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Best practice guidelines for studies of parasite community ecology

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Review Article

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Abstract

In recent decades, parasite community ecology has produced hundreds of studies on an ever-growing number of host species, and developed into an active sub-discipline of parasitology. However, this growth has been characterized by a lack of standards in the practices used by researchers, with many common approaches being flawed, unjustified or misleading. Here, in the hope of promoting advances in the study of parasite community ecology, I identify some of the most common errors or weaknesses in past studies, and propose ten simple rules for best practice in the field. They cover issues including, among others, taxonomic resolution, proper and justifiable analytical methods, higher-level replication, controlling for sampling effort or species richness, accounting for spatial distances, using experimental approaches, and placing raw data in the public domain. While knowledge of parasite communities has expanded in breadth, with more and more host species being studied, true progress has been very limited with respect to our understanding of fundamental general processes shaping these communities. It is hoped that the guidelines presented here can direct researchers away from the entrenched use of certain approaches flawed in design, analysis or interpretation, by offering a more rigorous and standardized set of practices, and, hopefully, a way forward.

Introduction

Animals are typically infected by a suite of ecto- and endoparasite species that form distinct assemblages with well-defined physical boundaries, replicated units (host individuals or populations), and a total species richness that can usually be quantified precisely. Parasite communities therefore provide good model systems to explore various questions regarding interspecific associations or interactions, community structure, and the determinants of species richness or diversity. Since its establishment as a research sub-discipline within ecological parasitology (Holmes, 1973; Holmes & Price, 1986; Esch *et al.*, 1990), parasite community ecology has expanded and generated hundreds of studies on an ever-increasing number of animal host species, mostly vertebrates.

A limited set of basic questions are frequently addressed in parasite community ecology. What individual host properties (size, age, sex, dietary preferences, etc.) influence the richness of infracommunities, i.e. all parasites within an individual host? Are infracommunities structured following some recognizable pattern, or do they represent random assemblages of the parasites locally available? What role do intra- and interspecific interactions among parasite species play in structuring their communities? How do different parasite species partition niche space within the host? What environmental characteristics correlate with the richness of parasite component communities, i.e. all parasites in a host population, across different localities? How do parasite community measures vary in time, i.e. across seasons or years? These have been the main recurring questions driving most research in this area.

However, the increasing number of studies on parasite communities has been accompanied by a lack of standards in the practices used by researchers. A diversity of approaches and perspectives is generally good for scientific advances, but can also impair progress if certain approaches are flawed, unjustifiable or misleading. In this short essay, based on my reading of the literature and years of acting as a reviewer and editor, I offer ten simple rules for best practice in research on parasite community ecology, and more generally research in ecological parasitology. This list is certainly not exhaustive, and other researchers will no doubt disagree with certain recommendations and think of others that should have been included. Yet I believe that the guidelines provided below can serve to avoid some of the common errors and misconceptions I have seen proliferating in the literature in the past several years, as well as encourage alternative ways of addressing the key issues. My hope is that they will serve to standardize the practices that should be standard, and weed out the use of some of the more questionable approaches that have plagued progress in the field.

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Achieve the highest possible taxonomic resolution

In community ecology, the basic units of study are genetically distinct populations that exist in sympatry and potentially interact. In other words, community ecology deals with distinct species. In recent years, surveys of parasite communities in vertebrate hosts have achieved relatively poorer taxonomic resolution than older surveys. Indeed, a greater proportion of parasite taxa have been identified only down to genus level, sometimes only to family level, in surveys published since the year 2000 than in previous decades (Poulin and Leung, 2010). Identification problems are an issue particularly for larval stages, such as juvenile cestodes or trematode metacercariae; these are often left out of ecological studies for this reason, though when properly identified their inclusion can greatly affect estimates of diversity (Vidal-Martinez *et al.*, 2012). Parasite species identification is not easy; it demands time and taxonomic expertise. Yet, resolving patterns in community structure requires that the basic units of study be delimited as precisely as possible. Poor taxonomic resolution in parasite community studies can result in underestimated species richness, inaccurate diversity estimates, overestimated similarity between distinct communities, and/or underestimated host specificity. Cryptic species that are morphologically indistinguishable without molecular markers will plague parasite community ecology for the foreseeable future (Pérez-Ponce de León and Nadler, 2010; Pérez-Ponce de León and Poulin, 2018). However, this is no excuse for the recent trend to conduct analyses on parasite communities with inadequate taxonomic resolution.

Treat intensity/abundance of infection as count variables

The number of parasites per host is a count variable that can take the values 0, 1, 2, 3…*n*, where *n* represents the maximum observed number of parasites in an individual host. It is totally inappropriate to force these counts into arbitrary categories (except perhaps in figures, for illustrative purposes). For instance, some authors create infection classes by lumping together hosts with certain numbers of parasites, e.g. those with light infections (1–5 parasites per host), moderate infections (6–10 parasites) and heavy infections (>10 parasites). This may seem convenient to determine, for example, whether host parameters, such as body condition, vary between infection classes. However, there is no biological justification to use 5 or 10 parasites per host as threshold boundary values between classes. Using different values (for example 1–3, 3–8, and >8 parasites) could change the outcome of statistical comparisons between classes. The only acceptable approach is to always treat count variables as they should be, and analyse them using appropriate statistical tools, such as non-parametric tests (because of their typically skewed distribution), or better yet, generalized linear models in which intensity data have quasi-Poisson, negative binomial or zero-inflated error distributions.

Do not combine parasite taxa

Several studies report prevalence or intensity of infection based on all parasite species pooled together. That is completely inappropriate. Terrestrial ecologists would never report the density of individual vertebrates in an area by pooling together elephants, mice, birds, frogs and snakes; that would make no sense. Yet it is not uncommon for parasitologists to pool helminths belonging to different phyla for an overall value of intensity, for example in order to determine whether intensity of infection correlates with host body size. Different parasite taxa have different sizes and virulence, they are acquired and lost in different ways, and they should be treated as different entities. Prevalence, abundance, intensity or any other measure of infection should always be calculated separately for each parasite taxon. The only instance when pooling species to calculate total intensity of infection (or total parasite biovolume/biomass) would be acceptable is when (1) the question posed justifies pooling and (2) the species being combined are either taxonomically very close (i.e. congeneric species) or ecologically equivalent in their body sizes, mode of feeding, and/or known pathological effects.

Choose the analytical approach that best suits the hypothesis

Several studies of parasite communities are presented as descriptive surveys, without a clear hypothesis and testable prediction(s). Sometimes the hypothesis is implied, but ideally it should always be stated explicitly. Descriptive studies continue to be highly valuable, of course, and they do not need to test any explicit hypothesis. Regardless of whether a hypothesis is actually mentioned or not, authors of parasite community surveys generally test for possible patterns derived from some *a priori* expectation. However, there is often a lack of justification for the routine analyses conducted or the particular measures reported. For example, it has become common to report one or more of the following: the proportions of core and satellite species, or of autogenic and allogenic species, or of specialist and generalist species, in the parasite community; diversity indices (e.g. the Brillouin index); a species dominance index; or a subjective statement regarding whether the community is isolationist or interactive. These and other practices have become *de rigueur* in the field, followed not because they are relevant to the main question addressed in the study but simply because others have applied these concepts in the past. This achieves nothing; it merely muddles things up. Parasite community ecology will move forward faster if new studies abandon this formulaic approach and instead either (1) test explicit hypotheses with only the most relevant and appropriate analytical tools, or (2) focus on high-quality descriptive data (made publicly available; see later section) with no accompanying analysis lacking justification.

Consider the influence of species richness on measures of community structure

The search for non-random assembly in parasite communities requires demonstrating that patterns of species presence/absence or relative abundances depart from those expected from stochastic processes guided by chance alone. For instance, the sets of parasite species co-occurring in individual hosts (parasite infracommunities) may not be random subsets of the entire pool of parasite species infecting the host population (component community). Non-random patterns of co-occurrence, like those corresponding to nestedness (e.g. Worthen and Rohde, 1996; Norton *et al.*, 2004), can be tested by comparing observed patterns to those predicted by null models. Alternatively, non-random covariation between the abundances of different parasite species among individual hosts can be detected by testing for significant positive or negative correlations between the intensities of pairs of parasite species. However, the probability of observing significantly nested patterns of co-occurrence is influenced by the

species richness of the total pool of species or that of the infracommunity subsets (Wright and Reeves, 1992; Hu *et al.*, 2011). Similarly, the likelihood of obtaining significant correlations between the intensities of pairs of parasite species across infracommunities is also dependent on how many pairs of species are tested, i.e. it depends on species richness (Poulin, 2005). The reason is that when testing all possible pairwise correlations in abundance across host individuals among three or more parasite species, the magnitude of individual correlation coefficients is mathematically constrained, i.e. the actual range of possible coefficient values is less than −1 to +1 (see Brown *et al.*, 2004). Thus, in many cases conclusions derived from analyses of parasite community structure, especially for species-poor parasite communities, need to be drawn with greater caution than they have been in numerous past studies.

Aim for an experimental approach

Correlation does not imply causation. In the context of parasite community ecology, a statistically significant association between two parasite species in natural infections does not automatically imply an interaction between the two species. For example, a negative correlation between the abundance of two species does not necessarily mean they are competing for resources within the host. The only way to ascertain that low abundance of one species is caused by the presence of the second species would be to demonstrate it experimentally. The logistics of obtaining infective stages of two or more parasite species and experimentally infecting hosts to create parasite communities make this approach impossible in many cases. However, it should not be dismissed without serious consideration. Experimental parasite community ecology is not a new approach (e.g. see Holmes, 1961), but has received renewed attention lately (Benesh and Kalbe, 2016; Budischak *et al.*, 2016). Whether it is to confirm the existence of pairwise competitive interactions or to test for priority effects, in which the final community structure depends on the order in which parasite species arrive in a host, experimental infections represent the most powerful approach in the parasite community ecology toolkit. Their use needs to become more common for the field to make serious progress.

Achieve appropriate higher-level replication

When testing for seasonal differences in the richness or composition of parasite communities in a host population (e.g. comparing wet versus dry seasons, or summer versus winter), the unit of replication is the season. Thus, when a study conducted over a single year contrasts the parasite community in a host population between two distinct seasons, there is no true replication. Even if the host sample sizes are large in each season, the comparison still involves only one dry and one wet season. Individual hosts are not truly independent of each other in this kind of study, and only data from other years would provide replication. The same applies to comparisons between populations of the same host species living in different habitats, such as forested versus cultivated habitats, or pristine versus polluted aquatic habitats. Replication in these cases would require multiple independent host populations (i.e. host samples from different localities) representing each habitat type. Without proper replication at the higher spatial or temporal level (and not just at the individual host level), the inferences one can draw from the results are very limited.

Consider distances between sampled host populations

Obtaining samples from multiple host populations in each category being compared (e.g. from several fish populations in polluted habitats and several in pristine habitats) provides replication, but does not guarantee that the replicate populations are independent. The geographical distance between two host populations influences their similarity in terms of the composition of their parasite communities (Poulin, 2003; Poulin *et al.*, 2011). Because the similarity decays exponentially with increasing distance, two populations can be considered independent in a statistical sense only if they are separated by a distance greater than some system-specific threshold, typically in the order of tens or hundreds of kilometres. Studies involving comparisons of parasite communities among different host populations should either (1) confirm that the pairwise similarity in the composition of parasite communities does not vary significantly with inter-population distance, (2) test the independence of the sampled host populations via spatial autocorrelation (e.g. using geostatistical tools such as correlograms and Moran's index) or (3) include inter-population distance as a covariate in the analysis testing for the effect of the main variable(s), for instance using multiple regressions on distance matrices.

Account for variable sampling effort

Many studies aim to compare parasite communities between different samples of hosts, such as samples of the same host population but from different seasons, or samples from different host populations. These samples should not only be random and representative subsets of individual hosts, i.e. capturing the sex ratio and age/size distribution of the host population at large, but ideally they should also include comparable numbers of hosts. Estimates of many variables that can be compared between host samples, such as the species richness of the communities (Walther *et al.*, 1995), and the prevalence, mean intensity or mean abundance of infection of particular parasite species (Gregory and Blackburn, 1991; Gregory and Woolhouse, 1993; Poulin, 2007) are well known to be influenced by host sample size. If the number of hosts examined is the same in all samples, then there is no problem. However, sample sizes usually vary among samples, sometimes substantially, and this is regularly ignored during analysis. The only time raw species richness can be used in comparisons among samples is when it can be shown that species accumulation curves have reached a plateau for the given number of hosts examined in each sample (Dove and Cribb, 2006). Otherwise, either sample size is included as a covariate in the analyses, or non-parametric estimates of true richness must be used instead of raw species richness values (Poulin, 1998). Similar adjustments are needed for among-sample comparisons of prevalence, mean intensity or abundance of infection, or any other community parameter.

Place raw data in the public domain

Animals have to die for us to obtain data on their parasites. This sacrifice should yield as much valuable information as possible. In addition, funding agencies often require data generated through their grants to be released publicly, because at its source the funding comes either from taxpayers or from philanthropic donations. An increasing number of scientific journals also demand that the raw data from articles they publish be fully

available to their readers. If these are not good enough reasons, making data publicly available also benefits scientific progress. For example, following the publication of a study on the parasites of a particular host species, other researchers may be able to address more specific questions using parts of the original data, or use the dataset in a meta-analysis. It is a simple process to make the data available either as supplementary material linked with the published article, or in a digital repository such as Dryad (https://datadryad.org/), to allow its use long after the study is finished.

Conclusions

Parasite community ecology has the potential to make important contributions to many research areas, from the search for the underpinnings of biodiversity to the design of control strategies for parasitic diseases of wildlife. Parasite communities are also used as biological markers to distinguish between harvested host populations (Cantatore and Timi, 2015; Poulin and Kamiya, 2015) and as sentinels of environmental change (Sures, 2004; Marcogliese, 2005; Vidal-Martinez *et al.*, 2010). Yet in spite of thousands of individual animals sacrificed specifically to survey their parasite assemblages, what have been the major advances in the field of parasite community ecology over the past 10 or 20 years? This may sound harsh, but I cannot think of any. Our knowledge is expanding in breadth (more and more host species being studied) but not moving forward. On one hand, this may be because patterns in parasite communities are so idiosyncratic and unpredictable that general principles are difficult to uncover. But on the other hand, without some lucky break, only rigorous and appropriate methodology can lead to important new discoveries. The process of dealing with samples from a new parasite community has become entrenched, with errors in design, analysis or interpretation being repeated endlessly. The guidelines presented here can remedy some of the most common flaws plaguing many studies of parasite communities, as well as emphasize different approaches; on their own, they will not lead to major breakthroughs, but hopefully they offer a way forward.

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