Wallis test, $p = 0.008$) and *B. paedophthorum* infected host density (B, Kruskal–Wallis test, $p = 0.01$). Letters denote significant pairwise comparisons (see Table S1 for full list of significant host species comparisons). For each panel, the y-axis is arranged from host species with the highest median centrality (top) to the lowest (bottom), so the order of host species changes. See Figure S1 for eigenvector centrality calculated based on prevalence vs. infected host density.

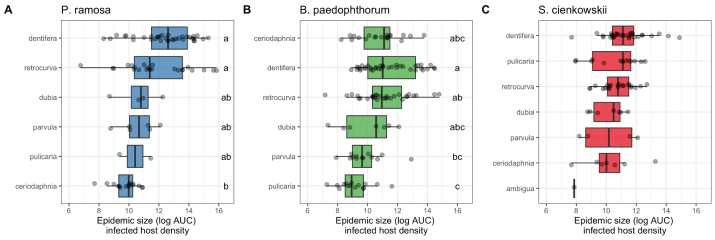

Figure 5: Size of a single epidemic of three common parasites in each host species varied for infected host density of certain parasites. Each point is the area under the curve of prevalence of infected host density for (A) *P. ramosa*, (B) *B. paedophthorum*, and (C) *S. cienkowskii* in a single site, in a single year. For *P. ramosa* and *B. paedophthorum*, there were significant differences in the area under the curve of infected host density across species (Kruskal–Wallis test, $p < 0.001$ for both parasite species; letters denote significantly different pairwise comparisons).