Tracking the assembly of nested parasite communities: using β-diversity to understand variation in parasite richness and composition over time and scale

Running header: Parasite community assembly

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ABSTRACT

1. Community composition is driven by a few key assembly processes: ecological selection, drift, and dispersal. Nested parasite communities represent a powerful study system for understanding the relative importance of these processes and their relationship with biological scale. Quantifying β-diversity across scales and over time additionally offer mechanistic insights into the ecological processes shaping the distributions of parasites and therefore infectious disease.
2. To examine factors driving parasite community composition, we quantified the parasite communities of 959 amphibian hosts representing two species (the Pacific chorus frog, *Pseudacris regilla,* and the California newt, *Taricha torosa*) sampled over three months from 10 ponds in California. Using additive partitioning, we estimated how much of regional parasite richness (γ-diversity) was composed of within-host parasite richness (α-diversity) and turnover (β-diversity) at three biological scales: across host individuals, across species, and across communities (ponds). We also examined how β-diversity varied across time at each biological scale.
3. Differences among ponds comprised the majority (40%) of regional parasite diversity, followed by differences among host species (23%) and among host individuals (12%). Host species supported parasite communities that were less similar than expected by null models, consistent with ecological selection, although these differences lessened through time, likely due to high dispersal rates of infectious stages. Host individuals within the same population supported more similar parasite communities than expected, suggesting that host heterogeneity did not strongly impact parasite community composition and that dispersal was high at the individual-host level. Despite the small population sizes of within-host parasite communities, drift appeared to play a minimal role in structuring community composition.
4. Dispersal and ecological selection appear to jointly drive parasite community assembly, particularly at larger biological scales. The dispersal ability of aquatic parasites with complex life cycles differs strongly across scales, meaning that parasite communities may

predictably converge at small scales where dispersal is high, but may be more stochastic and unpredictable at larger scales. Insights into assembly mechanisms within multi-host, multi-parasite systems provide opportunities for understanding how to mitigate the spread of infectious diseases within human and wildlife hosts.

Key Words: community assembly, β-diversity, trematode, multi-scale, amphibian disease, coinfection, disease ecology, infectious disease

INTRODUCTION

Parasites comprise more than one-third of known species on Earth and can be an important driving force in ecosystem dynamics, the maintenance of biodiversity, and evolutionary change (Hatcher et al. 2006, Hudson et al. 2006, Dobson et al. 2008, Paterson et al. 2010). Although epidemiology has traditionally focused on one-host, one-parasite interactions, parasites typically interact with rich assemblages of hosts and other symbionts (Rigaud et al. 2010). Moreover, interactions between host and parasite communities can combine to shape ecological processes across multiple biological scales (Telfer et al. 2010, Seabloom et al. 2015). For instance, co-infecting parasites can dramatically alter disease outcomes for individual hosts (Griffiths et al. 2011, Johnson and Hoverman 2012) as well as transmission rates within populations (Jolles et al. 2008, Susi et al. 2015). Scaling up even further, parasite diversity within ecosystems can functionally dictate food web linkages and patterns of energy flow (Lafferty et al. 2006), underscoring the importance of characterizing parasite community composition at multiple scales – from across individual hosts to entire regions (Pedersen and Fenton 2007).

A key challenge in the ongoing study of parasite community ecology is to understand the ecological processes underlying community structure and how these processes vary with scale (Holmes 1987, Sousa 1990). Historically, many studies have employed comparative approaches to quantify compositional differences in parasite communities (e.g. Sousa 1990, Vidal-Martínez and Poulin 2003, Krasnov et al. 2011, Altman and Byers 2014). This work has accumulated valuable information as to how parasite communities are structured at different biological scales: e.g. how parasite communities differ across host individuals (Guégan and Hugueny 1994), across host populations (Holmes 1990), across host species (Locke et al. 2013, Fecchio et al. 2017), and across environmental or spatial gradients (Poulin 2003, Thieltges et al. 2010). However, fewer studies have integrated multiple scales into the same study (but see Krasnov et al. 2010, Dallas and Presley 2014), for example, to compare whether host species identity or habitat represent a stronger structuring force. Comparisons of community composition across scales can reveal where and by what mechanisms species distributions are most strongly limited and identify whether assembly is non-random (Crist et al. 2003a). Identifying these core structuring processes and their scale-dependence in natural host-parasite systems offers essential opportunities to test hypotheses about the drivers of parasite distributions and better inform strategies for disease mitigation (Rynkiewicz et al. 2015). For instance, if parasite communities are strongly structured across environmental gradients, managing habitat features might be an important disease control strategy, whereas high among-individual or among-species variation might require a targeted vaccination program (Paull et al. 2012). More broadly, due to their hierarchically nested structure (Guégan et al. 2005), parasite communities offer potential for understanding how assembly processes vary over scale (Mihaljevic 2012) and therefore represent a powerful study system for addressing one of the major challenges in community ecology (Levin 1992, Chase and Myers 2011).

Community ecology theory advances that variation among ecological communities is driven by four key processes: dispersal, ecological selection, drift, and speciation (Vellend 2010). Dispersal is the process by which parasites colonize a site from a given species pool and operates at multiple biological scales; for instance, dispersal structures which parasites arrive to a particular habitat patch from the regional species pool, as well as the parasites reaching a given host within that habitat patch (Guégan et al. 2005). Ecological selection is a niche-based process, whereby abiotic or biotic conditions filter which parasites can persist following dispersal. Selection acts at the habitat-level (if habitat features influence the species able to persist), at the host species-level (if species vary in their susceptibility to different parasites), and at the individual host-level (if hosts differ in susceptibility or if parasite interactions occur). Finally, drift is the change in community composition resulting from stochastic fluctuations; this can occur across all biological scales but is expected to occur most strongly for small communities (Chase and Myers 2011), such as those at the within-host level.

To better understand at how the relative importance of these mechanisms varies with scale, metrics of community dissimilarity (β-diversity) can be compared through approaches such as additive partitioning (Veech et al. 2002, Johnson et al. 2016). For instance, if β-diversity is higher across host species than across habitats, ecological selection at the host-species level could be more important than dispersal among habitats or selection due to environmental variables. Moreover, because assembly processes are often temporally dynamic (Penczykowski et al. 2016, Fitzgerald et al. 2017), quantifying β-diversity across a temporal window can further inform how composition shifts with host development or environmental change (Cohen et al. 2015), offering further insight into process. Decreasing β-diversity over time indicates convergence among communities, due to high dispersal rates or similar selection regimes (Leibold et al. 2004), whereas increasing β-diversity between similar sampling units often indicates an important role of drift (Chase 2007). Studies examining β-diversity over time and scale have revealed that assembly processes and community compositions are temporally and spatially dynamic (Soininen et al. 2007, McIntire and Fajardo 2009) and have reinforced the idea that temporal studies yield a more process-based understanding of community assembly. For example, by partitioning variance in community composition across scale and over time, Costello and others (2009) showed that temporal variation in gut microbiomes within the same individual was smaller than among-individual variation, even on the same day, suggesting that different hosts selected particular microbial communities that remained distinct from other hosts' microbiota over time. Temporal analyses of parasite community composition are still relatively rare (but see Vidal-Martínez and Poulin 2003, Fallon et al. 2004, Cohen et al. 2015, Budischak et al. 2016), and even fewer studies have longitudinally tracked parasite community composition across multiple distinct scales to infer the general processes driving assembly.

Here, we compared variation in parasite communities across multiple, discrete biological scales and over time to better characterize the processes driving community composition. We quantified parasite communities (trematodes, nematodes, and protists) within two species of amphibian hosts (Pacific chorus frogs *Pseudacris regilla* and California newts *Taricha torosa*), sampled across 10 different ponds within the California Bay Area. By re-visiting each pond throughout host development, we generated a unique dataset on parasite accumulation and primary succession. Using an additive partitioning approach (Figure 1), we tested the extent to which parasite community composition was driven by differences among host individuals within the same population (β_{host}), among host species within the same community ($\beta_{species}$), or among host communities (β_{pond}). We next examined how β-diversity at each of these scales varied across time to gain further insight into how structuring processes changed over the course of primary succession. We also modelled temporal variation in richness (α-diversity) at each biological scale to better infer whether changes in community composition were operating through species losses (consistent with ecological selection or drift) or through species gain (consistent with dispersal). We expected that, if ecological selection at the community or species scale is a major structuring force, we would observe large differences among host species ($\beta_{species}$) and host ponds (β_{ponds}) relative to null models (Müller and Goßner 2010). If ecological drift is an important structuring processes, among-host variation (β_{host}) would be consistent with or larger than null models, and would increase through time (Püttker et al. 2015). Finally, we expected that if dispersal was not limiting at a particular scale, α-diversity would increase while β-diversity would decrease through time at that scale (Leibold et al. 2004). Overall, we intend that the application of a consistent framework and terminology can help to identify the drivers of parasite assembly across a range of multi-host, multi-parasite systems, thereby facilitating future comparisons focused on scale-dependent (or invariant) assembly processes related to disease.

MATERIALS AND METHODS

Field surveys and dissection

Over the summer of 2017 (May – August), we characterized parasite communities within the larvae of two amphibian species (the Pacific chorus frog, *P. regilla,* and the California newt, *T. torosa*). Hosts were sampled from 10 ponds in California Bay Area (Appendix S1: Figure S1). All ponds were small habitats containing both species of focal amphibian host and the snail species *Helisoma trivovlis* and *Physa* spp. (*P. acuta* or *P. gyrina*), which are intermediate hosts for a diversity of trematode species found in amphibians (McCaffrey and Johnson 2017). Each pond was visited four to five times across the summer, during which we sampled 10 to 20 individuals of each host species, representing juvenile life stages from post-hatching to peri-metamorphosis (Appendix S1: Figure S2). Previous research in the same system has shown that this sample size is sufficient for estimating parasite richness within a site (Johnson et al. 2012; 2013). After euthanizing hosts with MS-222, we examined their organs and tissues under an Olympus SZX10 dissection microscope following standard methods (Johnson et al. 2018) to characterize the presence and identity of parasites (Schell 1985, Sleigh 1991, Gibson et al. 2002, Duszynski et al. 2007, Anderson et al. 2009). For trematode and nematode infections, we quantified both parasite identity and load (number of parasites per host), whereas for protozoans we noted only presence or absence. We also measured host size (snout-vent length) and developmental stage (Gosner 1960, Wong and Liversage 2004).

Richness and composition across biological scale and time

To examine how parasite richness varied with biological scale and sampling date, we quantified host-level richness (α_{host}) as the number of parasite taxa within an individual host, population-level richness ($\alpha_{population}$) as the number of parasite taxa across all individuals of a given host species at a given pond, and community-level richness (α_{pond}) as the number of parasite taxa across both host species at a given pond. Regional richness (γ) was estimated as the total number of parasite taxa across all ponds. We quantified α_{host}, $\alpha_{population}$, α_{pond} and γ separately for each time point and modelled richness as a function of time separately for each biological scale, using generalized linear mixed models (GLMM). We included polynomial terms for sample date to test whether richness accumulation was linear or unimodal and selected the model that minimized AIC (Appendix S1). We used indicator species analysis in the package *indicspecies* for R (De Caceres and Legendre 2009), to determine whether certain parasite species were associated with particular time points ("indicator species analysis"), including early or late summer. The code for this analysis and all subsequent statistical analyses is included in Appendix S2.

β-diversity across biological scale and time

To quantify how differences among parasite communities (i.e., turnover or β-diversity) changed with time, we estimated pairwise dissimilarity metrics using Jaccard's distance (Jaccard 1912), which is based on presence-absence data (Appendix S1). We estimated between-host turnover ($\bar{\beta}_{\mathrm{J,\ host}}$) as the average pairwise dissimilarity between individuals of the same species collected in the same pond on the same visit. Between-species turnover ($\bar{\beta}_{\mathrm{J,\ species}}$) was the average difference between *P. regilla* and *T. torosa* parasite communities at a given site-visit, and between-community turnover ($\bar{\beta}_{\mathrm{J,\ pond}}$) was the average difference in parasite communities among ponds. We calculated all three metrics separately at each time point. Using a separate linear mixed model for each biological scale, we quantified the relationship between Jaccard's β-diversity and sample date (Appendix S1).

Because Jaccard's β-diversity metric is sensitive to total taxonomic richness and thus should not be used to compare turnover across scales, we used an additive partitioning approach to compare β-diversity among hosts, species, and communities (Lande 1996, Crist et al. 2003b, Gering et al. 2003, Veech and Crist 2010, Johnson et al. 2016). This approach partitions regional parasite diversity (γ) into scale-specific values of turnover (β diversity) and richness (α diversity) using the formula:

$\gamma = \alpha_{\mathrm{host}}$ (within host) + β_{host} (between hosts) + β_{species} (between species) + β_{pond} (between communities (Figure 1). We used the function *adipart* in the R package *vegan* (Oksanen et al. 2018), which implements random permutations of species occurrence data to produce estimates of significance relative to a null model. The null distribution was generated using 1000 random permutations which hold site-level richness constant and randomly distribute parasite species across sites relative to their abundance (Crist et al. 2003). We present the difference between observed values ($\bar{\alpha}_{\mathrm{host,\ obs}}$ and $\bar{\beta}_{\mathrm{host,\ obs}}$) and mean null simulation values ($\bar{\alpha}_{\mathrm{host,\ sim}}$ and $\bar{\beta}_{\mathrm{host,\ sim}}$) to evaluate whether observed diversity differed from random chance and then assessed how the proportional contribution of each component to total γ diversity varied with time as hosts progressively developed and infections accumulated.

We evaluated the sensitivity of the above analyses to the inclusion of rare species by re-running the analyses without rare species, as well as using the Bray-Curtis abundance-based dissimilarity metric (Appendix S1).

RESULTS

Field surveys and parasite community

We quantified the richness and community composition of parasites from 959 individual hosts (445 *T. torosa*, 514 *P. regilla*) from 10 ponds across three months of sampling. The majority of hosts (n = 795; 82%) were infected by at least one parasite. On average, ponds contained 6.2 parasite taxa per visit. We identified 18 distinct taxa of parasites, 11 of which were present in >1% of hosts (six digenetic trematodes, two nematodes, and three protists; Appendix S1: Table S1). The most common parasite was the trematode *Ribeiroia ondatrae* (present in 44% of *P. regilla* and 48% of *T. torosa*), followed by the trematode *Echinostoma* spp. (59% of *P. regilla* and 16% of *T. torosa*). Some parasites were specialists (e.g., the trophically-transmitted nematode *Chabaudgolvania* sp. was only found in *T. torosa,* whereas two protists [*Opalina ranarum* and *Nyctotherus cordiformus*] and the nematode *Gyrinicola batrachiensis* were only found in *P. regilla*). Other parasites (e.g. the trematodes *R. ondatrae* and *Manodistomum syntomentera*) infected both host species at roughly equal proportions.

Richness and composition across biological scale and time

Richness showed a non-linear relationship with time at both the host- and population-levels (Appendix S1: Figure S3). The top model for predicting host-level richness (α_{host})

included significant terms for host species, an interaction between visit and species, and a quadratic term for visit. This model showed improved fit over the next best model, which used visit as a linear term (Δ AIC = -10.47). Therefore, hosts accumulated parasites non-linearly with respect to time, with richness peaking in late June to mid-July. The median richness within *P. regilla* hosts was three parasites, which was significantly higher than *T. torosa* (median = 1; estimate = -1.65 ± 0.15; $P < 0.0001$; Appendix S1: Figure S3). The best performing model for predicting population-level richness ($\alpha_{population}$) included significant terms for host species, an interaction between visit and species, and a second-order polynomial term for visit. Similarly, within-population parasite richness was higher in *P. regilla* populations (median = 5) than in *T. torosa* populations (median = 2, estimate = -1.42 ± 0.34; $P < 0.0001$). While community-level richness (α_{pond}) exhibited a similarly unimodal relationship, the best model was a null model, indicating that visit was a poor predictor of parasite richness within ponds. Regional richness (γ; aggregated over all ponds) did not change over time (estimate [visit] = 0.009 ± 0.09; $P = 0.92$)

Of 11 parasite taxa, four were strongly associated with a particular time point or group of time points based on indicator species analysis (Appendix S1: Figure S4). The protozoan *Tritrichomonas augustus* was associated with the first and second visits ($P = 0.003$), and the nematode *Gyrinicola batrachiensis* was associated with the first through third visits ($P = 0.001$). Two trematode parasites, *Echinostoma* spp. and *Gorgoderina* sp., were “late season” parasites, associated with the third through fifth visits ($P = 0.001$ and $P = 0.02$, respectively).

β-diversity across biological scale and time

Additive partitioning of diversity (Figure 1) revealed that differences among communities (β_{ponds}) had the strongest influence on regional parasite diversity (γ), and were considerably greater than the effects of differences between host species ($\beta_{species}$) or among individual hosts (β_{host}; Figure 2). Among-community turnover averaged over all visits ($\bar{\beta}_{pond, obs}$) accounted for 40% of regional parasite diversity, and ponds were significantly less similar than expected from a null model ($\bar{\beta}_{pond, obs} - \bar{\beta}_{pond, sim} = 0.16$; $P < 0.001$). When rare species were removed, the same overall pattern remained, but $\bar{\beta}_{pond, obs}$ declined to 0.29. Within-host parasite richness ($\bar{\alpha}_{host}$) was the second largest contributor to regional diversity, accounting for 25% of regional parasite diversity, which was slightly higher than expected ($\bar{\alpha}_{host, obs} - \bar{\alpha}_{host, sim} = 0.040$; $P < 0.001$). Differences among host species ($\bar{\beta}_{species}$) accounted for 23% of regional parasite diversity and were only slightly less similar ($\bar{\beta}_{species, obs} - \bar{\beta}_{species, sim} = 0.08$; $P < 0.001$) than predicted by null models. Finally, differences among host individuals within the same population ($\bar{\beta}_{host}$) accounted for just 12% of regional diversity, and individuals were much more similar in their parasite communities than predicted by null models ($\bar{\beta}_{host,obs} - \bar{\beta}_{host,sim} = -0.29$; $P = 0.001$). Therefore, turnover between communities (ponds) accounted for the highest percentage of regional diversity, followed by within-host richness, between-species at the same site, and between-hosts of the same population (Figure 2).

Differences in parasite community composition between host species ($\bar{\beta}_{J, species}$) weakened over the summer (estimate = -0.06 ± 0.02 $P = 0.005$), such that species at the same site became more similar in their parasite communities over time. Between-host differences ($\bar{\beta}_{J, host}$) did not change over time (estimate = -0.02 ± 0.01; $P = 0.20$), indicating that individuals within the same population did not become more or less similar in their parasite communities over the season. Overall, *T. torosa* individuals had lower $\bar{\beta}_{J, host}$ values than *P. regilla* (estimate =

-0.16 ± 0.03; P < 0.0001); therefore, *T. torosa* individuals shared more parasites with other members of their population than did *P. regilla.* Differences among communities ($\bar{\beta}_{J,\ pond}$) did not change appreciably over time (estimate= -0.01 ± 0.02; P = 0.64). These patterns were robust to the dissimilarity metric used, as well as the inclusion of rare species (Appendix S1).

DISCUSSION

Despite increasing interest in describing how parasite community composition changes across habitats, host species, and individuals (Pedersen and Fenton 2007, Johnson et al. 2015, Seabloom et al. 2015), comparatively few studies have investigated the drivers of compositional differences (i.e. “parasite community assembly”) especially across both biological scale and time. In this study, we compared patterns of parasite composition across nested biological scales and used repeated sampling over a temporal window of host development to derive insight into the potential roles of drift, dispersal, and ecological selection. This integration of scales indicated that parasite communities were more different across habitat patches than across host species, and that individuals within the same population contained highly similar parasite communities. Differences among communities (i.e., ponds) were consistent with dispersal limitation, whereas within communities, high rates of dispersal appeared to erode individual and species-level differences. Our study illustrates that, as a whole, dispersal processes can play a strong role in structuring parasite communities, but that rates of dispersal strongly differed across scales.

Based on an additive partitioning of regional diversity, hierarchical differences in parasite community between communities (β_{ponds}) comprised the majority (40%) of regional level diversity, more than differences between host species ($\beta_{species;}$ 23%) or individuals ($\beta_{host;}$ 12%). Community-level differences (β_{ponds}) were significantly greater at each time point than expected by chance (Figure 2), suggesting that parasites were distributed non-randomly across ponds. These differences were, in part, driven by the occurrence of rare parasites; when they were removed, the contribution of β_{ponds} was lower (28%). Differences among ponds could be the result of ecological selection if sites filter out certain parasite taxa based on abiotic or biotic conditions. For example, the only two ponds that lacked a common trematode parasite (*Cephalogonimus americanus*) were also the only ponds with American bullfrog (*Rana catesbeiana*) larvae, which are a less competent host that could reduce *C. americanus* abundance (Johnson et al. 2013). Consequently, the host community could represent one possible selection mechanism driving parasite community composition, as previously reported in similar studies (Krasnov et al. 2005, Johnson et al. 2013, Maestri et al. 2017, Mihaljevic et al. 2017). More broadly, our results are consistent with a large body of work indicating that ecological selection can strongly structure parasite communities across localities (e.g. Dallas and Presley 2014, Krasnov et al. 2015, Warburton et al. 2016). To further elucidate the role of ecological selection, interannual variation could be assessed; differences among ponds that are stable across years and correlate strongly to environmental factors could point to important selection mechanisms shaping parasite community composition.

Differences among ponds could also be generated by dispersal limitation; for instance, ponds lacking certain parasites may have not been colonized, even if suitable conditions for that parasite's survival exist. Differences among communities were consistent over time, using both Jaccard's distance and additive partitioning approaches (Figure 2), and community-level richness remained stable over time (Figure S3), suggesting that dispersal did not occur during the sampling window at levels high enough to attenuate differences in pond parasite communities. As such, lack of dispersal during the sampling season created stable differences among sites.

Parasites are spread from site-to-site through the movement of hosts (Price 1980). Despite the large dispersal potential of parasites within vagile hosts, host activity may be seasonal or infrequent, limiting colonization opportunities. Indeed, variation in definitive host activity is known to limit dispersal (Kuris and Lafferty 1994), and parasite communities are often strongly structured by this dispersal limitation at landscape scales (Smith 2001, Thieltges et al. 2009; Hartson et al. 2011). Moreover, habitat-level factors can strongly influence the activity of hosts and therefore the probability of dispersal (Sousa and Grosholz 1991, Hartson et al. 2011) making parasite dispersal across sites a non-neutral process. Consequently, relationships between geographic distance and community dissimilarity, which are commonly used to infer dispersal limitation in free-living communities (Cottenie 2005, Soininen et al. 2007), may not be expected for parasite communities structured by dispersal (Poulin 2003, Thieltges et al. 2010). On the other hand, a relationship between environmental attributes and community composition, commonly interpreted as evidence of *selection* in free-living community ecology (Smith et al. 2015, Vellend 2016), may actually be evidence of a system driven by non-neutral dispersal limitation. Therefore tracking parasite dispersal more directly, for instance by monitoring or manipulating host movement (Wood et al. 2019), could be an important tool for further evaluating the role of dispersal limitation in driving among-site variation in parasite communities.

In addition to high variation across sites, host species also demonstrated significant and non-random differences in parasite community composition. Differences between the parasite communities of *P. regilla* and *T. torosa* (β_{species}) were greater than expected by chance (Figure 2). Therefore, ecological selection appeared to play a role in structuring the parasite communities among different host species, consistent with other systems (e.g. Friggens and Brown 2005, MacColl 2009, Dallas and Presley 2014). Indeed, almost half (5/11) of the parasite taxa in this study were strict specialists on one host (Appendix S1: Table S1) and previous studies (Johnson et al. 2016) have demonstrated that host diversity is an important driver of regional parasite diversity in this same system. Intriguingly, differences among host species ($\beta_{J,\ species}$) decreased over time, revealing that *P. regilla* and *T. torosa* within the same site became more similar in their parasite communities. This pattern appeared driven by a combination of decreasing selection and increasing dispersal pressure over time. Specialist parasites (protozoans and nematodes) tended to peak earlier in the season (Appendix S1: Figure S4); this observation was supported by indicator species analysis showing that both early season parasites were specialists with direct lifecycles. On the other hand, generalist parasites in this study were all digenetic trematodes requiring a snail intermediate host, meaning that infections occurred later in development, after snails began shedding infectious trematode stages around June (Paull and Johnson 2014). Host species became more similar due to the accumulation of infections by generalist parasites, which reached high prevalences later in summer. Therefore, high dispersal of generalist parasites homogenized host species over time, and parasite specialization, a form of selection, attenuated through time. This differs from free-living communities, wherein specialist taxa often appear to colonize later in succession (Piechnik et al. 2008, Raevel et al. 2012, Helsen et al. 2016).

We found little evidence of heterogeneity among individual hosts; hosts within the same population shared significantly more parasites than expected by chance (Figure 2). Low β-diversity at this scale can result from high dispersal rates (“propagule pressure”), which act to homogenize communities (Leibold et al. 2004, Qian 2009), and it is well-established that dispersal to individual hosts can be high in pond ecosystems given the high biomass of infectious forms produced by trematodes (Preston et al. 2013; Lambden and Johnson 2013). Many parasites reached high prevalences within host populations, including numerous taxa that reached 100% prevalence (Appendix S1: Figure S4), indicating a lack of dispersal limitation to individual hosts. Our evidence differs from previous studies documenting high heterogeneities among hosts (Paull et al. 2012), driven by selection (e.g. host traits influencing susceptibility or exposure risk; Johnson et al. 2005), dispersal (e.g. spatial variation in exposure risk; Calabrese et al. 2011) or interactions between dispersal and drift (e.g. priority effects; Budischak et al. 2016). Overall, parasite interactions and variation in host competency or exposure risk did not appear to be important processes in this system. We note, however, that by sampling similarly-aged, non-reproductive hosts within similar habitats, our approach effectively limited several of the sources of intra-host variability in parasite communities. In natural populations containing a mixture of different larval cohorts, differences among individuals would likely be larger, with younger individuals supporting more protozoan infections, and older individuals supporting more trematode infections. Hosts at intermediate stages of development supported the most diverse parasite communities, as evidenced by the unimodal relationship between richness and time (Figure S3). Finally, the low divergence among host individuals indicated that drift was not a strong structuring force, since drift is expected to increase divergence over time (Gilbert and Levine 2017). Drift can represent a powerful stochastic force driving community dissimilarity (Hubbell 2001) and is expected to operate more strongly when community sizes are small, such as the within-host scale (Vellend 2010). The most prevalent parasites in this system, digenetic trematodes, do not reproduce within our focal amphibian hosts, requiring a definitive host to complete reproduction; thus demographic fluctuations and thus the influence of drift were likely dampened at the within-host scale. Therefore, unlike free-living species, the effect of drift may be more important at larger scales which encompass the entire parasite life cycle. The role of drift has not received as much attention in parasite community ecology (Seabloom et al. 2015) but its scale dependency may represent an important future avenue of research.

Empirical data from hierarchically nested parasite communities provide opportunities to understand how ecological processes vary over biological scale and enable syntheses across community ecology. However, key differences among these study systems present challenges for integrating free-living and parasite community ecology. For example, within parasite community ecology, the use of multiple, alternative sets of terminologies to describe assembly have led to both confusion and a disconnect from community ecology generally. For instance, parasitologists may refer to parasites as being limited by “encounter filters” or “compatibility filters” (Combes 2001) or evaluate the role of “host-level” processes such as host age, sex, or body size in driving community composition (Blaylock et al. 2011). Yet mechanistically such traits can affect community composition through dispersal (e.g., if larger hosts experience greater colonization Kuris et al. 1980), selection (e.g., if body size correlates with immune function; Venesky et al. 2012), or drift (e.g., if larger hosts contain larger parasite communities more resistant to stochastic extinctions; Guégan and Hugueny 1994). Additionally, the same *process* may be referred to by multiple names depending on the scale of study – for instance, dispersal may be called “transmission” when parasites disperse to hosts, but may be referred to as “colonization” when that host or population is previously uninfected (Bush et al. 1997). The concept of “transmission” further tends to combine both selection and dispersal-based processes (McCallum et al. 2017), making it challenging to compare with assembly processes in free-living ecology. As empirical data on parasite communities accumulates, using a common language about the underlying processes will further facilitate comparisons across studies and biological scales, improving links with free-living community ecology.

Studies of parasite community ecology have generated numerous fundamental insights into how parasite community composition alters disease dynamics (Johnson et al. 2015), but understanding how those communities arise is still difficult with observational studies in natural populations. Owing to the inherently nested nature of parasite communities, implementing cross-scale comparisons can more accurately capture the drivers of parasite community composition (Esch 2002, Penczykowski et al. 2016). Using additive partitioning is one way to make direct, cross-scale comparisons in nested communities, and we have used this approach herein to compare processes acting at the within-host, within-population, and within-pond level. We additionally demonstrate that comparing communities over a temporal window, especially over primary succession, can aid in disentangling process and pattern. Community assembly mechanisms influence important facets of infectious disease-causing agents: where they are, when they arrive, and at what scales they are most limited. A process-based approach therefore improves our ability to predict where parasites will spread and at what scales they may be appropriately managed.

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AUTHOR CONTRIBUTIONS

WEM and PTJ designed the study; WEM, TMG, and DMC collected the data; WEM analysed the data; WEM led the manuscript writing. All authors contributed to editing and reviewing the manuscript and gave final approval for publication.

DATA ACCESSIBILITY

Upon acceptance, individual dissection data and R code necessary to reproduce analyses will be archived in the Dryad Data Repository.

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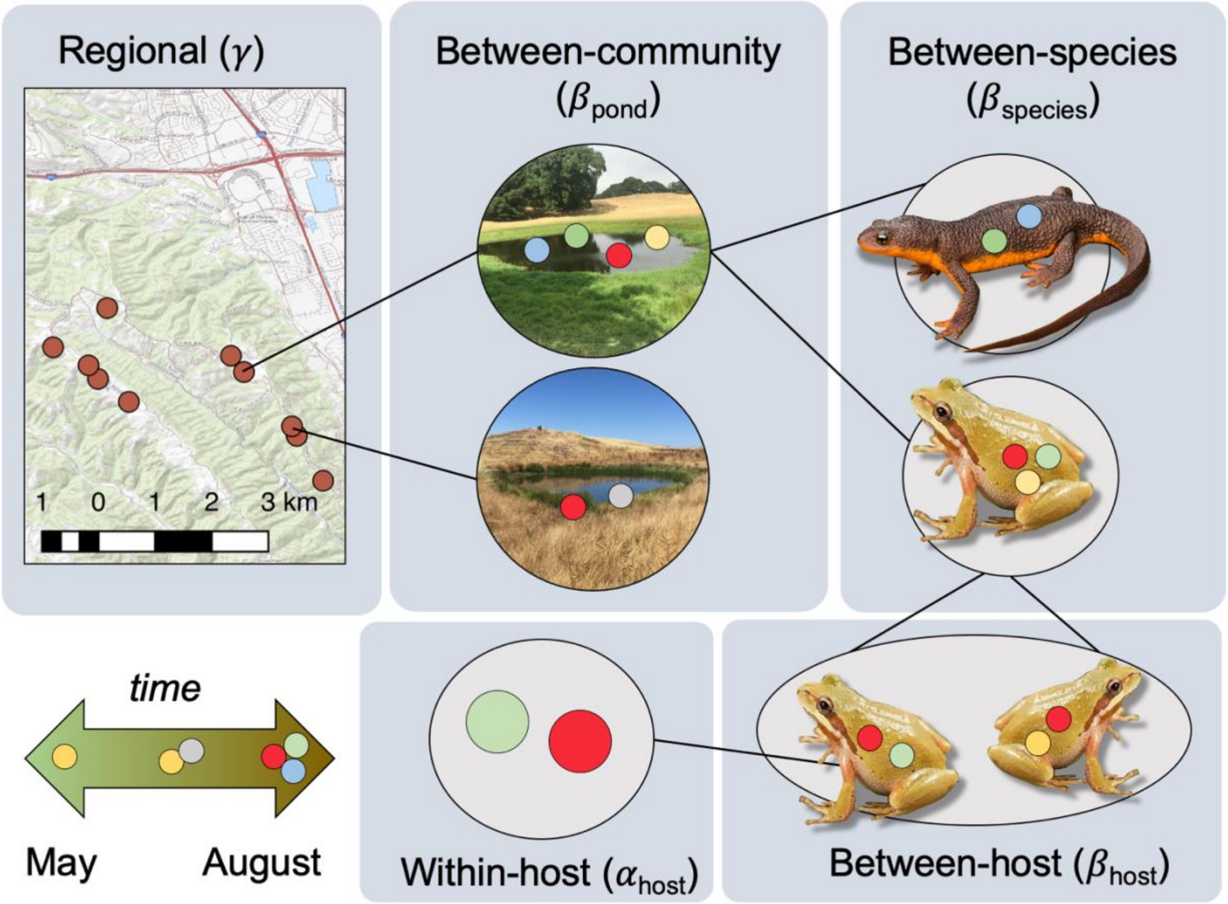
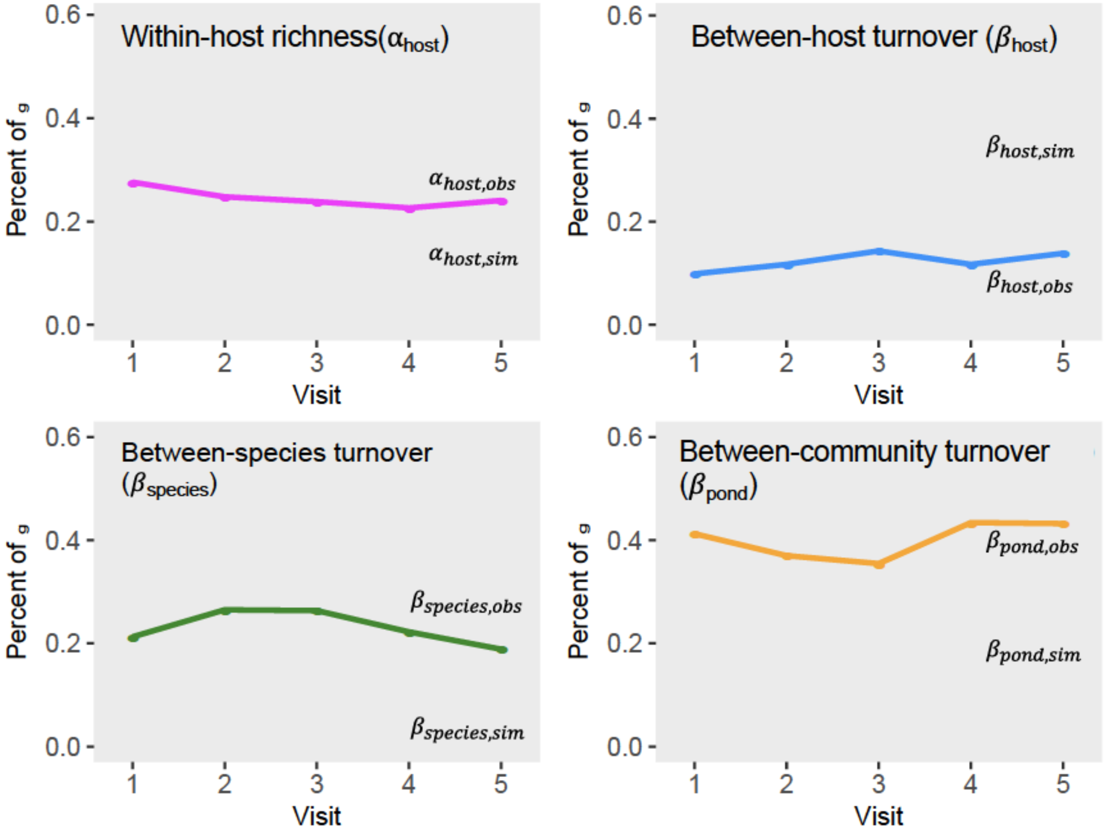
FIGURE LEGENDS

Figure 1. Contribution of different biological scales to regional parasite diversity. At the largest scale, regional richness (γ) is the total number of parasite species within all ponds at any given point in time. Regional richness can be partitioned additively into within-host richness (α-diversity) and turnover at each biological scale (β-diversity) using the equation:

$\gamma=\alpha_{host}$(within host)+β_{host}(between hosts)+ $\beta_{species}$(between species)+ β_{pond}(between pond communities). We compared the relative contributions of each component across time.

Figure 2. Additive partitioning of regional parasite richness (γ). Each panel shows the observed contribution of each level to overall regional parasite richness (solid line), compared to null simulations where parasites are distributed randomly (illustrated by a shaded ribbon showing the 95 % CI of simulations). Differences among communities or ponds (β_{pond}) represented the largest contribution to regional parasite richness and variation among individual hosts in the same population (β_{host}) represented the smallest contribution to regional parasite richness.


Regional (γ)
1 0 1 2 3 km
Between-community (β_pond)
Between-species (β_species)
time
May
August
Within-host (α_host)
Between-host (β_host)


Within-host richness(α_{host})
$\alpha_{host,obs}$
$\alpha_{host,sim}$
Between-host turnover (β_{host})
$\beta_{host,sim}$
$\beta_{host,obs}$
Between-species turnover ($\beta_{species}$)
$\beta_{species,obs}$
$\beta_{species,sim}$
Between-community turnover (β_{pond})
$\beta_{pond,obs}$
$\beta_{pond,sim}$
Percent of g
0.6
0.4
0.2
0.0
1
2
3
4
5
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