

Examining network analysis methods and the knowledge gained of bipartite host-parasite networks through fish-parasite studies (total word count: 2446)

Background (401 word count):

Fish and parasites are ancient taxonomic groups. Fish parasites constitute of helminths and various microorganisms, the first group vastly studied. The long relationship between the fish and their parasites makes them great candidates for intimate co-evolutionary processes studies. Furthermore, fish are a bridge to parasites which trophically transmit to land animals, i.e., piscivorous birds and mammals, making fish an important link for ecological networks of parasitism and food networks. This crowns the link between fish and their parasites as an example of a bipartite network which has evolutionary and veterinary importance. The veterinary aspect becomes more significant when considering the fish farming industry – understanding the networks of fish and their parasites has agricultural implications, by disease control within and between fish farms. Studying the connection has economic values for the same matter, as fish are a major, commercially farmed food resource. Anthropogenic interactions with fish, without fully understanding relationship with parasites, could also promote disease spread and transmission to other organisms. In addition, freshwater fish which are not commercially farmed can still be unintentionally affected by anthropogenic activities, promote invasion of new parasites in populations which were not exposed to them before, and add rapid selective stress on local species and fish-parasite interactions.

The fish-parasite network, being two trophic levels, is an example for a bipartite network. Bipartite network studies open the door to taxonomic relations and evolutionary processes between groups of intimately connected organisms which affect each other directly. This intimate relationship is sometimes referred to in evolutionary ecology as a powerful force driving to speciation, enhancing either modular networks or prompts generalists and more nested networks. Bipartite networks are a powerful tool for understanding ecological processes. Studying the fish-parasite system can promote development of new tools in this growing field, which can then be used for other applications too, like in epidemiology and economics.

Understanding the mechanisms of the bipartite network of freshwater fish and their parasites is thus crucial for responsible and sustainable fish farming, parasite control between farmed, wild

and invasive fish populations, as management guidelines for invasion and disease control, and as an important host-parasite system for studying evolutionary processes.

For these reasons, this project will review research papers which used network analyses of different types to explore the gained knowledge in the important field of fish and their parasites as well as in bipartite network applications which were used and developed while studying this system.

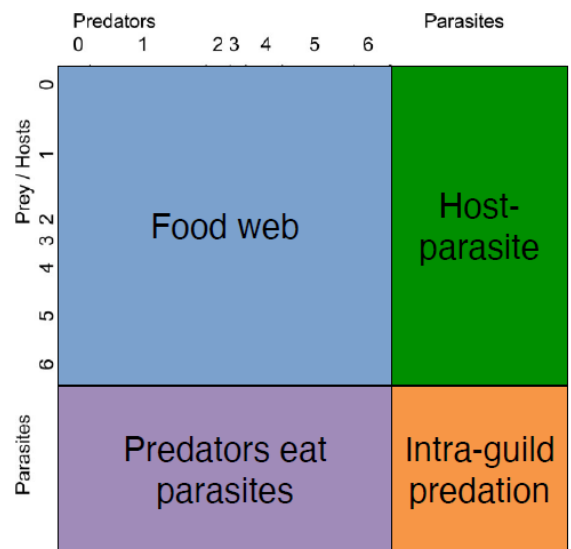


Figure 1: merging multiple types of interactions, Lafferty 2006

Research goal (26 word count):

In this review I will compare the methodology used in each paper for analyzing the host-parasite network and summarize the gained knowledge regarding the fish-parasite system.

Brief description of data (83 word count):

The data is consisted of 8 research papers and one review, written between the years 2005-2020. The papers all explore the host-parasite relationships of freshwater fish and their parasites, using network analysis tools and methods. The focus was on bipartite networks, but two papers were more generalized and looked at an entire food web. The studies cover a wide range of questions, from evolutionary perspectives like specialization, to invasion dynamics and universal host-parasite interactions, to practical management of fish disease transmission and conservation.

1. Methodology in papers (641 word count)

Four papers out of the nine were written by the same authors and discussing the same systems with different research questions, once even using the exact same methods with a small twist. The methods for analyzing the networks were diverse, though the main components shared by all of them were null models along with some sort of permutation test like Monte-Carlo or bootstrapping. Three papers also used a similar gap-minimizing model, originating in niche theory – in a nutshell, rearranging hosts in some sort of way to get the most continuous parasitism line as possible which connects all hosts. An open gap in the line means an open niche for a new parasite to enter the system. Three papers (all in which Lafferty was a co-author) used the Lafferty quadrics system, which separated interactions into "subwebs": predator–prey web, parasites and hosts and

predator-prey webs, everything together including predator–parasite and parasite–parasite links (Fig.1, class material).

The tools for analyses were diverse, from softwares like Binmatnest to programming languages like Matlab and R and programmable softwares like GIS (Table 1). For the most part, the math was suitable for readers who have studied basic calculus (for example, Calculus 1C in BGU), but the late papers started becoming more and more interdisciplinary. The papers from 2017 and 2018 included coauthors which were statisticians, engineers and mathematicians. For ecology advocates, this is wonderful – environmental questions and problems are expanding beyond the realm of biologist "tree-huggers", other scientific realms are gaining interest in ecological systems and finding new perspectives and solutions for questions which previously could not be answered without cooperation with other fields of science. However, understanding such methodology and following the math can be more difficult the more interdisciplinary the paper is. This may be problematic, instead of making network analysis more widespread, it end up becoming a bottleneck topic limited to researchers with higher mathematical skills. On the other hand, this could indicate that higher math and possibly basic engineering (like hydrology or civil engineering) might have to become part of basic biological studies in universities, to keep the field of network ecology open. This is important since the mathematical solutions provided in the 2017 and 2018 papers are remarkable but could remain purposeless without biological context.

Another factor was the use of binary networks in all the papers until 2020. The binary approach was used in the papers to determine trends in networks made of many taxonomic groups. Focusing on taxonomic relations was used as justification for using binary matrices. Arguably, weighted networks could have been adding noise to an already complex system, making results unreadable, which was also the reasoning for most papers. This could also have been due to most papers analyzing previously collected datasets, and information was not necessarily collected for weighted network analyses purposes. However, it is arguable that binary networks overlook real interactions between hosts and their parasites, since some interactions are rarer than others, and should be taken with a grain of salt through a binary prism. Some of the papers tried to overcome this bias by eliminating single interactions, but even two interactions in a large system could still be in the margin of very rare yet still counted, while many interactions which are a main network component thinned down. I conclude that indeed, a binary matrix was useful for the purposes of the written papers, but adding the layer (when data is available) of weighted interactions could give closer-to-reality results and could be completely different than binary networks. The

standalone 2020 paper would not have been in-depth and complete without the weighted network, since it was the only paper discussing two specific fish host species, and losing the information gained by the weighted network would have indeed left them with very weak results.

Table 1: Methodology and data origin of the papers reviewed

| Paper | Tool/s | Method | Null model | Data type – collected specifically for paper? |
|--------|---------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| 2005 | Matlab | 1. Monte-Carlo permutations of actual data against null models 2. Path analysis | 1. Completely randomized interactions 2. randomized but chance for interaction proportional to species abundance | No |
| 2008i | Matlab | Monte-Carlo permutations of actual data against null models | Minimal gaps: rearranging the hosts on the x axis from different starting points to get minimal gaps. 1. Random 2. Constrained to genus, family, order. | No |
| 2008ii | Review, irrelevant | | | |
| 2009 | Binmatnest software | Building a food web | Quadrants (subwebs) of interactions and including parasites into the food web | No |
| 2012 | Aninhado software Netcarto software | Pairwise testing for each pair of species according to different attributes (abundance/biomass or size...) | Random interactions | Yes |
| 2013 | Binmatnest software | Building a food web | Quadrants (subwebs) of interactions and including parasites into the food web, and removing according to before/after invasion | No |
| 2017 | ArcMap R spatial stream network packages | Akaike's Information Criterion for model selection Spatial Stream Network framework and models Leave-one-out cross validation | X | Unspecified, probably yes - collected 7 years prior to publishing, but by one of the authors |
| 2018 | 1. Optimal channel network models | Generating river system, changing parameters like fish | X | No |

| | | | | |
|------|-------------------------------------------------------------------------|-----------------------------------------------------|----------------------------------|---------------------------|
| | 2. Metacommunity prevalence and data 3. Local epidemiological models | movement, water velocity, temperature, initial hubs | | Rest is program generated |
| 2020 | R bipartite package Becket algorithm | Swap-web Bootstrapping | 1000 randomly generated networks | No |

2. The gained knowledge about fish-parasite interactions (1075 word count):

Both fish-trematode and rodent-flea systems were consistent in showing that abundant hosts will harbor higher numbers of parasites, making the hosts generalists, while the parasites tended to be specialists for those abundant hosts. If the same patterns are found both in the completely different land and in sea systems, it could be a basis for other hypotheses regarding host-parasite systems and their similarities (Mouillot, Krasnov, and Poulin 2008; Vázquez et al. 2005). For example, if one finds a new pattern or explanation for a terrestrial or avian system, it shows that assuming the same pattern in freshwater is reasonable. It sets a general rule that could be an explaining factor or ground for hypotheses for other researchers and for understanding systems.

Both terrestrial and freshwater systems were interval, meaning that there were more "open niches" for potential invasion than expected by chance. Hence it is expected that new trematode-borne diseases could always emerge, even in well-established systems. This has conservation implication for aquacultural systems which are close to natural systems – if there is always an open niche for a parasite, it should mean that the control and flow between the farmed and natural systems should be well monitored to prevent invasion of parasites from the one to the other, even if the natural system is presumably undisturbed (Lafferty et al. 2008; Lima et al. 2012; Mouillot, Krasnov, and Poulin 2008). Ecologically, it may seem like a trivial conclusion, but it could impact regulation and decision-makers.

The two systems also showed that phylogeny was the main explanation for the structure of the network when compared to behavioral traits of the animals which did not significantly explain it (Lima et al. 2012; Mouillot, Krasnov, and Poulin 2008). The taxa being the main driving force explained the modularity or nestedness and found consistent for freshwater and terrestrial as well as native and non-native host systems.

Additionally, there were similar nestedness and modularity patterns specific for fish and trematode networks. High nestedness in a system caused a decrease in the resilience and

persistence of the entire food web (Amundsen et al. 2009, 2013; Lafferty et al. 2008; Llopis-Belenguer et al. 2020), while modularity of a system increased resilience and persistence of the food web. The nestedness was found for endoparasites, complex life-cycle parasites and for native hosts. Nestedness was not found for ectoparasites, for non-complex life cycle parasites or for invasive hosts. Native hosts and their parasites were significantly nested, while non-native hosts were less nested. However, native and non-native hosts shared less parasites than expected, only 10% of parasite species. It is important for aquacultural systems since they suffer greatly from endoparasites with complex life cycles (Savaya et al. 2020; Yizhar et al. 2009), which are difficult to detect among fish. Thus, veterinary efforts for detection of endoparasites should be meticulously done for fish farms, or prior to reintroductions of hybrid hosts to natural systems and vice-versa, since they can transport with them parasites which decrease the entire food-web resilience. For example, salmonid hybrids, popular in commercial fishing, when introduced to a fish farm or releasing hybrids to a natural system, if the introduced fish are not sufficiently sampled it could cause outbreaks of disease in systems which were previously not infected by a specific parasite in the wild types. Since finding endoparasites or complex life cycle parasites is very difficult (requires intense invasive sampling of fish or other intermediate hosts and essentially kills the sampled animal), it opens a clearly needed future research direction for identification of such parasites easily and without causing mortality. Also, the results imply that ectoparasites and non-complex life cycle parasites are not as problematic for the entire system, both because of the reduced shared parasites and the lack of nestedness for ectoparasites. Thus veterinary efforts for fish farms or conservation efforts for natural system should focus more on sampling of endoparasites for the systems stability.

However, parasites which originated from a non-native host were shown to slowly spread to native hosts in three different systems (Amundsen et al. 2009, 2013; Llopis-Belenguer et al. 2020). This was true for ectoparasites, which were not significantly nested in these systems, and that introduction of trophically transmitted parasites (usually passive and non-complex life cycled parasites) have a dominant role in changing invaded systems. Both types of parasites tend to be more generalists than actively transported and endoparasites. This means that even though the former do not seem to be as "harmful" to the system as the latter, they should still be considered when transporting fish or equipment between systems, since they do have a rippling effect on the entire food web.

Anthropogenic activity, ranging on the urban-agricultural-natural gradient and included human-induced climate change, showed to negatively affect the host and the parasite abundances (Carraro et al. 2018; Lois and Cowley 2017). The overlap between the habitats of the parasites and the fish hosts was shown to be important for parasitism control – when there was less overlap between them, the fish were parasitized less. This has two conservation implications – first, any anthropogenic activity is expected to have a negative effect on fish abundances, even when they are not fished. This means that fishing in natural areas is expected to negatively affect the entire system, as also discussed previously, and further reassures conservation and regulation efforts around river fishing industries. Secondly, parasites could live in the system, but the parasitism can be mediated by the overlap in the habitats of the fish and the parasites. This is crucial for fish farming areas: selected river zones should be picked if the parasite density there is low, or establishing a fish farm far away from areas where the parasite is naturally abundant. Obviously these are not completely air-tight solutions, but could be good indicators for farming and for parasitism control.

Furthermore, when the fish movement was negligible, sites remained as they are – sites with high or low prevalence remained as they were. When fish mobility increased, the prevalence was lower as distances between the site and the outlet was greater. This pattern (Carraro et al. 2018) is very similar to observed largely during the human Covid19 pandemic, and also agrees with the findings discussed in the early papers (Mouillot, Krasnov, and Poulin 2008; Vázquez et al. 2005). The implications for stocked fish which have low to no mobility between sites as they are confined to a fishpond, a single infected fish means a rapid spread to the entire fishpond, requiring meticulous surveillance to prevent trematode outbreaks. If the fish are harvested from a river and have higher mobility, it might be better to fish as close as possible to outlets, where the prevalence is lower. If fish are protected, it may be better to fish far from the river outlet to increase the chances that sick fish are taken out of the system. This could be embedded into disease control in river fish, borrowing similar strategies from conservation-poaching in mammals.

Summary (220 word count):

All papers discussed the importance of incorporating parasites into food webs and research in general. The information gained focuses on the effects of invasions and open niches available in fish-parasite systems, indicates that there are mechanistic similarities between bipartite systems

in land and water, and the general structure of networks before and after invasion and implying the dangers and approaches of invasion.

The main future directions should be invested in conservation and preventing invasion by borrowing tools used in terrestrial animal population and disease control. Furthermore, efforts should be put into finding efficient sampling methods for endoparasites which are a significant factor in changing food webs and could cause disease outbreaks which are difficult to manage once erupted.

There are many ways for looking and analyzing systems and opens a door to multidisciplinary research incorporating tools from hydrology, civil engineering and statistics into solving ecological questions. The variety of tools indicates how varied the questions asked and answered in the papers are, showing that network analyses methods are powerful tools which encourage different approaches to similar datasets.

Although the main purpose of this review was to study bipartite networks, there was a large emphasis on food webs, which concludes that even intimate interactions between two taxa cannot be isolated from their environment and a more holistic approach should be encouraged.

References (papers for review are numbered, miscellaneous are bulleted)

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