A macroecological approach to understanding parasite aggregation

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Abstract

A few hosts have many parasites while many hosts have a few parasites - this axiom of macroparasite aggregation is so pervasive it is considered a general law in disease ecology, with important implications for the dynamics of host-parasite systems. To understand the factors affecting the distribution of parasites across hosts, the traditional approach has been to use a "bottom-up" strategy in which aggregation mechanisms are sequentially added to a null model until the observed level of aggregation is achieved. Macroecology has recently taken an alternative approach by implementing a variety of "top-down" modeling techniques that attempt to predict observed aggregation using only a set of known constraints. Using an extensive dataset of 842 amphibian host-trematode parasite distributions, we show that the top-down approach provides a robust null hypothesis for host-parasite aggregation, correctly predicting observed parasite aggregation where bottom-up null models fail. Moreover, we show that when a top-down model fails it can be extended to account for known aggregating mechanisms such as host-heterogeneity and disaggregating mechanisms such as parasite-induced host mortality. This allows for a coherent and flexible synthesis of the top-down approach with the more commonly used bottom-up approach, thereby offering a powerful tool for understanding parasite aggregation.

Introduction

The discipline of disease ecology has traditionally emphasized mechanistic descriptions of infection patterns (Anderson and May, 1978; Duerr et al., 2003; Poulin, 2007). One particular pattern observed in macroparasites, such as parasitic helminths and arthropods that do not directly reproduce within their host (Anderson and May, 1979), is that many hosts in a population tend to have few parasites and a few hosts tend to have many. In statistical parlance this means that parasites tend to be aggregated within their hosts. This pattern is so ubiquitous in parasites that is has been called one of the few general laws in disease ecology (Poulin, 2007).

Canonical models of host-macroparasite dynamics have illustrated that a balance between parasite pathogenicity (α) and parasite aggregation plays an important role in the ability of a parasite to regulate a host population (Anderson and May, 1978; Tompkins et al., 2002). If parasites are highly aggregated and highly pathogenic, they cannot regulate a host population because parasites will be concentrated in a few hosts who will eventually experience parasite-induced mortality, extirpating the parasites from the host population. Similarly, models show that when parasites are nearly uniformly distributed among hosts and highly pathogenic they also cannot stably regulate a host population (Anderson and May, 1978). In general, the stability of a host-parasite system and the regulation of a host population by parasites requires some level of parasite aggregation and that parasite pathogenicity is not too high. Because of the importance of parasite aggregation, much empirical and theoretical work has been devoted to understanding both the mechanisms that can lead to aggregation in host-macroparasite systems (Anderson and Gordon, 1982; Wilson et al., 2002; Raffel et al., 2011) and how to infer the dominant mechanisms structuring a host-parasite system from observed aggregation patterns (Duerr et al., 2003; Grear and Hudson, 2011; Wilber et al., 2016).

Traditionally, studies of macroparasite aggregation have solely relied on explicitly modeling various mechanisms that affect aggregation patterns (henceforth the "bottom-up" approach, Anderson and Gordon, 1982; Isham, 1995; Chan and Isham, 1998; Pugliese et al., 1998; Rosà

and Pugliese, 2002; Rosà et al., 2003; Grear and Hudson, 2011). While the bottom-up approach has usefully illuminated various aggregating and disaggregating mechanisms in host-parasite systems (summarized in Wilson et al., 2002), it presents a number of difficulties. First, it is often challenging to empirically measure these aggregation mechanisms in host-parasite systems, making it difficult to compare predicted levels of aggregation with those empirically observed. Second, the bottom-up approach for describing aggregation suffers from the "many-to-one problem" inherent in much of ecology (Frank, 2014): there are many bottom-up models that can result in similar levels of parasite aggregation making it impossible to identify the specific processes leading to aggregation from patterns alone.

In contrast to disease ecology, the field of macroecology has often used various statistical rules and constraints to predict ecological patterns (henceforth a "top-down approach", Conlisk et al., 2007; McGill and Nekola, 2010; Harte, 2011; Marquet et al., 2014). The top-down approach does not propose that more traditional biological mechanisms are not acting in a system; it contends that the combination of all of these mechanisms leads to patterns of aggregation that have predictable statistical properties (Frank, 2009; McGill and Nekola, 2010; Frank, 2014). The predictions from various top-down approaches have had much success in describing levels of aggregation observed in empirical free-living populations and communities without any explicit mechanistic assumptions (White et al., 2012; Locey and White, 2013; Newman et al., 2014; Xiao et al., 2015; Harte et al., 2015). This is important because these models can then be used as robust null models (i.e. models that do not trivially fail) upon which other theories and predictions can be built. For example, Kitzes and Shirley (2015) use top-down, maximum entropy models of species abundance distributions and species spatial distributions to predict biodiversity loss due to dam construction. Despite these advantages, this top-down approach has not been used to predict the aggregation patterns in host-parasite systems.

It is, however, important to highlight a few disadvantages of the top-down approach. First, many successful top-down approaches used in macroecology are not dynamic and their predictions are limited to static patterns (Harte, 2011; White et al., 2012; Locey and White, 2013).

However, this is not a limiting feature of the top-down approach *per se*, rather a limitation of the current top-down approaches available. Dynamic top-down approaches to classical ecological problems are currently and will continue being developed (Neill et al., 2009; Zhang and Harte, 2015). Second, because these top-down approaches do not explicitly invoke biological mechanism in their predictions, they can not provide ecological insight as to why a particular pattern emerges. This is not as limiting as it may initially appear for three reasons. First, the reliance on using ecological and biological mechanism as sole descriptors of a system's patterns can ignore the fact that less traditionally considered statistical mechanisms can also play a role in structuring pattern (McGill and Nekola, 2010). For example, the pattern of free-living individuals being less aggregated in larger areas may be more a result of the central limit theorem than any particular biological mechanism (Conlisk et al., 2012). Second, there are many instances in ecology where the mechanisms leading to a particular pattern observed are neither unique nor of direct importance, but rather what is important is the behavior of the system given the observed pattern. For example, while substantial work has explored the particular mechanisms leading to host-parasite distributions, a negative binomial distribution is a common phenomenological assumption that makes it easy to develop rules of thumb for treatment strategies without explicitly understanding the mechanisms leading to this distribution (Anderson and May, 1991). Finally, while the success of a top-down model might not shed light on biological mechanism, the failure of a robust top-down model can provide important information on biological mechanisms that may be disproportionately affecting a system (Harte and Newman, 2014).

In this study, we extend the top-down approach previously used on free-living individuals to describe the aggregation patterns of parasites across hosts. We use a dataset consisting of 22 unique amphibian host-trematode parasite pairings with over 8000 amphibians sampled at 205 sites over 5 years to show that 1) the top-down approach provides a robust null model for describing parasite aggregation across hosts and 2) upon failing to describe the aggregation in a host-parasite distribution, the top-down approach can be complemented with the traditional bottom-up approach to generate hypotheses as to which aggregation mechanisms are dispropor-

tionately affecting a host-parasite system. This synthesis of top-down and bottom-up approaches for describing parasite aggregation provides a new paradigm for exploring host-parasite aggregation and identifying the dominant mechanisms structuring host-parasite systems.

Methods

The bottom-up approach to parasite aggregation

The classic bottom-up approach for understanding parasite aggregation begins by recognizing that the degree of parasite aggregation in a system can theoretically range from all hosts having exactly the same number of parasites (a perfectly even distribution) to a single host having all of the parasites (a completely uneven distribution) (Anderson and Gordon, 1982). Using this approach, the null hypothesis for parasite aggregation falls somewhere in between these two extremes and is nearly ubiquitously assumed to follow a Poisson distribution (Anderson and Gordon, 1982; Wilson et al., 2002).

The bottom-up approach then determines whether observed distributions are more or less aggregated than the predicted Poisson distribution. Depending on the direction of deviation from the Poisson null hypothesis, different aggregating mechanisms (e.g. host heterogeneity, clumped parasite infections, etc.) or disaggregating mechanisms (e.g. parasite-induced host morality, parasite negative density dependence, etc.) are invoked to explain the distribution of parasites across hosts (Anderson and Gordon, 1982; Rosà and Pugliese, 2002; Rosà et al., 2003).

The top-down approach to parasite aggregation

The top-down approach to parasite aggregation differs in that it starts with a non-Poisson null model for parasite aggregation. In this study, we examine two different top-down null models that have been recently used in the macroecological literature. The first model is based on the concept of feasible sets (Haegeman and Loreau, 2009; Locey and White, 2013; Locey and McGlinn, 2013) and begins by identifying two primary state variables of the system: here the total number

of hosts H and the total number of parasites P. The feasible set is defined as all possible configurations in which P parasites can be divided amongst the H hosts such that every configuration has a mean of P/H (see Appendix 1). A configuration $\mathbf{x}$ is one realized distribution resulting from distributing P parasites among H hosts. The center of this feasible set is the most likely configuration given the constraints on the system (SI Figure 1). A common assumption is that all configurations are equally likely (Locey and McGlinn, 2013; Locey and White, 2013), but by weighting configurations differently one can obtain very different feasible sets (Xiao et al., 2015). In practice, feasible sets can be intractably large and instead of enumerating the entire feasible set one draws a large number of random samples and uses them to estimate of the center of the feasible set (SI Figure 1, Locey and McGlinn, 2013; Xiao et al., 2015).

The second top-down model is conceptually related to the feasible set approach and has most recently been derived using the principle of maximum entropy. The maximum entropy approach finds the distribution $p(\mathbf{x})$, where $\mathbf{x}$ is a configuration of P parasites amongst H hosts, that maximizes the entropy equation $H = -\sum_{\mathbf{x}} p(\mathbf{x}) \ln \left(p(\mathbf{x}) / p_0(\mathbf{x}) \right)$ given a set of constraints on $p(\mathbf{x})$, where $p(\mathbf{x})$ is the probability of observing a host-parasite configuration $\mathbf{x}$ and $p_0(\mathbf{x})$ is a prior weight on the configuration $\mathbf{x}$ (Frank, 2009; Haegeman and Etienne, 2010). Finding the maximum entropy distribution is equivalent to finding the distribution that is the most uniform given a prior distribution and a set of constraints. For example, one may assume that each possible configuration is equally likely (i.e. $p_0(\mathbf{x})$ is uniform) and $\mathbf{x}$ is constrained such the total number of parasites any configuration is P. By assuming that individual parasites are indistinguishable, one could then derive the top-down model $p(\mathbf{x})$ that contains the least amount of information given these constraints (Jaynes, 1982; Haegeman and Etienne, 2010). The relationship between the feasible set and maximum entropy top-down models as well as their relationship with the bottom-up model are discussed in Appendix 1.

After specifying a null model, the top-down approach proceeds very similarly to the classic bottom-up approach. The observed parasite distribution is compared to the distribution predicted from the maximum entropy or feasible set approach and if it fails to fit the predicted

distribution then one has reason to believe that additional constraints (potentially in the form of known aggregating and disaggregating mechanisms in host-parasite systems) are disproportionately affecting the system (Harte and Newman, 2014). In contrast to the bottom-up Poisson null model that is trivially rejected in almost every host-parasite system (Shaw and Dobson, 1995; Shaw et al., 1998; Wilson et al., 2002), we show that the top-down approach provides a robust null model in which failures can be used to direct further investigation.

Defining the top-down and bottom-up null models

Without any prior reason to choose otherwise, we assumed that all configurations of parasites across hosts were equally likely for both the maximum entropy approach ($P_0(\mathbf{x})$ is uniform) and the feasible set approach. We also assumed that the constraints on the host-parasite distribution predicted by the two top-down approaches were that any configuration of parasites across hosts had to have P parasites and a mean parasite load of P/H. It has been widely shown that mean parasite abundance is a significant predictor of other higher order moments of the host-parasite distribution, such as the variance (Poulin, 2013; Johnson and Hoverman, 2014; Lagrue et al., 2015). Therefore, we hypothesized that constraining the mean of the distribution would provide enough information to predict the shape of the host-parasite distribution.

Given these constraints, we computed the predicted feasible set host-parasite distribution by randomly sampling from the full feasible set using the algorithms provided by Locey and McGlinn (2013). We drew 1000 samples from the full feasible set and used the median of this feasible set as the predicted host-parasite distribution. To compute the maximum entropy solution, we used the result that the univariate maximum entropy distribution with a uniform prior distribution and a constraint on the mean is a geometric distribution (Haegeman and Etienne, 2010; Harte and Newman, 2014). Because our constraints ensured that any top-down host-parasite distribution could not have more than P parasites, both our top-down models had finite support such that they could only take on values from 0 to P (Haegeman and Etienne, 2010; Zillio and He, 2010). For the maximum entropy model, this solution is given analytically by a finite neg-

ative binomial distribution with an aggregation parameter $k = 1$ (Haegeman and Etienne, 2010; Zillio and He, 2010). For comparison with the top-down null models, we also considered the bottom-up, Poisson null model with finite support (i.e. a Binomial distribution). Thus, all of the null models were two parameter distributions defined by the mean number of parasites per host P/H and the total number of parasites P in the system.

Empirical data and null model analysis

To test these models of host-parasite distributions, we used an extensive dataset of all macroparasites found in 8099 amphibian hosts across 205 ponds (sites) in the East Bay region of California (Alameda, Contra Costa and Santa Clara counties) from 2009-2014. In this field study, we sampled recently metamorphosed amphibians, as these provide a reliable and standardized indicator of infections acquired during aquatic development. Sampling amphibians as they reached metamorphosis - rather than as adults - also helped to ensure that any detected infections reflected conditions of the system in which the animal was collected. To measure parasite abundance, we collected at least 10 of each host species as they approached metamorphosis, and performed a systematic examination of all major tissues and organs for parasites (Hartson et al., 2011). The sampled amphibians consisted of *Pseudacris regilla* (Pacific Tree Frog, $n = 4431$), *Anaxyrus boreas* (Western Toad, $n = 1309$), *Lithobates catesbeianus* (American Bullfrog, $n = 410$), *Taricha torosa* (California Newt, $n = 1568$), and *Taricha granulosa* (Rough-skinned Newt, $n = 381$).

We focused the following analyses on the five most common macroparasites in the system in terms of both prevalence and abundance. These were the larval trematodes *Ribeiroia ondatrae* (RION), *Echinostoma* sp. (ECSP), *Alaria* sp. (ALAR), *Cephalogonimus* sp. (CEPH), and *Manodistomum* sp. (MANO). All of these trematodes have complex life cycles in which their first intermediate hosts are pulmonate snails, their second intermediate host can be amphibians, snails or fish, and their definitive hosts are water-associated vertebrates (reptiles, amphibians, birds, or mammals) (Johnson and McKenzie, 2008).

We fit the top-down and bottom-up models to the distribution of parasites across hosts for

each combination of host species and parasite species at each site during each year. We included a year-by-site-by-host-by-parasite distribution only if it had at least 10 parasites and 10 hosts. Given this criterion, we were able to fit the top-down and bottom-up models to 842 host-parasite distributions. For each of these, we extracted the total number of individuals of a given amphibian species (H) and parasites of a given trematode species (P) and calculated the corresponding rank abundance distribution (RAD) for the feasible set, maximum entropy and Poisson model (Fig 1A). The RAD is a commonly used tool in macroecology that gives the predicted parasite abundances from a given distribution for H hosts and assigns a rank of 1 to the host with highest abundance and a rank of H to the host with the lowest abundance (Harte, 2011; White et al., 2012).

We measured the success of a top-down or bottom-up model in predicting a host-parasite distribution using two criteria. The first criterion was plotting the observed RAD versus the predicted RAD and calculating the R^2 value based on a fit to the 1:1 line (Fig 1B, White et al., 2012; Locey and White, 2013). If a model could exactly replicate the observed RAD then the R^2 value from the 1:1 line would be unity. If the model was a poor fit, the R^2 value will be less than unity and possibly negative if the 1:1 line was a worse fit than assuming that each host had a parasite abundance equal to mean of the observed distribution (White et al., 2012). We calculated R^2 values for each distribution independently as well as for all distributions combined.

The second criterion we used was an Anderson-Darling test to determine whether a predicted RAD was significantly different from the observed RAD for a given host-parasite distribution. The null hypothesis of this test is that the two distributions are the same. The Anderson-Darling has more power to test this hypothesis than the more traditionally used Kolmogorov-Smirnov test (Engmann and Cousineau, 2011). To account for the discrete nature of the data, we used a bootstrapped Anderson-Darling test with 10,000 bootstrapped samples using the function `ad.test` in the R package `kSamples` (Scholz and Zhu, 2015). We considered two distributions to be significantly different if the p-value of the Anderson-Darling test was less than 0.1. We chose this cutoff value as a conservative measure as to when a model did not fit a dataset. When a top-

down or bottom-up model did not fit a given empirical distribution, we determined whether this fit was due to the observed distribution being underaggregated or overaggregated relative to the predicted model. We considered a model to be overaggregated (underaggregated) if the variance-to-mean ratio of the observed data was greater than (less than) the variance-to-mean ratio of the predicted model.

Accounting for the failure of top-down and bottom-up null models

When a top-down null model fails, this may provide evidence that additional constraints/mechanisms beyond just P and H are disproportionately affecting the system (Harte and Newman, 2014). To account for this possibility of additional constraints, we extended the top-down models to include classic aggregating and disaggregating mechanisms in host-parasite systems as described below.

Accounting for disaggregating mechanisms

Disaggregating mechanisms such as parasite-induced host mortality and parasite density-dependence can play an important role in structuring empirically observed host-parasite distributions (Anderson and Gordon, 1982). The parasite *Ribeiroia ondatrae* is known to have a strong, intensity-dependent effect on the survival of some amphibian hosts where increased parasite intensity leads to increased limb-malformations and decreased survival (Johnson, 1999). This this means that the hosts with large parasite burdens are removed from the system, thereby making the parasite distribution more uniform. Therefore, *Ribeiroia*-induced host mortality may be an important mechanism constraining host-*Ribeiroia* distributions that needs to be incorporated into top-down and bottom-up models.

To include *Ribeiroia*-induced host mortality into the top-down and bottom-up predictions we used laboratory-derived survival curves that describe how *Ribeiroia* intensity affects amphibian host survival probability (Johnson et al., 2012). We focused on the amphibian species *Pseudacris*

regilla because *Ribeiroia*-induced mortality and malformations in this species have been documented in the field and in the lab (Johnson, 1999; Johnson and McKenzie, 2008), an intensity-dependent *P. regilla-Ribeiroia* survival curve has been experimentally derived in the lab (Johnson, 1999), and there were a large number of *P. regilla-Ribeiroia* distributions in the dataset on which to test the extended models ($n = 133$). We assumed that the intensity-dependent survival curve, which specifies the probability of an amphibian host surviving from larva to recent metamorph with some observed parasite load, followed a logistic function and estimated the shape of this function from the laboratory data given in Johnson (1999) (see Appendix 2 for more information).

We then used this laboratory-derived survival curve to constrain the top-down and bottom-up predictions by assigning each possible host-parasite configuration (i.e. distribution of parasites among hosts) a likelihood based on the estimated survival function (Appendix 2). For each top-down and bottom-up model, potential configurations were then weighted by this likelihood such that configurations with small likelihoods were less likely to be observed than configurations with large likelihoods. Using this weighting scheme, we were able to sample from top-down and bottom-up models that were constrained on *Ribeiroia*-induced host mortality. We provide a description of how we sampled from these constrained models in Appendix 2. Once we obtained estimates of the constrained top-down and bottom-up predictions, we compared the resulting predictions to the observed *P. regilla-Ribeiroia* distributions using the methods described in *Empirical data and null model analysis*.

Accounting for aggregating mechanisms

Host heterogeneity, whether it be in susceptibility, parasite encounter rates, behavior or other factors, is a strong mechanism leading to aggregation in host-parasite systems (Cornell, 2010; Raffel et al., 2011). We accounted for this aggregating mechanisms by extending top-down and bottom-up models to included empirically observed levels of host-heterogeneity. In particular, we explored discrete host heterogeneity where we assumed that overaggregation relative to the

predicted model was a result of mixing discrete groups of hosts (Grafen and Woolhouse, 1993; Wilson et al., 2002). This approach is conceptually and practically distinct from the standard practice of fitting a negative binomial distribution to overaggregated host-parasite distributions. Under one derivation, the negative binomial distribution assumes that a population of hosts has some aspect of continuous, gamma-distributed heterogeneity (e.g. host susceptibility, Calabrese et al., 2011). It is well known that fitting this type of flexible, continuous heterogeneity model provides an excellent fit to many overaggregated host-parasite distributions (Shaw et al., 1998). However, this approach does not provide immediate insight into the potential host attributes leading to this overaggregation. Considering discrete heterogeneity, as is done here, can provide more specific information as to the relative importance of different levels of host-heterogeneity in structuring a host-parasite distribution.

We used 5 observed host attributes by which we could bin hosts into groups of heterogeneity. The first attribute was host body size (i.e. snout-vent length), which is a well-known attribute affecting parasite exposure and aggregation (Grutter and Poulin, 1998; Poulin, 2013). The other 4 host attributes were the parasite intensities of other larval trematodes. For example, if we were examining the host parasite distribution of the parasite *Echinostoma* in the host *P. regilla*, the host attributes that we considered were the individual body-sizes of *P. regilla* and the intensities of *Ribeiroia*, *Alaria*, *Cephalogonimus*, and *Manodistomum* in *P. regilla* individuals at that site. Co-infection can potentially increase aggregation by increasing heterogeneity in host susceptibility to the focal parasite (Cattadori et al., 2008), but can also decrease aggregation by increasing intra-host parasite negative density dependence (Pacala and Dobson, 1988). Here we consider co-infection as a mechanism leading to increased aggregation.

Using these 5 host attributes, we used a regression tree analysis in which the response variable was the focal parasite abundance and the predictor variables were body size and the larval trematode intensities of a given host, excluding the focal parasite. Separate regression trees were run for each of the 842 host-parasite distributions. For a given host-parasite distribution, we found the best regression tree with 2-5 of groups of host heterogeneity (Fig. 2). We restricted

each group to have at least 2 hosts. Within each of these i groups, we determined the total number parasites P_i and the total number of hosts H_i and calculated the relative importance of each predictor variable base on how much they reduced the sum of squared error compared to the other predictor variables (Fig. 2). We chose to use a regression tree analysis to analyze parasite abundance because it makes fewer assumptions than commonly used generalized linear models and it could be easily combined with the top-down feasible set model that does not have an analytically defined likelihood. However, using regression trees prevented us from determining the direction of the effect of any particular host-heterogeneity predictor on mean parasite load and only allowed us to assess its importance relative to other predictors in the regression tree.

After running the regression tree analysis, we then assumed that each predicted group followed either a top-down or bottom-up model and defined the predicted host-parasite distribution for a given host by parasite by year by site as the following mixture model

$$g(x) = \sum_{i=1}^{G} \frac{H_i}{H} f(x, \frac{P_i}{H_i}) \tag{1}$$

where x is a parasite abundance between 0 and P, G is the number of groups between 2 and 5, $f(x, \frac{P_i}{H_i})$ is the probability mass function for a given top-down or bottom-up model with mean $\frac{P_i}{H_i}$, and $\frac{H_i}{H}$ is the probability of drawing a host from group i. To generate a predicted RAD from this mixture model, the RADs for each group i were computed with P_i and H_i and the predicted RAD was given by the concatenation for these i vectors (Fig. 2). This predicted mixture RAD could then be analyzed using the various methods described above. Our assumption that each ith heterogeneity group follows the same top-down or bottom-up model is roughly analogous to the result of Pacala and Dobson (1988) who used a stochastic host-parasite model to show that, in the absence of density-dependent effects, host-parasite distributions following a negative binomial distribution should have a constant aggregation parameter k across host age/host body size groups. Finally, we also employed a randomization test to ensure that any improvement in model fit after including host heterogeneity was due to the host attributes considered, rather

than just the act of grouping itself. All analyses were performed in R and Python and the code to replicate the analysis can be found at X.

Results

Top-down and bottom up null models

Overall, the top-down null models both fit a larger fraction of the 842 host-parasite distributions considered and described a larger portion of the variation in these distributions than the bottom-up null model. Of the 842 year-by-site-by-host-by-parasite distributions that we considered, 88% were not significantly different from a feasible set model, 85% were not significantly different than a maximum entropy model, and 40% were not significantly different than a Poisson model. The median R^2 for all empirical distributions compared to the feasible set, maximum entropy, and Poisson predictions were 0.78, 0.76 and 0.25, respectively.

Examining the models with regard to host-by-parasite combinations, the top-down models predicted the host-parasite distributions as well or better than the bottom-up model for all host-parasite combinations. The two top-down models typically fit over 70% of the distributions, with some notable exceptions for the host *Lithobates catesbeianus* and the parasites *Alaria* sp. and *Cephalogonimus* sp. (SI Fig 2-4). In contrast, the bottom-up models fit less than 50% of the distributions examined for most host-by-parasite combinations (SI Fig 2, 5). The median R^2 for the top-down models tended to be close to 80% for the various host-by-parasite combinations, while the median R^2 for the bottom-up model was typically below 50% (Fig. 3).

How do top-down and bottom-up null models fail?

When top-down null models failed to fit a given host-parasite distribution, they failed by being both overaggregated and underaggregated relative to the empirical distribution (89 and 120 distributions more aggregated and 13 and 7 distributions were less aggregated than predicted by feasible sets and maximum entropy, respectively). In contrast, the bottom-up null model al-

ways failed by predicting less aggregation than the empirical distribution (501 distributions more aggregated than the bottom-up model).

Ribeiroia, a parasite known to cause intensity-dependent host mortality (Johnson et al., 2012), accounted for 11 of the 13 underaggregated distributions relative to the top-down model. Considering the empirical distributions that were overaggregated relative to the top-down models, *Alaria* had the highest proportion that were overaggregated (39%), followed by *Cephalogonimus* (26%), *Manodistomum* (25%), *Echinostoma* (13%) and *Ribeiroia* (4%).

Accounting for disaggregating and aggregating mechanisms

Parasite-induced host mortality

Including independently-estimated *Ribeiroia*-induced parasite mortality into the top-down models improved the overall fit of the models to *Pseudacris regilla-Ribeiroia* distributions (bootstrapped 95% confidence interval for the difference in overall R^2 between the mortality top-down model and the null top-down model from 1000 resamples: feasible set model, [0.019, 0.033]; maximum entropy model, [0.018, 0.035]; Fig 4A-D). This improvement in fit can be visualized by observing the tightening of the points to the 1:1 line when *Ribeiroia*-induced mortality was included in the model (Fig. 4A-D). This result contrasts with the bottom-up model where including *Ribeiroia*-induced mortality did not improve the fit of the model (bootstrapped 95% confidence interval for the difference in overall R^2 between the mortality bottom-up model and the null bottom-up model from 1000 resamples: Poisson, [-0.005, -0.003]; Fig 4E-F).

Host-heterogeneity

There were 124 unique host-parasite distribution that were overaggregated relative to one of the top-down models. We considered only these overaggregated distributions when examining how host heterogeneity may affect parasite aggregation. The regression tree analysis showed that host body size (snout-vent length) was relatively the most important predictor in delineating groups of host heterogeneity for the 124 unique host-parasite distributions that were overaggregated

relative to one of the top-down models, followed by *Echinostoma* and then *Ribeiroia* abundance (Fig. 5). Moreover, the mixture models predicted by the regression tree analysis improved the fit of the top-down and bottom-up models to the empirical data beyond what would be expected by the inevitable increase in fit by simply grouping hosts (overall R^2 greater than the 95% interval from randomly permuting hosts into groups; Fig. 5). The improvement in fit can be visualized in Figure 5 by noting how the data points compress to the 1:1 line as more groups of heterogeneity are included. In particular, the overaggregation relative to a given model becomes less pronounced (though not absent) as host heterogeneity is included. Finally, all three models showed the largest increase in R^2 when two groups of heterogeneity were added and the increase in R^2 was greater when body-size was included as a level of host-heterogeneity (Fig. 6).

Discussion

The top-down approach developed here provides a more robust null model for describing parasite aggregation and subsequently investigating aggregating mechanisms than the commonly used bottom-up approach. Using the same number of parameters, the top-down models significantly outperformed bottom-up null models when predicting the level of parasite aggregation from 842 host-parasite distributions across 22 unique host-parasite combinations. These results suggest that, given no other information about the system, top-down models provide a better null hypothesis regarding parasite aggregation than the often trivially-rejected Poisson model. Because of both the breadth and standardized nature of the dataset used to test these models and the fact that the aggregation levels displayed by the amphibian-trematode systems tested here are within the range of aggregation displayed by many other macroparasites (Shaw and Dobson, 1995; Shaw et al., 1998), these top-down models should be widely applicable as robust null models for describing macroparasite aggregation.

Moreover, these top-down models can also fail in a more informative way than bottom-up models, providing interesting insight into the dominant mechanisms and constraints affecting a

given system (Haegeman and Loreau, 2008; Harte and Newman, 2014; Marquet et al., 2014). The classic bottom-up inference paradigm is built on the assumption that host-parasite distributions can fail by being both overaggregated and underaggregated relative to a Poisson distribution (Anderson and Gordon, 1982). However, host-parasite distributions that are underaggregated relative to a Poisson distribution are exceedingly rare (Shaw and Dobson, 1995; Shaw et al., 1998). This would lead to the conclusion that disaggregating mechanisms such as parasite-induced host mortality and negative density-dependent interactions between parasites are almost always swamped out by aggregating mechanisms such as host heterogeneity. However, this conclusion is inconsistent with the fact that disaggregating mechanisms such as parasite-induced host mortality can potentially be identified in empirical host-parasite distributions that show substantial aggregation (Crofton, 1971; Wilber et al., 2016). In contrast, because top-down models can fail by being overaggregated and underaggregated, one could reasonably hypothesize that disaggregating mechanisms are important for structuring the parasite distribution even when the observed distribution is aggregated.

This advantage of the top-down approach is supported by our inclusion of *Ribeiroia*-induced amphibian mortality into the top-down and bottom-up models. Including this disaggregating mechanism into the bottom-up models provided no evidence for the importance *Ribeiroia* pathogenicity in structuring the host-parasite distribution of *P. regilla*. However, including *Ribeiroia*-induced mortality in the top-down models improved the overall fit of the models to *P. regilla*-*Ribeiroia* distributions. This improvement in model fit is, of course, not proof that *Ribeiroia*-induced host mortality is shaping these distributions, but it does provide evidence that accounting for the well-described negative effects of *Ribeiroia* on these amphibian hosts (Johnson, 1999; Johnson and McKenzie, 2008) can help predict observed aggregation patterns. Moreover, because we achieved this improvement in fit using a survival curve estimated from an independent dataset (Johnson, 1999), this result provides strong evidence that parasite-induced mortality is influencing the *P. regilla*-*Ribeiroia* distributions. This conclusion is more consistent with our knowledge about the effect of *Ribeiroia* on these amphibians than the conclusion we would have

drawn using only a bottom-up approach. However, we stress that the top-down approach does not allow us to conclude that parasite-induced mortality is not important in structuring a host-parasite distribution if, after including this mechanism, the model fit is not improved. In this case, we can only conclude that the parasite-induced mortality relationship provided no additional information about aggregation beyond what was already provided by P and H.

In addition to describing patterns of underaggregation, we also found that extending top-down models to include host-heterogeneity helped explain observed overaggregation in parasite distributions. Including host heterogeneity through host attributes such as host body size and co-infection with other trematode parasites improved the fit of top-down and bottom-up models to overaggregated parasite distributions. In particular, we found that, in this system, host body size was generally a more important predictor of parasite aggregation than a host's level of co-infection with other trematodes. This result is consistent with previous studies which have shown the importance of host age/body size heterogeneity for increasing parasite aggregation due to changes in host immunity and/or exposure to parasites with host age/body size (Pugliese et al., 1998; Poulin, 2013). Moreover, while previous work has shown that co-infection can act as a type of host heterogeneity and increase parasite aggregation (Cattadori et al., 2008), this same work has also shown that host characteristics such as age/body size, sex, and breeding status can often be more important factors affecting parasite aggregation and host-parasite dynamics than co-infection. We acknowledge that this analysis simplifies the complex effects of co-infection as both an aggregating and disaggregating mechanism in host-parasite systems (Wilson et al., 2002; Cattadori et al., 2008) and future work should look to extend top-down models to more robustly account for the possible effects of co-infection on parasite aggregation. However, the null top-down models discussed here should still provide the starting point and we have shown that even simple extensions to these top-down models can help explore the importance of various aggregating and disaggregating factors affecting host-parasite distributions.

While the top-down models do provide a more robust null hypothesis than the bottom-up model, we did observe differences between the two top-down models that we used in this anal-

ysis. In general, the feasible set approach had a higher median R^2 than the maximum entropy approach and fit a greater proportion of the parasite distributions for all host-parasite combinations. In addition to being a better fit to the empirical data, the feasible set approach has a number of characteristics that make it appealing as a null model. In contrast to the maximum entropy model used in this study which predicts a constant level of aggregation regardless of the number of hosts or parasites in the system ($k = 1$ in terms of the aggregation parameter of the negative binomial distribution), the feasible set approach allows aggregation to vary as a function of both the total number of hosts and parasites in a system (SI Fig 6, Locey and McGlinn, 2013). In particular, the feasible set approach predicts that as the mean number of parasites per host increases the aggregation of parasites across hosts decreases (SI Fig 6). This decrease in aggregation with increasing abundance has been empirically observed in both parasites and free-living individuals (Taylor, 1984; Poulin, 1993; Condit, 2000; Conlisk et al., 2012). That being said, there have been theoretical results that have shown that the assumption of constant k across time and/or mean parasite abundance is not unrealistic for some host-parasite systems (McCallum, 1982; Pacala and Dobson, 1988). If this is the case, one advantage of the maximum-entropy predicted distribution over the feasible set approach is its simple, analytical form. In contrast, the feasible set approach currently relies on intensive computation (Locey and McGlinn, 2013).

The general success of top-down models in describing host-parasite distributions has important implications for understanding the dynamics of host-macroparasite systems. Many macroparasite models explicitly model the state variables H and P (Anderson and May, 1978) and examine, in addition to other biological factors, how either fixed (Anderson and May, 1978) or dynamic aggregation (Kretzschmar and Alder, 1993; Rosà et al., 2003) influences host and parasite dynamics. Top-down models in turn predict that aggregation is largely determined by exactly these state variables. Therefore, a top-down approach to parasite ecology can be directly linked back to a more familiar mechanistic framework by examining the implications of top-down predictions on dynamics of the total number of hosts and parasites in a system.

For example, assuming that parasite aggregation follows a feasible set would allow the aggre-

gation parameter k to change over time as this aggregation measure tracks the total number of hosts and parasites in a given system. While there is no closed form solution for how k depends on P and H in a feasible set, it could be easily approximated via simulation and the resulting model dynamics examined. Linking top-down models for describing aggregation to dynamic equations for the state variables of a system has often been alluded to in macroecology (Supp et al., 2012; White et al., 2012), but has been difficult to implement (Harte, 2011). The rich empirical and theoretical understanding of biological factors affecting the total number of hosts and the total number of parasites in a system (Kretzschmar and Alder, 1993; Hudson et al., 1992; Dobson and Hudson, 1992) makes disease ecology an ideal field in which to make this connection.

Top-down models have seen little use in disease ecology despite their success in describing the population and community-level distributions of free-living individuals (Conlisk et al., 2007; White et al., 2012; Newman et al., 2014; Xiao et al., 2015). We show that these top-down models 1) can provide a robust null model for host-parasite distributions and 2) can be extended to explore dominant mechanisms structuring host-parasite systems such as host-heterogeneity or parasite-induced host mortality. While it is important to understand how multiple aggregating and disaggregating mechanisms interact to generate host-parasite distributions, it is the resulting shape of this distribution that dictates host-parasite dynamics in models (Rosà and Pugliese, 2002; Tompkins et al., 2002). If top-down models can provide a good approximation to this distribution then they provide a means to explore the dynamics of host-parasite systems without needing to elucidate the magnitude of every aggregating and disaggregating mechanisms affecting the system.

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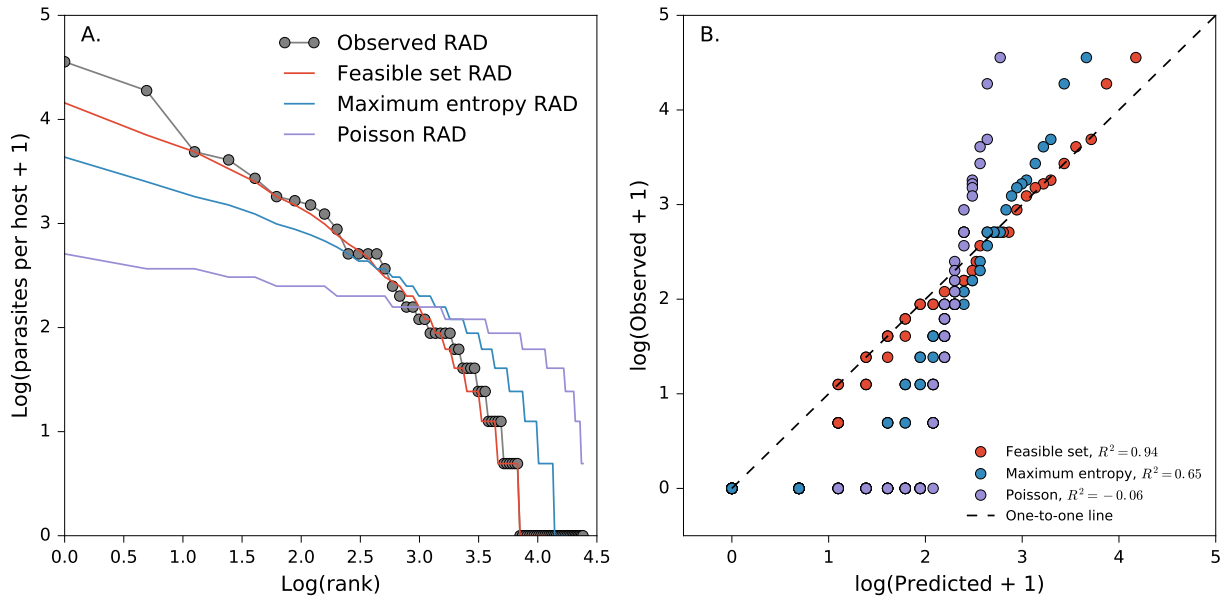

Figure 1: For a single observed host-parasite distribution with, for example, $H = 80$ hosts and $P = 579$ parasites, we A) plot the observed rank abundance distribution (RAD) against the rank abundance distribution predicted from the two top-down models (feasible set and maximum entropy) and the bottom-up model (Poisson). B) When comparing the observed RAD to the predicted RADs, we plot the predicted values against the observed and calculate the R^2 value to the 1:1 line. If the predicted RAD equals the observed RAD then $R^2 = 1$. If the predicted RAD is worse than just assuming that each host has the mean parasite abundance, $R^2 < 0$. In this case, the feasible set is the best fitting model and generally hugs the 1:1 line. The maximum entropy model is the next best model, but predicts less aggregation than is observed in the empirical distribution. The Poisson distribution severely underestimates the observed aggregation and does worse than assuming that each host has a parasite abundance equal to the mean of the observed distribution.

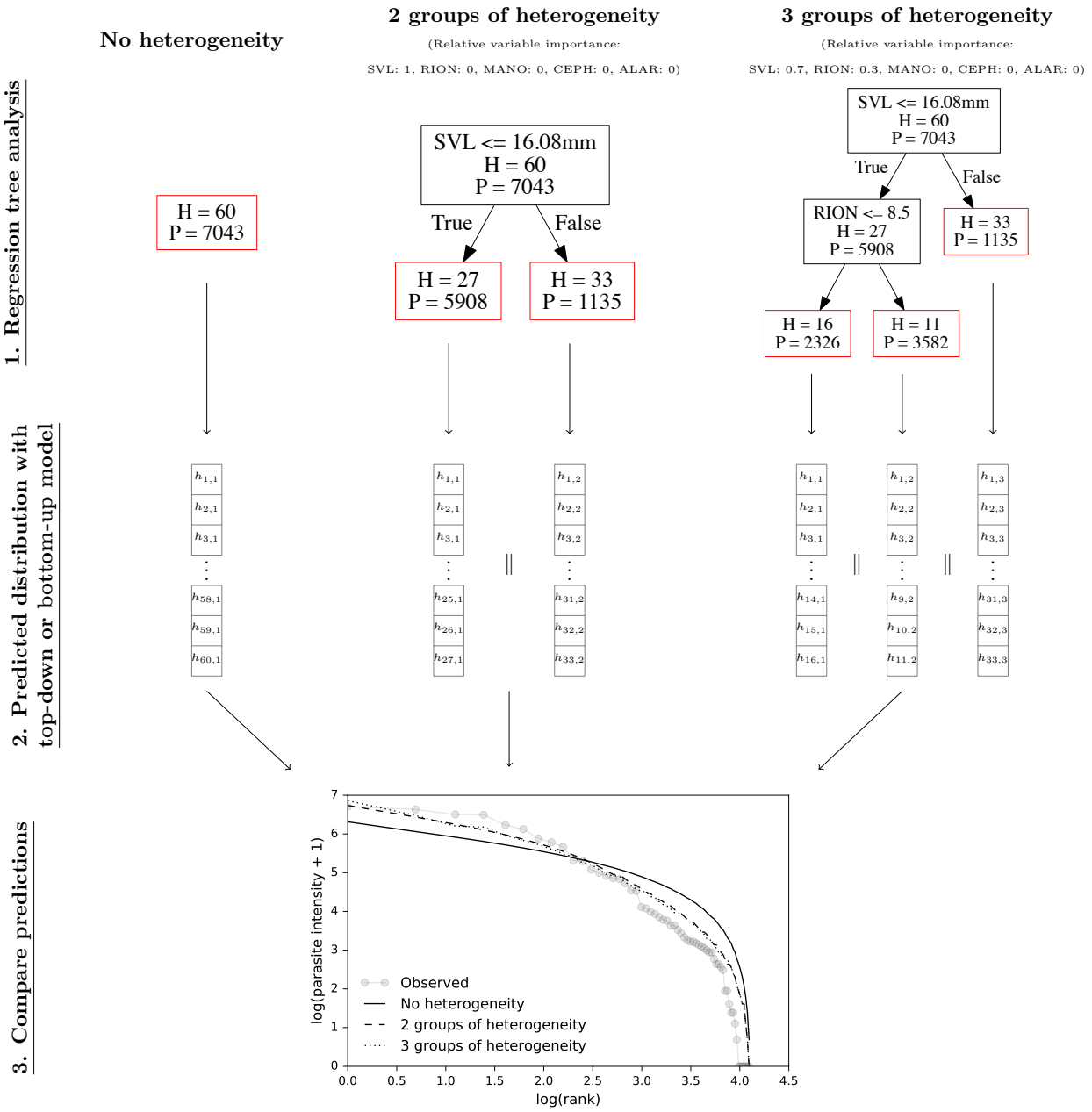

Figure 2: A diagram showing how host-heterogeneity can be incorporated into top-down and bottom-up models. **Step 1:** Consider, for example, a distribution for the parasite *Echinostoma* sp. in the host *Pseudacris regilla* with $H = 60$ hosts and $P = 7043$ parasites. When no host heterogeneity is included, the predicted host-parasite distribution can be computed directly from H and P using a top-down or bottom-up model. To include groups of heterogeneity, a regression tree analysis is performed in which the response variable is *Echinostoma* abundance and the predictor variables are *Pseudacris regilla* body size (snout-vent length, SVL) and the abundance of *Ribeiroia ondatrae* (RION), *Alaria* sp. (ALAR), *Cephalogonimus* sp. (CEPH), and *Manodistomum* sp. (MANO) in a particular host. In the example above, the regression tree analysis shows that the "best" way to make two groups of heterogeneity given the predictor variables is to split the 60 *P. regilla* individuals into those with SVL ≤ 16.08 mm and those with SVL > 16.08 mm. To make three groups of heterogeneity, *P. regilla* individuals with SVL ≤ 16.08 are again split into individuals with RION abundance ≤ 8.5. For each of these regression trees, we can determine the relative importance of each variable in building the regression tree by how much they decrease the sum of squared error compared to the other predictors. **Step 2:** We can then predict the resulting top-down or bottom-up predictions from each of these groups of heterogeneity (the red boxes above) using the total number of hosts and parasites in each heterogeneity group. Each prediction generates a rank abundance distribution with $h_{j,i}$ being the jth ranked host with some number of parasites in the ith heterogeneity group. Concatenating ($\|$) these predictions together and re-ordering the resulting vector gives the predicted top-down or bottom-up model after including host heterogeneity. **Step 3:** These predicted distributions can then be compared to the observed host-parasite distribution.

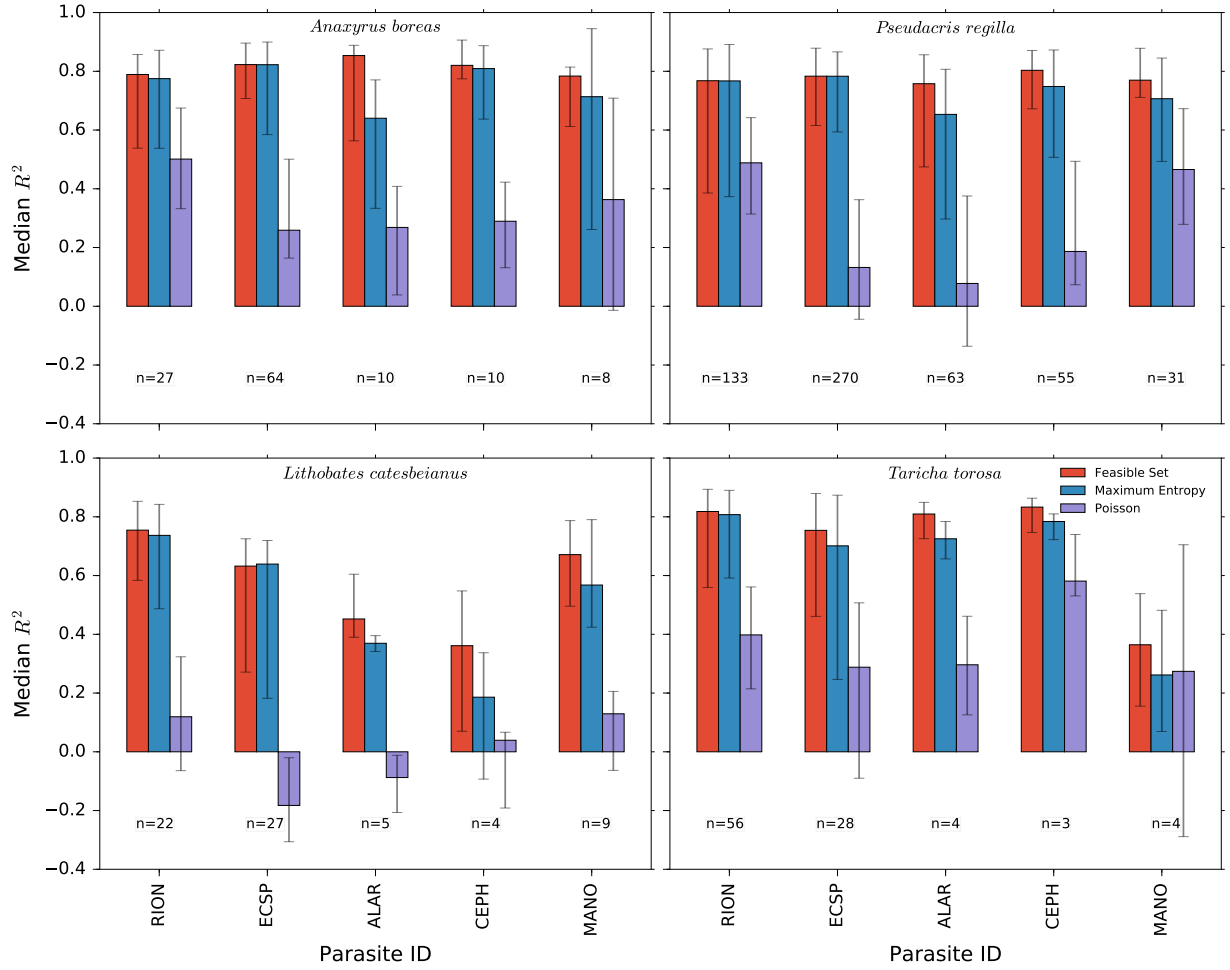

Figure 3: The height of each bar gives the median R^2 about the 1:1 line comparing the observed and predicted rank abundance distributions for various host-parasite combinations. The error bars give the first and third quartiles of the distribution of R^2 values for each distribution of a species host-parasite distribution. The number of a distributions for each host-parasite combination that were used to compute this median R^2 are shown in the figure. The x-axis gives the 5 trematode parasites examined in this analysis: *Ribeiroia ondatrae* (RION), *Echinostoma* sp. (ECSP), *Alaria* sp. (ALAR), *Cephalogonimus* sp. (CEPH), and *Manodistomum* sp. (MANO). *Taricha granulosa* is not shown in this plot as it was never infected with ALAR, CEPH or MANO.

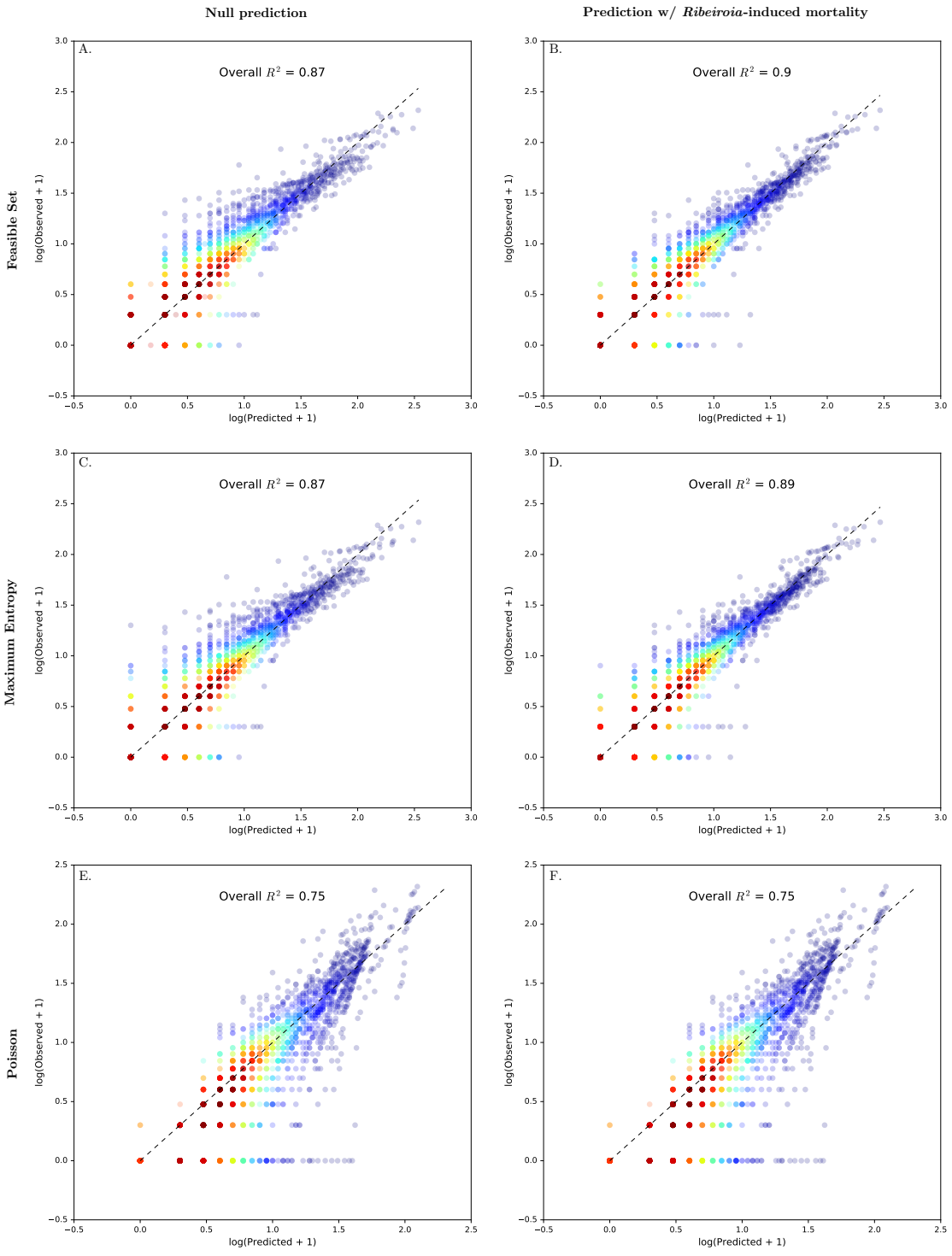

Figure 4: The figure shows the effect of including empirically-estimated *Ribeiroia*-induced *Pseudacris regilla* mortality (Johnson, 1999) into the top-down (feasible set and maximum entropy) and bottom-up models (Poisson) models. The first column in this plot (A, C, and E) compares 133 observed rank abundance distributions (RAD) of *Ribeiroia*-*P. regilla* with the RADs predicted by the top-down and bottom-up models they were constrained on parasite-induced host mortality.The second column (B, D, and F) compares the observed and predicted RADs after they were constrained on parasite-induced host mortality. The overall R^2 gives the fit of these distributions to the 1:1 line (black, dashed line) where an R^2 of 1 indicates a perfect fit to the 1:1 line and an R^2 of less than 0 indicates that 1:1 line fit is even worse than assuming that each host has a parasite abundance equal to the mean of the observed distribution. Each point represents a single host with a given predicted and observed parasite abundance. "Hotter" colors indicate a higher density of points in the region than "cooler" colors. Including empirically-estimated *Ribeiroia*-induced mortality improves the fit of both top-down models to the *P. regilla*-*Ribeiroia* distributions, but does not improve the fit for the bottom-up, Poisson model.

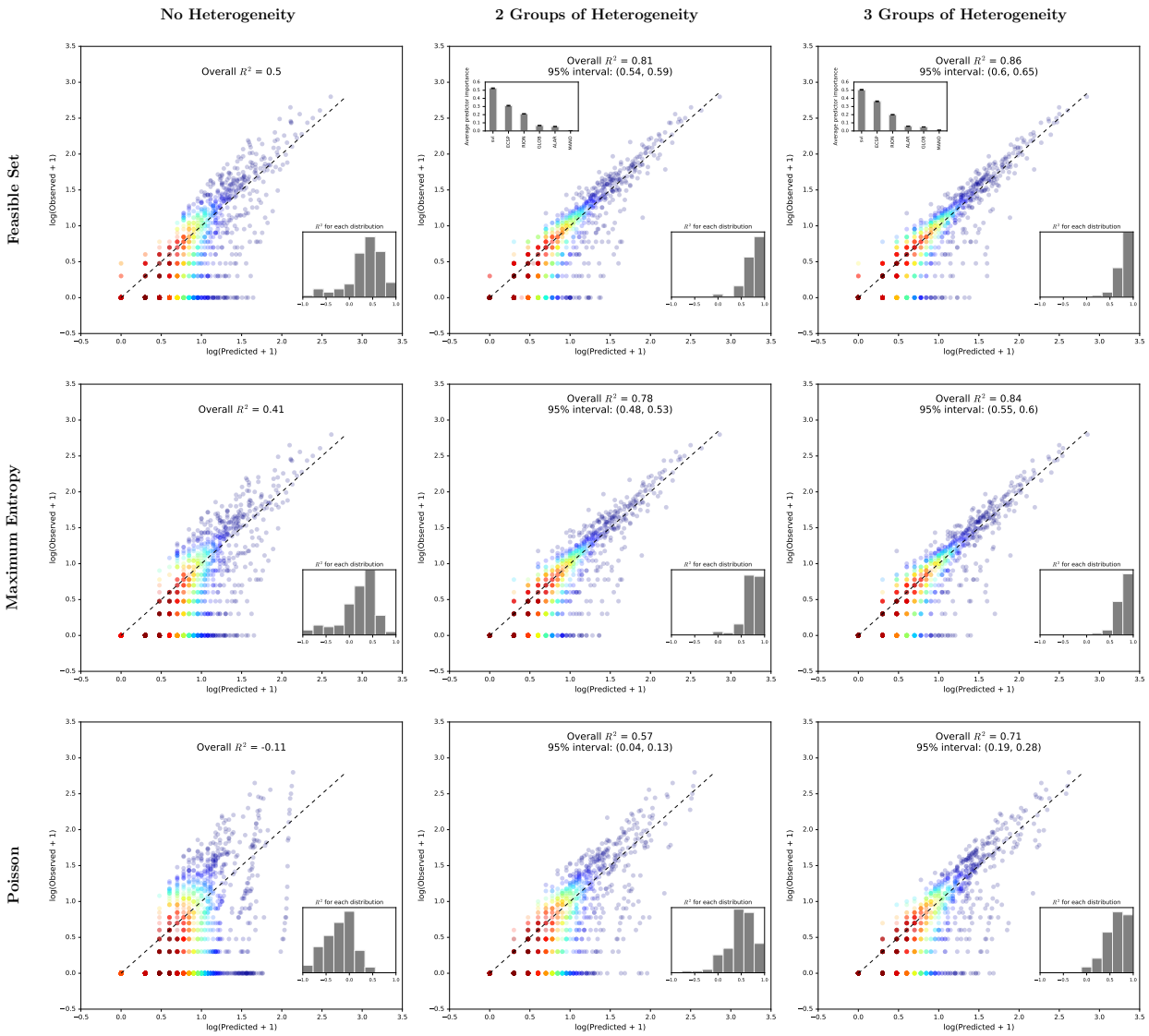

Figure 5: The figure shows the effect of discrete heterogeneity on the host-parasite distributions that were overaggregated relative to the top-down models (all hosts and parasites shown together). The first column in this plot shows the predicted rank abundance distributions (RAD) compared to the observed RADs when no host heterogeneity was included in the model for the two top-down models (feasible set and maximum entropy) and the bottom-up model (Poisson). The overall R^2 gives the fit of these distributions to the 1:1 line (black, dashed line) where an R^2 of 1 indicates a perfect fit to the 1:1 line and an R^2 of less than 0 indicates that 1:1 line fit is even worse than assuming that each host has a parasite abundance equal to the mean of the observed distribution. Each point represents a single host with a given predicted and observed parasite abundance. "Hotter" colors indicate a higher density of points in the region than "cooler" colors. The histogram in the lower right hand side gives the distribution of R^2 values for each particular host-parasite distribution (124 distributions). The second and third columns in this plot show the effect of adding 2 and 3 groups of host heterogeneity, respectively, on the predicted host-parasite distributions based on the results from a regression tree analysis on known host attributes in the dataset. The plots in the upper left hand corner show the mean importance of a given host attribute in structuring the regression tree for all the 124 overaggregated host-parasite distributions. The predictor variables were body-size (svl), *Echinostoma* sp. (ECSP), *Ribeiroia* (RION), *Cephalogonimus* (CEPH), *Alaria* (ALAR), and *Manodistomum* (MANO). The predictor importance was the same for all models within a heterogeneity group and are therefore only displayed once for each group. Finally, the 95% interval displayed in the plot gives the 95% quantiles of overall R^2 values based on randomly permuting parasites into the groups predicted by the regression tree analysis. If the overall R^2 is greater than the interval, it shows that the increase in R^2 from the regression tree predicted is a result of the predictors used in the regression tree analysis, rather than just grouping itself.

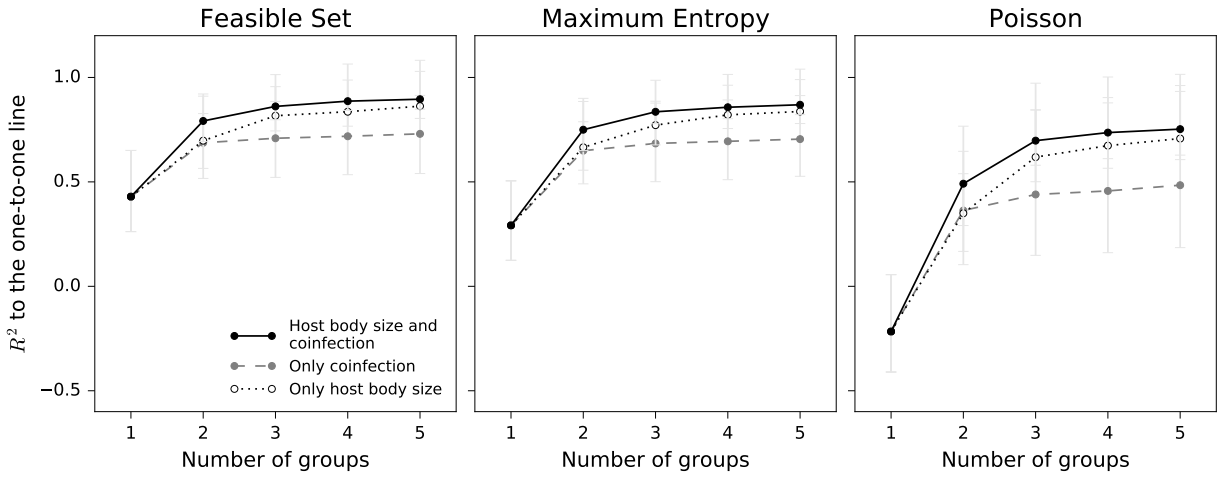

Figure 6: The effect of adding groups of heterogeneity to the overall R^2 for the two top-down models (feasible sets and maximum entropy) and the bottom-up model (Poisson). The black points and solid line gives the resulting median R^2 of the 124 overaggregated distributions when host heterogeneity was assumed to be predicted by host body size and/or co-infection with other larval trematodes. The white points and dotted line gives the median R^2 when host heterogeneity was assumed to be only predicted by host body size (i.e. co-infection was not included). The gray points and dashed line gives the median R^2 when host heterogeneity was assumed to be only predicted by co-infection with other larval trematodes (i.e. host body size was not included). The error bars give the first and third quartiles of the distribution of R^2 for each group number.