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## Identifying the Achilles' heel of multi-host pathogens: The concept of keystone “host” species illustrated by *Mycobacterium ulcerans* transmission

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### Abstract

Pathogens that use multiple host species are an increasing public health issue due to their complex transmission, which makes them difficult to mitigate. Here, we explore the possibility of using networks of ecological interactions among potential host species to identify the particular disease-source species to target to break down transmission of such pathogens. We fit a mathematical model on prevalence data of *Mycobacterium ulcerans* in western Africa and we show that removing the most abundant taxa for this category of pathogen is not an optimal strategy to decrease the transmission of the mycobacterium within aquatic ecosystems. On the contrary, we reveal that the removal of some taxa, especially Oligochaeta worms, can clearly reduce rates of pathogen transmission and should be considered as a keystone organism for its transmission because it leads to a substantial reduction in pathogen prevalence regardless of the network topology. Besides its potential application for the understanding of *M. ulcerans* ecology, we discuss about how networks of species interactions can modulate transmission of multi-host pathogens.

### 1) Introduction

Multi-host pathogens represent an increasing concern for human public health [1]. Among the infectious diseases considered as emerging, more than 60% use at least three different host species [2]. The increasing burden of these generalist infections, i.e., that can infect a wide range of host at each stage of life cycle, is especially threatening considering their sensitivity to current environmental fluctuations [3–5], e.g., biodiversity alteration [6,7].

Consequently, mitigating transmission of these pathogens is a research priority [1]. Nevertheless, such control efforts are known to be especially difficult and complex [8] since each host species can serve as a “refuge” for the pathogen, allowing its re-emergence after the end of a specific host species control effort. During the last several decades, public health consortiums have even claimed that pathogen eradication is not possible in the presence of complex cycles in wildlife, like for sylvatic cycles in Yellow Fever [8]. Today,

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the only attempt, and success, of elimination of these infections has concerned disease transmitted between domestic animals [9] where it was possible to target, by vaccination and/or by culling, the host species that are the most responsible for pathogen transmission. However, such a strategy does not seem possible for wildlife diseases, especially those embedded in speciose communities or in the environment [10,11].

An interesting alternative to control these infections would be to identify “critical point(s)” in the dispersal of these pathogens, *i.e.*, a species or a group of species that is mandatory in the transmission chain. Such critical point(s) exist for some diseases, especially those that are vector-borne where the insect life cycle requires blood meals. Identifying the critical point(s) in other disease systems offers the opportunity of control for pathogens [12]. It would be then especially useful to identify this kind of *Achilles’ heel* for multi-host pathogen infections involved within complex and heterogeneous networks of transmission.

The increasing recognition of ecological theory in infectious diseases literature is perfectly timed to address this problem. Indeed, parasitism is one of the possible types of ecological interaction between two species. Today, it is well demonstrated that many networks of ecological interactions contains some “critical points” [13,14], which are required species to sustain the whole connectivity within the community. Applying this concept of “keystone” species to the network of pathogen transmission would be especially relevant in an epidemiological context. A pathogen transmitted by many different host species could go through a given number of hosts that do not amplify the transmission, but are still required to connect the amplifying hosts between them.

Here, we aim to test the hypothesis that such multi-host systems can entertain one or several “keystone species” for pathogen transmission that can represent an *Achilles’ heel* for controlling disease. To reach this goal, we analyze the case of *Mycobacterium ulcerans*, a pathogen transmitted in aquatic environments through complex networks of ecological interactions [15], in 27 distinct aquatic communities in Ghana, western Africa [16]. We develop a mathematical model that we fit against the prevalence observed for 68 aquatic invertebrate taxa within each of these communities in order to estimate the networks of pathogen transmission for each of them. Then, we show that removing almost half of host taxa from our model is required to observe a significant decrease in pathogen prevalence in most aquatic communities. On the contrary, we show that removing just one host taxon, *i.e.* Oligochaeta worms, within and across aquatic localities, can decrease by almost 50% the prevalence predicted by our model. Thus, regardless of the network topology, removing of this taxon leads to a substantial decrease in prevalence. We conclude that this taxon can play a role of “keystone” hosts for *M. ulcerans* transmission. We then discuss the implications of these results for the control of such environmentally-persistent microbes and conclude that characterizing disease webs using community ecology can help to improve the knowledge of such pathogen transmission, and then contribute to mitigate their undesirable effects and stimulate future epidemiological studies.

## 2 Materials and Methods

### 2.1) Biological model

*Mycobacterium ulcerans* causes the disease Buruli ulcer that leads to serious skin ulcerations in humans. Despite the fact that this mycobacterium was first described 60 years ago [17], its ecology and life-cycle is still a matter of debate [15,18,19]. The path of *M. ulcerans* transmission to humans remains unclear and two main hypotheses have been proposed [20–23]. The first one implies that the microbe is transmitted through the aquatic environment, and *M. ulcerans* could infect humans who have frequent contact with contaminated water through swimming or through body injuries that may facilitate the

introduction of the microbe into the skin [20,24]. The second hypothesis, which has received particular attention in recent years despite the absence of ecological evidences to support it [21], suggest that *M. ulcerans* could be transmitted through the bite of aquatic bugs [19,22,25]. The accumulation of many controversies in the characterization of different potential host species for *M. ulcerans* transmission with time [15] suggest that this mycobacterium could be transmitted among both a large host spectrum, which are embedded within networks of heterospecific interactions within aquatic environments, and a diversity of more or less heterogeneous species communities.

## 2.2) Data

The study sites, invertebrate field sampling, and *M. ulcerans* detection methods have been detailed elsewhere [26], and are briefly described hereafter. In June 2004 and August 2005, 27 waterbodies associated with reported Buruli ulcer human communities in southern Ghana were sampled (summary of this dataset is given in supplementary materials S5).

Within each waterbody, two 10 – 20 m transects were measured parallel to the shoreline and positioned through the dominant macrophyte plant community. Along each transect we randomly placed two 1m<sup>2</sup> PVC quadrats and collected invertebrates by sweeping within the quadrat with a 500  $\mu$ m mesh dipnet. Three sweeps of the dipnet were performed from the water surface to the bottom substrate for comprehensive sampling of specimens in the water column. The two quadrats were combined into a single composite sample. All contents were washed through a 500  $\mu$ m sieve and preserved in 100% ethanol for laboratory identification and PCR assays.

Samples were analyzed in a two-step procedure described in detail by Williamson et al [26]. Small invertebrates were analyzed in pools of 3–15 individuals, whereas larger specimens were tested individually. DNA was extracted using a protocol adapted from Lamour and Finley [27]. Presumptive identification of *M. ulcerans* in invertebrates was based on PCR detection of the enoyl reduction domain (ER) in *mlsA* that encodes the lactone core of the mycolactone toxin, the major virulence determinant of *M. ulcerans*. All samples were screened for the presence of the ER gene, which has been evaluated for *M. ulcerans* by Williamson et al. (2008).

Hereafter, we use the terminology of host taxon to refer to host “carriage” taxon that may participate in *M. ulcerans* life cycle. They may, or may not, allow mycobacteria proliferation in their body, or simply house those cells onto or inside the body as a passive material without any duplication.

## 2.3) Epidemiological model

If the assumption of that transmission of *M. ulcerans* is well described by a Susceptible, Infectious and Recovered (SIR) within the aquatic community, in which individuals are classified according to their infectious status [28,29], the following multi-host model can be used to analyze its transmission among a network of different hosts:

$$\frac{dS_i}{dt} = b_i N_i - \sum_{j=1}^n \beta_{ij} I_j S_i - d_i S_i \quad (1)$$

$$\frac{dI_i}{dt} = \sum_{j=1}^n \beta_{ij} I_j S_i - (d_i + \gamma) I_i \quad (2)$$

$$\frac{dR_i}{dt} = \gamma I_i - d_i R_i \quad (3)$$

Where index  $i$  represents a given taxon, and  $n$  is the number of taxa in the local community. This model follows the tradition of mathematical modeling of micro-parasites infecting different kind of hosts [28,29]. Each (host or carrier) taxon has specific birth and death rates ( $b_i$  and  $d_i$ ) that are quantified through allometric laws (see Supplementary materials and [30]). Since the presence of immune reactions against *M. ulcerans* in invertebrate aquatic organisms is still enigmatic [31], our model analysis explores the possibility of no immunity ( $\gamma=0$ ) or short immunity ( $\gamma=7 \text{ days.ind}^{-1}$ ).

#### 2.4) Network of disease transmission

Our model assumes that pathogen is spreaded through a transmission network, characterized by the matrix  $\beta_{ij}$ , which is different for each local ecological community studied. These networks consider only pathogen transmission, and thus do not involve other direct interaction between each taxa (i.e., abundance of each taxa are assumed to be independent from each other).

Our networks are designed through two parameters (schematized at the top of figure 1): number of different levels in the network ( $p$ ) and number of inter-connected levels ( $\delta$ ). First, we assume that the different aquatic taxa are distributed along  $p$  different levels according to their abundance (less abundant taxa are at the top of network). The number of present levels,  $\rho$ , goes from 1 to  $n$ , allows to produce a gradient between the case where each taxon is situated at an unique level to the case where each taxon is situated at different levels. Within a given level, we assume that each taxon is connected to all other taxa of  $\delta$  lower and upper levels. Then, the number of inter-connecting levels,  $\delta$ , goes from 1 to  $\rho$  to yield a gradient between a situation where each taxon is connected to only the levels closest to it to the situation where all levels are connected together. Finally, for the sake of simplicity, we assume that all inter-taxon transmission rates are equal between them.

This network generation can yield a very wide range of networks topologies wherein food web is a very specific case [32]. However, it is worth mentioning here that we consider “disease transmission web” as being completely independent of the “food web”. Indeed, predation is definitely not the only possibility of pathogen transmission between two host species [33], so mycobacterium spillover will not necessarily match with energy fluxes between species from prey to predator.

#### 2.5) Fitting the model to empirical data

Taxon abundances are given by our dataset, allometric laws are used to estimate birth and death rates (Supplementary materials) and two cases of recovery are considered (with and without immunity). Consequently, for each locality present in our study, we have to estimate four parameters relative to the construction of transmission networks: (i) inter-taxon contact rate ( $\beta_{ij}$ ), (ii) intra-taxon contact rate ( $\beta_{ij}$ ), (iii) number of levels within the network ( $\rho$ ), and (iv) number of inter-connected levels ( $\delta$ ). Then, we compare iteratively the maximal pathogen prevalence predicted for each aquatic community by our model with the prevalence observed in the field to estimate the correct network of pathogen transmission for each locality (see Supplementary materials for the estimation algorithm).

### 3) Results

#### 3.1 Model fitting

Networks of pathogen transmission for each location in Ghana have been estimated through our epidemiological model (Figure 1). The resulting prevalences across aquatic taxa are strongly correlated with the observed ones, whatever the assumption about immunity (with immunity:  $r=0.9761$ ,  $p\text{-value}<0.0001$ , and without:  $r=0.9975$ ,  $p\text{-value}<0.0001$ ). Nevertheless, considering a likelihood error structure in order to derive an AIC (see supplementary materials) does not allow rejecting any hypothesis regarding immunity or not. Consequently, both situations are considered in the present work.

#### 3.2) The influence of removing many host taxa on pathogen transmission within each community

Here, we aim to understand how many host species have to be removed in order to significantly decrease pathogen prevalence within aquatic localities. We use the ratio between disease prevalence with all (host) taxa and disease prevalence without a proportion of the less abundant (host) taxa from each local community. We analyze especially how the complexity of transmission networks, characterized by the ratio between the number of levels  $\rho$  and the number of connected levels  $\delta$ , acts on the number of host taxa that have to be removed.

Figure 2 shows that local aquatic communities with many transmission pathways between host taxa (i.e. that would occur with high species number in localities) are more robust when facing removal of host taxa. Indeed, a higher number of links within this category of network allows maintaining an efficient transmission despite lower community abundance due to the removal of the less abundant taxa. In other words, the higher complexity within this type of network allows mycobacterial transmission through “secondary” pathways even though an important (host) taxon was removed from the system.

#### 3.3) The influence of removing one host taxon on pathogen transmission over all aquatic communities

Here, we wish to understand whether the loss of some specific (host) taxa could significantly decrease *M. ulcerans* prevalence across the different communities. To quantify this impact, we calculate, from the mathematical model detailed in the main text, the number of infectious individuals in each community with and without the focused host taxon, that we average across all communities. The resulting ratio gives the proportional decrease of prevalence due to the removal of this taxon.

We observe that the most frequent taxa, *i.e.*, present in many aquatic communities across the 27 different sites, are not necessarily those that have strongest influence on pathogen transmission (Figure 3A,C). This lack of relationship is somewhat surprising since removal of taxa that are very frequent can decrease prevalence within all communities while less frequent taxa do not. On the other hand, it shows that some other taxa (Figure 3B,D) are very important for *M. ulcerans* transmission since their removal within their host community leads to a very sharp decrease in pathogen prevalence.

Figure 3 also shows that one host taxon in particular has a strong impact on pathogen prevalence across the 27 different aquatic sites, whatever we accept or not an immunity against mycobacteria in invertebrates. The removal of the Oligochaeta taxon led to a 15% decrease of disease prevalence across communities in the model without immunity, and to a 55% reduction in the one with immunity.

#### 4) Conclusion and Discussion

In this paper, we have demonstrated that removing one “keystone” taxon, the Oligochaeta, can dramatically decrease prevalence of *M. ulcerans* across the different aquatic communities. Then, we have fitted a mathematical model against this dataset to estimate the networks of pathogen transmission (Figure 1). These parameterized networks have been used to show that many different host taxa have to be removed to significantly decrease the prevalence of the mycobacterium within each aquatic community (Figure 2). But, we have also shown that removing one single taxon, the Oligochaeta worms, can significantly decrease pathogen prevalence - by 15% to 55% - among all the aquatic communities (Figure 3).

Our study relies on the assumption that *M. ulcerans* is transmitted through a web of ecological interactions between potential host carriers in the aquatic environment. So, depending on the ecological complexity in space and time and changes in the composition of aquatic communities, transmission of *M. ulcerans* in nature should take different pathways. This first application of a mathematical model to the study of *M. ulcerans* transmission in aquatic ecosystems may be considered too simple and other routes of transmission may be important [15], especially direct acquisition from water or soil where the mycobacteria would be environmentally-persistent micro-organisms. In the supplementary materials, we show that considering this route of transmission, i.e., free mycobacterium particles in water, in our model shows significantly lower fidelity to prevalence values observed ( $r=0.90$ ,  $p\text{-value}<0.05$  without immunity, and  $r=0.30$ ,  $p\text{-value}=0.6851$  with immunity). Consequently, we consider the model with transmission within ecological webs, notably through organism-to-organism contacts, as the most likely scenario of transmission according to the field observations.

For the case of *M. ulcerans*, the keystone property of the worm taxon Oligochaeta makes sense from an ecological point of view. This taxon is one of the most abundant and most common taxon within and across studied localities, but some other taxa could have been intuitively better keystone taxa than the Oligochaeta, especially Baetidae or Chironomidae that are among the most abundant taxonomic groups. This finding underlines once again the high potential of network models in infectious disease ecology. Due to its major place within the network, the removal of Oligochaeta individuals led to a dramatic decrease of *M. ulcerans* prevalence in aquatic communities. Interestingly, this (host) taxon was the only one found to be infected in field conditions during microbiological trials in previous works [34]. More experimental studies are clearly needed, nevertheless our work suggests that this category of aquatic host organisms, and to a lesser extent some other taxa exhibiting a similar pattern, such as the Nematode roundworms, may play a central role in the transmission pathways of *M. ulcerans* in Africa.

Thus, ecological niche of Oligochaeta worms, and to a less extent of Nematodes, in disease webs allows these categories of host taxa to act as a major source of infection for other taxa in the web. Aquatic nematodes are generally grazers feeding upon bacterial films and free protozoans at the bottom of the water column, and aquatic Oligochaeta worms are limivorous organisms that feed upon algae and detritus found on the mud. These feeding styles might contribute to accumulate mycobacterial cells from mud and facilitate its transmission through concentration in disease-webs.

From a multi-host pathogens point of view, we have shown that most efficient target for removal, in terms of disease control, is not always the most common host species, nor the host species with the highest prevalence of the mycobacterium (Figure 3). This finding, despite still needing to be confirmed on other aquatic ecosystems and in both epidemic and

endemic sites for Buruli ulcer, is clearly encouraging because it opens new doors for mitigating epidemics of such systems. Even if controlling *Oligochaeta* in the case of *M. ulcerans* seems not to be applicable today, the environmental conditions that may favor this taxonomic group to flourish should do. In addition, some other host-disease systems like rodent-borne diseases could benefit from such an approach.

Our results have to be considered within the existing literature on pathogen transmission through networks [35]. Indeed, numerous studies have shown that pathogens are transmitted through networks at very different scales, from small communities [36] to a worldwide scale [37]. At the scale of ecological communities, a lot of work has been done regarding pathogen transmission within food webs [38]. In such a case, it has been demonstrated that some host species are critical to maintain parasite diversity and, consequently, host diversity because of the structuring role of parasites in food webs. In our study, we show that some host species are crucial for pathogen transmission, even without structuring host communities. We believe that this is an important result because it opens perspectives for disease control without, potentially, altering irreparably host diversity locally.

As shown here for the transmission of *M. ulcerans*, networks of species interactions could be a central component of disease transmission for many pathogens with an indirect life cycle or multiple host solutions. From an ecological perspective, disease transmission through these networks has, to our knowledge, never been demonstrated in previous studies. However, Lafferty et al. [38,39] have shown that parasite taxa are key components in the stability of salt-marsh communities as they improve the connectivity of "food-webs" in those ecosystems. Our study, on the other hand, demonstrated how the characteristics of the community of taxa (i.e., community organization and/or presence of keystone taxa) could affect the transmission of a pathogen within a network of species interactions. Another important aspect not studied in the two previous research works is the fluctuation in population dynamics of the different taxonomic groups. Further studies should combine these approaches to give a more global picture of the real interactions between parasites and their host taxa communities.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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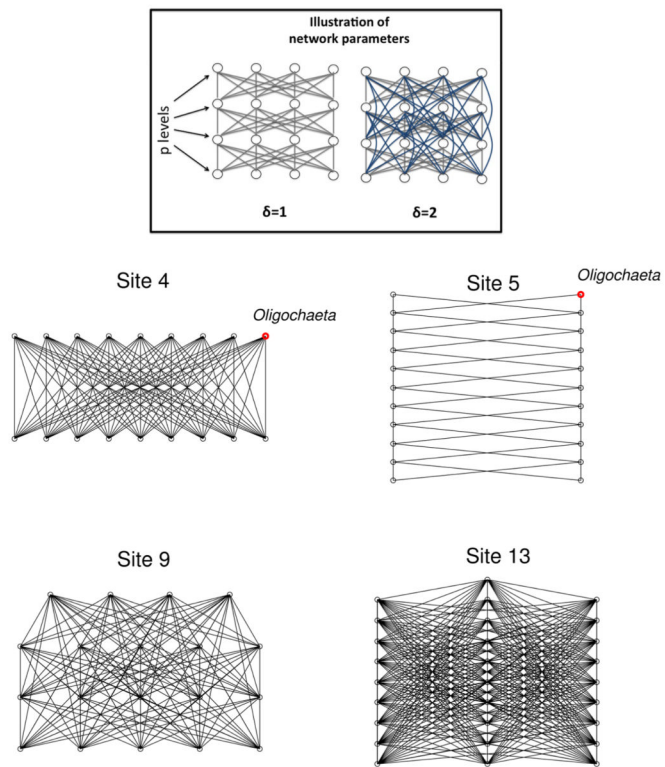
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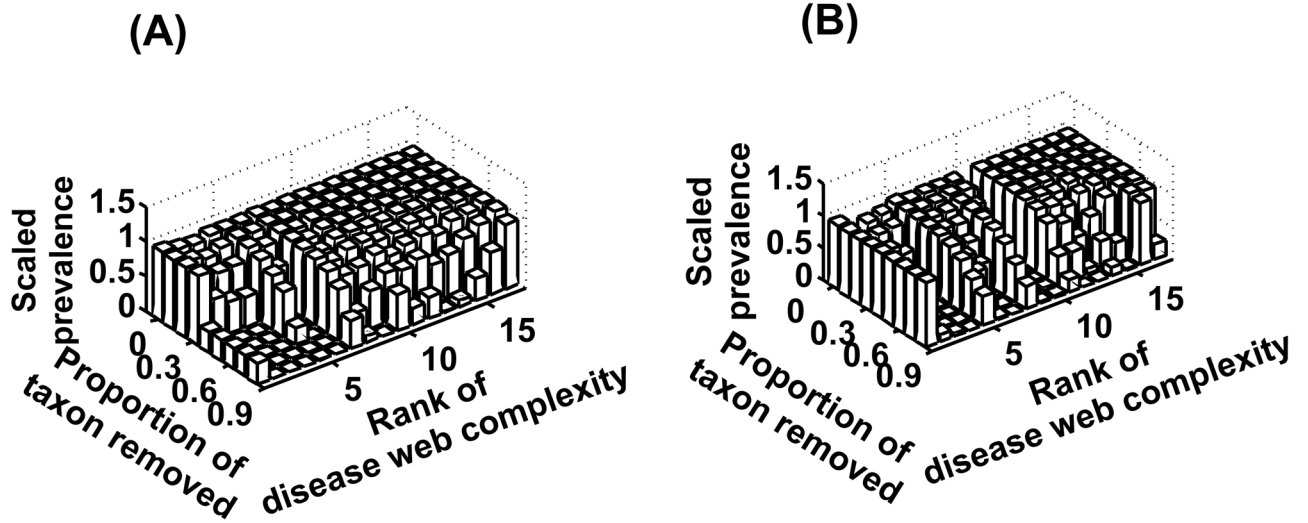
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**Figure 1.** Illustration of pathogen transmission networks. (Framed) Illustration of the network parameters used to construct network models ( $p$  levels and  $\delta$  number of inter-connecting levels). The blue lines represent the difference between  $\delta=1$  and  $\delta=2$ . (Bottom) Examples of pathogen transmission network between species estimated for four different sites selected because they represent a large variety of network topologies encountered in our data. (A)  $\rho = 2$ ,  $\delta = 2$ , (B)  $\rho = 20$ ,  $\delta = 1$ , (C)  $\rho = 4$ ,  $\delta = 4$ , (D)  $\rho = 12$ ,  $\delta = 11$

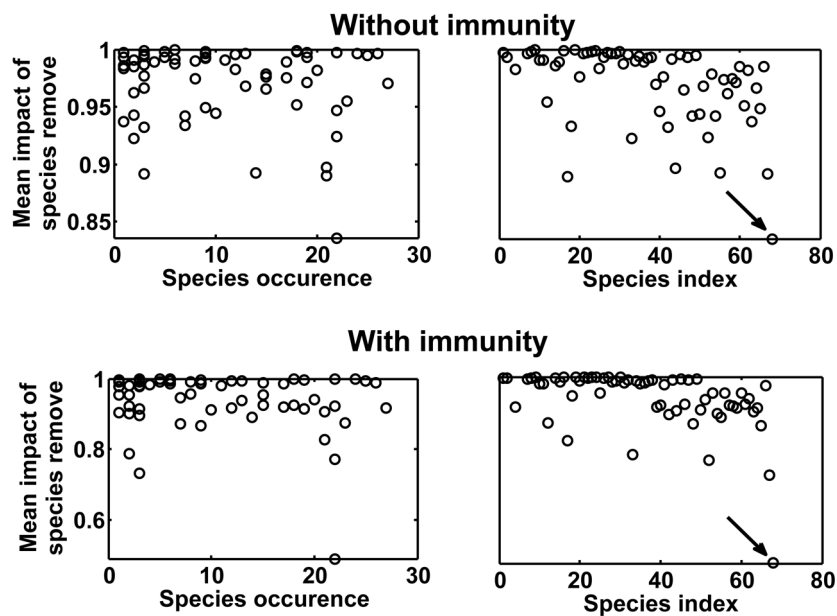
## Without immunity

## With immunity



**Figure 2.**

Stability of *M. ulcerans* transmission across the different aquatic communities in the network transmission models without (A) and with immunity (B). Scaled prevalence represents the decrease of pathogen prevalence within each local community after removing a given proportion of the less abundant host taxa.



**Figure 3.** Relationships between the “mean impact of taxon removal”, representing proportional decrease of disease prevalence across all communities, and (A,C) occurrence of host taxon (number of times where this taxon has been observed among the 27 aquatic communities) or (B,D) their identity. Arrows indicate the Oligochaeta taxon (see main text for further details).