



## Evaluating the role of regional and local processes in structuring a larval trematode metacommunity of *Helisoma trivolvis*

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Metacommunity theory has advanced our understanding of how local and regional processes affect the structure of ecological communities. While parasites have largely been omitted from metacommunity research, parasite communities can provide the large sample sizes and discrete boundaries often required for evaluating metacommunity patterns. Here, we used assemblages of flatworm parasites that infect freshwater snails (*Helisoma trivolvis*) to evaluate three questions: 1) what factors affect individual host infections within ponds? 2) Is the parasite metacommunity structured among ponds? And 3) what is the relative role of local versus regional processes in determining metacommunity structure and species richness among ponds? We examined 10 821 snails from 96 sites in five park complexes in the San Francisco Bay area, California, and found 953 infections from six parasite groups. At the within-pond level, infection status of host snails correlated positively with individual snail size and pond infection prevalence for all six parasite groups. Using an ordination method to test for metacommunity structure, we found that the parasite metacommunity was organized in a non-random pattern with species responding individually along an environmental gradient. Based on a model selection approach involving local and regional predictors, parasite species richness and metacommunity structure correlated with both local abiotic (pH and total dissolved nitrogen) and biotic (non-host mollusk density, and *H. trivolvis* biomass) factors, with little support for regional predictors. Overall, this trematode metacommunity most closely followed the predictions from the species sorting or mass effects metacommunity paradigm, in which community diversity is filtered by local site characteristics.

Over the last decade, metacommunity theory has advanced our understanding of how local and regional processes interact to structure ecological communities (Leibold et al. 2004, Holyoak et al. 2005). The metacommunity framework is based on four paradigms (species sorting, patch dynamics, mass effects, and neutral) that differ in the assumed rates of dispersal, the degree of heterogeneity in local habitat conditions, and the degree of species similarity in terms of niche requirements (Logue et al. 2011). Thus, these four paradigms vary in their proposed importance of local and regional processes, and recent studies have focused on evaluating the relative influence of these processes to the assembly of metacommunities (Cottenie 2005, McCauley et al. 2008). In a recent meta-analysis, Cottenie (2005) found that local, niche-based processes (i.e. species sorting) best characterized most published metacommunities followed by more regional, dispersal-based processes (i.e. mass effects). For instance, McCauley et al. (2008) reported that a larval dragonfly metacommunity was structured both by dispersal limitation and by predation, supporting both local and regional processes. However, Cottenie (2005) stressed the need for a larger diversity (both across habitats, dispersal types, and spatial scales) of metacommunity studies that include both local and regional

processes. The relative importance of local versus regional processes across differing metacommunity characteristics is still uncertain and additional research is needed.

Although few studies have explored the application of metacommunity theory to parasites, parasite communities are appropriate models for testing such theory (Mihaljevic 2012). Parasite communities match the definition of a metacommunity, with species interacting within hosts and dispersing among hosts (Leibold et al. 2004, Mihaljevic 2012). Broader landscape level concepts have been tested using parasite communities; for example, Poulin et al. (2011) applied a comparative approach, which accounted for phylogenetic relationships, to explore patterns of biogeography inherent to host-parasite communities. In community ecology, researchers have historically integrated parasites through the exploration of competitive interactions, leading to the identification of a continuum from isolationist (little to no species interactions) to interactive (highly competitive communities) (Holmes and Price 1986). Many macroparasite stages are non-interactive (e.g. resting stages); thus, the structuring mechanisms (e.g. biotic interactions) typical of niche-based models may be relatively weak for these stages (Poulin 2004). In contrast, some macroparasite stages (i.e. larval trematodes),

have been shown to be very interactive (Lafferty et al. 1994), and may potentially exhibit tradeoffs between competitive ability and productivity (Sousa 1993, Tilman 1994). The isolationist-interactive continuum can be expanded to evaluate metacommunity dynamics of parasite communities by broadening the perspective of species interactions to include environmental interactions and dispersal.

While parasites provide an interesting study system for exploring metacommunity theory, they have several key differences from free-living systems that may complicate their inclusion into the theory. In particular, hosts often have shorter life spans to accumulate parasite species compared to habitat patches of free living species, which may lead to higher turnover rates and additional effects of host size and age on parasite community composition (Kuris et al. 1980). Hosts are also mobile and thus have complex relationships with space (i.e. inter patch distance), leading to migration rates that are constantly in flux (Kuris et al. 1980, Dove 2006). Because of the hierarchical nature of parasite communities (i.e. communities within hosts and communities among hosts), hosts also have overlapping parasite community definitions, sharing species composition across organization levels (Zelmer and Seed 2004, Dove 2006). Despite these difficulties, applying metacommunity-based approaches to parasites could offer valuable insights into the dynamics of these ‘hidden’ communities. Given that, by some estimates, parasitic species outnumber free-living species (Bush et al. 2001), extending metacommunity theory to include parasites will also help to address and overcome challenges on the road toward developing a more comprehensive statistical and theoretical approach.

In this study, we used a trematode metacommunity within the first intermediate host, rams horn snails *Helisoma trivolvis*, to investigate the role of local and regional processes in structuring a parasite metacommunity. We used the hierarchical structure of parasite communities to investigate two levels: patch occupancy at the host level (i.e. parasite infections within individual snails) and species richness and metacommunity structure aggregated within infection levels individual ponds (i.e. the parasite metacommunity among ponds). We investigated three specific questions 1) are there effects of host size and pond infection levels on infection status within individual hosts? 2) Does the parasite metacommunity in *H. trivolvis* show non-random patterns of metacommunity structure among ponds? 3) If so, what are the relative contributions of local versus regional factors in metacommunity structure and parasite species richness? Over a two-year period, we sampled *H. trivolvis* for trematode infections in freshwater ponds across three counties in California.

We used a combination of metacommunity approaches to evaluate the role of local and regional factors in metacommunity structure. In particular, we used ordination methods to look for patterns in the site by species matrix. There are six idealized metacommunity structures (Gleasonian, Clementsian, evenly spaced, nested, checkerboard, and random), which reflect the varying importance of local and regional processes (Leibold and Mikkelsen 2002). Because parasites depend on the availability of their hosts and many parasites within our community share

host species, we expected the metacommunity to exhibit a nested structure (Zelmer et al. 2004). We then evaluated how the metacommunity structure (reflected by the ordination scores) correlated with abiotic, biotic, and environmental variables at each pond using generalized linear mixed models. Because trematodes have both free-living stages and parasitic stages, we expected that parasite communities would be sensitive to the availability of hosts and to water quality (Marcogliese 2004, Soldanova et al. 2010). Thus, we expected our metacommunity to fit the species sorting or mass effects paradigms with local factors sorting species along an environmental gradient.

## Methods

### Study system

In California pond assemblages, six groups of trematodes are commonly encountered (*Alaria* sp., *Echinostoma* sp., *Ribeiroia ondatrae*, *Cephalognathus* sp., *Halipegus occidualis*, and *Allasostomoides* sp.). Hereafter we refer to the individual parasite groups by their genera. All of these parasites depend on *H. trivolvis* as their first intermediate host; however, they differ in how many additional hosts are generally used (from 1 to 3) and in which host species are suitable. For instance, *Alaria* requires 3 hosts, *H. trivolvis*, amphibians, and mammals (canids and mustellids), but can use a 4th host if an unsuitable vertebrate (often a snake) eats an infected amphibian. In contrast, *Allasostomoides* requires only two hosts, *H. trivolvis* and turtles. Because of the necessity of transmission to multiple hosts, trematodes are dependent both on the local community for the availability of hosts (Carney and Dick 2000) and on the abiotic conditions that influence their hosts (Anderson and Sukhdeo 2010, Soldanova et al. 2010). Previous studies on trematode parasite communities have found significant correlations with biotic variables (snail host density), abiotic variables (water quality), and dispersal (Fernandez and Esch 1991, Urabe and Hinoue 2004, Anderson and Sukhdeo 2010).

### Field survey

We surveyed 120 freshwater ponds in five park complexes over two years for trematode infections in *Helisoma trivolvis*. All the ponds were part of the oak chaparral eco-region and were spread across three counties in the San Francisco Bay area of California (Santa Clara, Alameda, and Contra Costa) (Fig. 1). Parks were selected by choosing areas that were accessible from land owning agencies (East Bay Regional Parks, Univ. of California Reserves, Santa Clara County Parks, Contra Costa Watershed District, California State Parks, East Bay Municipal Utility District, and The Nature Conservancy), had a combined area  $> 10 \text{ km}^2$ , had  $> 20$  potential ponds, had continuous coverage of adjacent accessible property, and were surrounded by a dense urban matrix. We used  $10 \text{ km}^2$  as the minimum area because common definitive hosts (e.g. great blue herons and egrets) for our trematode guild have an estimated

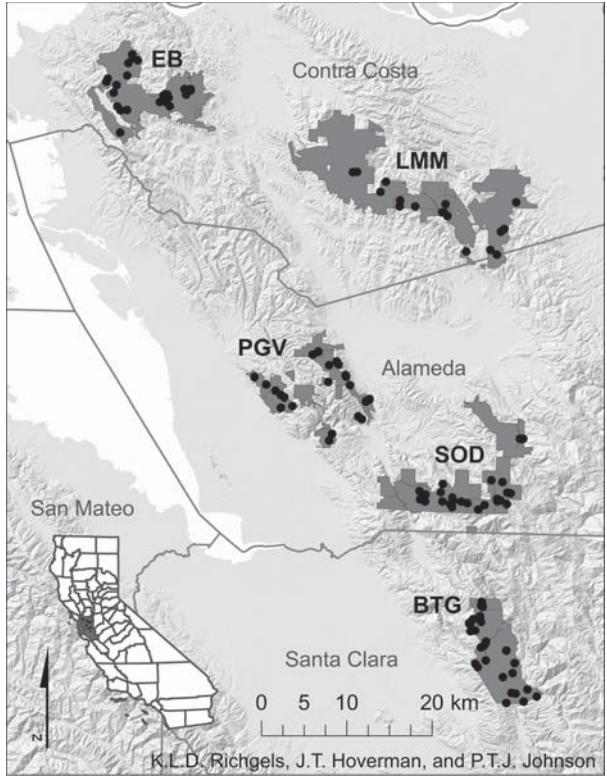


Figure 1. This map shows the study area in California, including the five park complexes (gray polygons) and the ponds sampled (black dots).

home range of 10 km (Kelly et al. 2008). We chose to have park complexes with a minimum of 20 ponds to ensure we had large enough sample sizes. Our five selected park complexes were 1) Briones Reservoir Watershed, San Pablo Reservoir Watershed, and Briones Regional Park (EB), 2) Los Vaqueros Watershed, Morgan Territories Regional Park, and Mount Diablo State Park (LMM), 3) Pleasanton Ridge Regional Park, Garin/Dry Creek Pioneer Regional Park, and Vargas Plateau Regional Preserve (PGV), 4) Sunol Regional Park, Ohlone Regional Wilderness, and Del Valle Regional Park (SOD), and 5) Blue Oaks Ranch Reserve, The Nature Conservancy's Rancho Canada de Pala, and Joseph Grant County Park (BTG) (Fig. 1). Within these park complexes, we randomly selected ponds that were <2 ha and were visible in Google Earth (ver. 4.3) imagery from August to October 2008. Of these randomly selected ponds, we sampled ponds containing *H. trivolvis* twice within the same year, with first visits between 9 May and 25 June 2009 and 17 May and 4 July 2010, and second visits between 26 June and 5 August 2009 and 5 July and 6 August 2010.

To assess parasite species richness, we collected and measured >50 *H. trivolvis* over 5 mm in shell length on each visit (for a total of approximately 100 snails per pond). We collected snails by conducting 10–15 dipnet sweeps (45.7 cm d-frame with 1200 µm mesh) every 10–20 m around the perimeter of the pond on the first visit and 2–5 seine hauls (1.2 × 1.8 m with 3000 µm mesh) within the interior of the pond on both visits. We chose to collect only snails >5 mm because trematode infections utilize the mature gonads of their hosts, and snails below this

threshold size usually do not have mature gonads and thus rarely support infections (Richgels unpubl.). Snails were placed individually into 50 ml centrifuge tubes for 24 h and checked every 12 h for the release of free-swimming parasite stages (cercariae). When free-swimming stages were present, we identified infections based on the free-swimming stages' morphology (Yamaguti 1971, Schell 1985). We dissected snails that did not release free-swimming stages to quantify immature infections. Infections in dissected snails without free-swimming stages present were recorded as unidentified, because identifications based on parasite stages within the snail are often unreliable (Schell 1985). When possible, we identified parasite species using a combination of known species lists in this region, molecular work, and relevant literature (Thomas 1939, Lang 1968, Brooks 1975, Schell 1985, Fried and Graczyk 2004, Johnson et al. 2004). If species identifications were not possible, we used free-swimming stage morphotypes or parasite genera (e.g. *Echinostoma* sp.). Rarefaction curves supported that our sampling methods were sufficient for estimating parasite species richness within a pond. Using a subset of 11 ponds, 10 reached an asymptote and all Chao 1 estimators were within 0.5 of the observed species richness (EstimateS, Colwell 2009).

We collected information on the abiotic (pH, conductivity, total dissolved nitrogen, trees within 15 m, surface area, and percentage of the pond surface vegetated) and biotic characteristics (non-host mollusk density, and *H. trivolvis* biomass) of each pond. For biotic variables, we assessed the density of *H. trivolvis* and other non-host mollusks (*Lymnaea* sp., *Physa* sp., and *Gyraulus* sp.), which represents host availability and potential dead end hosts for the parasites, from the dipnet and seine sampling as described above. Because the *H. trivolvis* population represents habitat patches, we accounted for variation in average size of the host population by converting the density to biomass using the average length to mass conversion found in Johnson et al. (2012) then multiplying that by the density. For abiotic variables, we assessed three water quality measurements. At each site, we measured pH and conductivity using a handheld meter (Yellow Systems Instruments 556 Multi Probe System). Conductivity (the ability to conduct electricity) is an indicator of the dissolved salts in the water while pH has been shown to impact both snail and trematode communities (Lodge et al. 1987, Soldanova et al. 2010). Water samples were collected and analyzed for total dissolved nitrogen concentration to represent pond productivity using standard methods (<<http://snobear.colorado.edu/Kiowa/Kiowaref/procedure.html>>). We also estimated three abiotic variables that impact available host habitat and vertebrate host use. We estimated the percentage of the water surface that was vegetated as a proxy for habitat of *H. trivolvis*. We also counted the number of trees taller than 3 m within 15 m of the pond. This variable represented cover and perching sites for vertebrate hosts and can affect parasite prevalence (Smith 2001). We calculated the surface area by walking the perimeter of the pond with a GPS unit (Garmin GPSMAP 60). Surface area is a proxy for habitat size, because parasite species richness often increases with pond size (Zelmer and Campbell 2011).

Along with local variables, we also calculated three variables that were used as proxies for dispersal (pond density, distance to nearest *H. trivolvis* occupied pond, and distance to nearest lake). Pond density was defined as the number of neighboring ponds within 1 km, irrespective of whether they supported *H. trivolvis* (in R statistical package ver. 2.13.2 call ‘dist’). We chose to use pond density instead of park complex size (e.g. number of patches in the metacommunity) because the boundaries of each park complex were artificially set. We also calculated the distance to the nearest *H. trivolvis* occupied pond and the distance to the nearest lake (defined as having a surface area >10 000 m<sup>2</sup>) to represent dispersal distances and major habitat for potential vertebrate hosts. Distance to nearest *H. trivolvis* occupied pond was created by spatially joining all pond locations with a layer including only *H. trivolvis* occupied ponds within ArcGIS 10 (ESRI). Distance to nearest lake was created by spatially joining the National Wetlands Inventory layer for California (NWI, USFWS) that had water bodies with surface areas >10 000 m<sup>2</sup> selected to our pond locations in ArcGIS 10 (ESRI).

## Statistical analyses

Our analyses focused on two different scales of organization. Within ponds, we evaluated the factors affecting infection within individual snail hosts. This analysis sought to evaluate the effects of snail size and pond infection prevalence (the number of infections divided by the total number of snails sampled within a pond) on individual infection status (yes or no) both for the community and for each parasite species individually. We chose to evaluate snail size because larger snails are often more likely to be infected (Kuris 1990), whereas increasing prevalence at the pond level should widen the range of sizes that are infected. Among ponds, we evaluated metacommunity structure and the role of local versus regional processes in predicting both parasite metacommunity structure and species richness. This analysis included all sampled ponds across park complexes, thereby capturing the trematode metacommunity at the scale of their vertebrate host metacommunity. We chose to evaluate the roles of local and regional processes among ponds because the parasite metacommunity is more complex at the pond scale (as opposed to individual host scale) and the larger perspective will capture factors affecting more mobile hosts.

We used generalized linear models (GLM) with a binomial distribution to test for factors affecting infection status in individual snails. The response variable was infection status (yes or no) and the explanatory variables were snail size and overall infection prevalence at the pond scale. We ran this analysis for overall infection (i.e. aggregating among all parasite species) and for each parasite species individually. For the latter, we excluded snails that were infected with other parasite species so as to determine whether the size relationship was consistent across parasite species. We excluded ponds without *H. trivolvis* ( $n = 24$ ) or without any parasites present ( $n = 24$ ) to reduce the likelihood of overdispersion (72 ponds were included in the analysis). We initially ran generalized linear mixed models

with a random effect of pond identity, but using generalized least squares and likelihood ratio tests, we found that the random effect of pond identity was not necessary ( $p = 0.99$ , Zuur et al. 2009). We squared the correlation coefficient of the fitted values versus the observed values to estimate R<sup>2</sup> (e.g. the models predictive power) and used the  $\chi^2$  statistic to test for overdispersion and goodness of fit (Mitchell et al. 2010). All models were run in R statistical package (call `glm()`).

We followed the methods of Leibold and Mikkelsen (2002) to identify the best-fit structure for the metacommunity (Presley et al. 2010). In brief, this method uses ordination by reciprocal averaging on the site by species incidence matrix and then tests the resulting ordinated matrix against randomized site by species matrices for a dominant axis of variation (coherence), species replacements (turnover or nestedness), and groups of species with similar ranges (boundary clumping). In more detail, reciprocal averaging places sites with the most similar species compositions and species with similar distributions close together. The ordinated matrix is then tested for a dominant axis of variation (coherence) using a null model that randomizes the species matrix while keeping row and column totals constant. The matrix was considered coherent if the ordinated matrix had significantly fewer embedded absences (e.g. species missing from a pond within their range) than the average of the randomized matrix (Leibold and Mikkelsen 2002). If the matrix was coherent, we examined the number of species replacements, which is when a species replaces another at ponds along the ordinated gradient such that their ranges do not overlap, after filling in species absences within each individual range (embedded absences). If the ordinated matrix had significantly less or more species replacements than the average randomized matrix, we considered it to be dominated by nestedness or turnover, respectively (Leibold and Mikkelsen 2002). We used Morisita’s index to test the matrix for species ranges with similar boundaries (boundary clumping, Leibold and Mikkelsen 2002). Index values >1 indicate clumped boundaries whereas values <1 indicate overdispersion. A chi-squared test was used to test for significance of the observed index value. For all tests, we compared our observed matrix to 1000 iterations of the randomized matrix and applied the range perspective for reciprocal averaging. Tests were conducted in Matlab, release 2010b with script files downloaded at <[www.tarleton.edu/higgins/EMS.htm](http://www.tarleton.edu/higgins/EMS.htm)> (Presley et al. 2009, 2010). We ran this analysis for the combined site by species matrix which included only ponds that had at least one parasite group present (among ponds; 72 sites  $\times$  6 species).

To test the contributions of local versus regional factors to both parasite species richness and metacommunity structure (the resulting ordination scores from reciprocal averaging), we used model selection on generalized linear mixed models (GLMM) (Burnham and Anderson 2002). We created eight possible models representing hypotheses based on potential local and regional variables that may be important for species richness and metacommunity structure and used Akaike’s information criterion (AIC<sub>c</sub>) corrected for sample sizes (Burnham and Anderson 2002) to compare among candidate models. The eight competing

hypotheses decomposed potential local and regional factors; local models included abiotic, biotic, and abiotic + biotic variables, the regional model included dispersal variables, the combined local and regional models included abiotic + dispersal, biotic + dispersal, and all variables (global model), and the null model included the intercept only (Supplementary material Appendix 1, Table A1 for a summary of the variables). We ran GLMM with a Poisson distribution for parasite species richness, including *H. trivolvis* occupied ponds ( $n = 96$ ), and a Gaussian distribution for the ordination scores, including ponds with at least one parasite group present and excluding 3 ponds with missing data ( $n = 69$ ). Park complex was included as a random effect using the R statistical package ('lme4' call glmer()). Predictor variables were transformed as necessary to help normalize their distributions. We assumed models within 4 AIC<sub>c</sub> were equally good fits and applied model averaging to get robust parameter estimates (Burnham and Anderson 2002). To test for model fit, we squared the correlation coefficient of the fitted values versus the observed values for the global model (including all variables) for both response variables to estimate R<sup>2</sup> and used the  $\chi^2$  statistic for species richness to test overdispersion and goodness of fit (Mitchell et al. 2010).

## Results

From the 120 ponds sampled, 96 ponds had *H. trivolvis* and 72 ponds had at least one parasite group present. Three ponds were excluded from the GLMM analyses because of missing environmental variables. From 10 821 dissected snails, we found 953 infections (including two double infections), and six parasite groups were commonly found in the study area: *Alaria* (47 ponds), *Echinostoma* (33 ponds), *Ribeiroia* (24 ponds), *Cephalogonimus* (22 ponds), *Halipegus* (21 ponds), and *Allassostomoides* (19 ponds). The parasite groups were found at different frequencies between the 5 park complexes, with the highest frequency for each parasite group occurring at PGV for *Alaria*, *Echinostoma*, *Ribeiroia*, and *Allassostomoides*, and LMM for *Cephalogonimus* and *Halipegus* (Supplementary material Appendix 1, Table A2). The average infection prevalence for each pond, combined among parasite species, was 0.094 (standard error of the mean = 0.015) and ranged from 0.00 to 0.87. The individual parasite species varied widely in prevalence between each pond, ranging from 0.00 to 0.78 (*Cephalogonimus*). The highest pond-level prevalence observed for each species was 0.78 for *Cephalogonimus*, 0.74 for *Echinostoma*, 0.27 for *Ribeiroia*, 0.25 for *Alaria*, 0.14 for *Halipegus*, and 0.04 for *Allassostomoides*. The average parasite species richness per pond was 1.63 and ranged from 0 to 6 species.

Snail size and overall pond prevalence were both positive predictors of infection status with a relatively good model fit and no evidence of overdispersion (GLM AIC = 4630, snail size unstandardized coefficient = 0.35, pond prevalence unstandardized coefficient = 3.95, R<sup>2</sup> = 0.22, deviance = 4624,  $\chi^2 = 0$ , p = 1, n = 9001), and was a better fit for the data than the intercept model (AIC = 6066, deviance = 6064). Correspondingly, infected snails were

larger (average = 13.28 mm, standard error of the mean = 2.54) than the overall average size of *H. trivolvis* dissected (average = 10.38 mm, standard error of the mean = 1.98). Snail size was a significant predictor for each of the individual parasites when excluding other infections, although the magnitude of the unstandardized coefficients varied by parasite species; *Ribeiroia* had the largest coefficient for size (0.55) while *Alaria* had the smallest (0.24) (Table 1). Four species had significant, positive unstandardized coefficients with overall pond prevalence (*Cephalogonimus*, *Echinostoma*, *Halipegus*, *Ribeiroia*) (Table 1). Overall, snails were more likely to be infected if they were larger or occurred in ponds with higher prevalence.

The site by species matrix exhibited a dominant axis of variation (e.g. significant coherence), indicating that it had fewer species absences from within the species' ordinated ranges (e.g. embedded absences) (73) than the average null model (117.65, p < 0.0001). The number of species replacements (1804) was not different from the average null model number of replacements (2262.4, p = 0.70), thus the species matrix exhibited non-significant turnover and non-significant nestedness. The species matrix did not have clumped species range boundaries (e.g. boundary clumping, Morisita's index = 0.00, p = 0.23). This suggests that the regional metacommunity displayed quasi-nested structure with stochastic species loss, which is defined as having significant coherence, fewer number of species replacements than the null model (but non-significant), and non-significant boundary clumping (Fig. 2, Presley et al. 2010). This quasi structure indicates that the larval trematode metacommunity was characterized by species-specific tolerances to the gradient identified by reciprocal averaging (Presley et al. 2010).

The global models for parasite species richness and metacommunity structure fit the data reasonably well and were not overdispersed (R<sup>2</sup> = 0.39 and 0.44, species richness  $\chi^2 = 1.14$ , p = 0.88). All GLMM models used to explain

Table 1. The results from the GLM of infection status by snail size and overall pond prevalence for each parasite group. The correlation estimate represents the unstandardized coefficient of the relationship with size or prevalence from the GLM results. The All species model has 9001 snails and represents 72 ponds.

Parasite species	Estimate	Standard error	Z value	p value
Relationship between size and parasite species				
All species	0.35	0.01	25.42	<0.0001
<i>Alaria</i>	0.24	0.03	7.88	<0.0001
<i>Cephalogonimus</i>	0.33	0.03	10.50	<0.0001
<i>Echinostoma</i>	0.39	0.03	14.80	<0.0001
<i>Halipegus</i>	0.30	0.03	9.24	<0.0001
<i>Ribeiroia</i>	0.55	0.03	15.72	<0.0001
<i>Allassostomoides</i>	0.29	0.09	3.41	0.0006
Relationship between site prevalence and parasite species				
All species	3.95	0.28	14.02	<0.0001
<i>Alaria</i>	0.77	0.67	1.15	0.251
<i>Cephalogonimus</i>	5.44	0.53	10.30	<0.0001
<i>Echinostoma</i>	4.73	0.44	10.81	<0.0001
<i>Halipegus</i>	2.14	0.65	3.31	0.0009
<i>Ribeiroia</i>	1.29	0.56	2.29	0.0219
<i>Allassostomoides</i>	2.74	1.57	1.74	0.0819

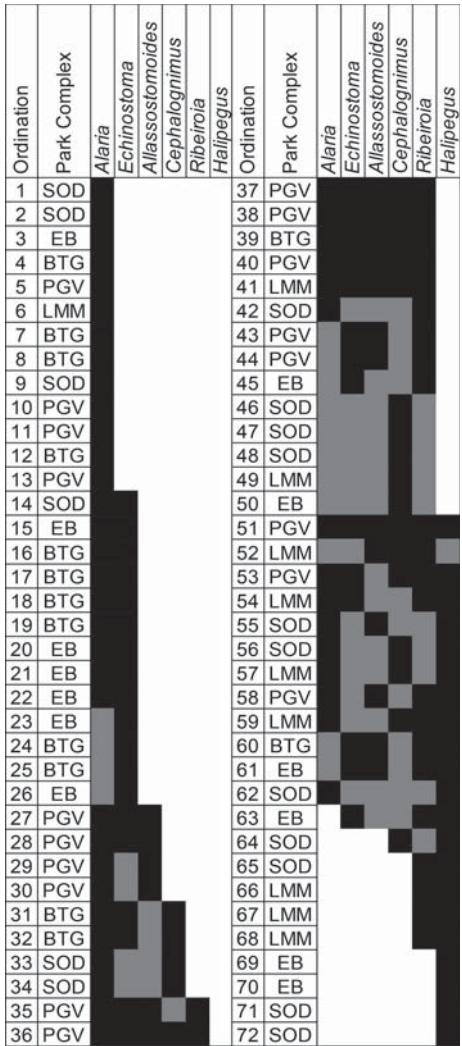


Figure 2. Incidence matrix for the 5 park complexes in San Francisco Bay area, California. The site by species matrix was ordinated using reciprocal averaging and following the methods of Leibold and Mikkelsen (2002). Black areas represent species presence during at least one sampling event during the survey and gray areas represent embedded absences within a species range. Ordination number and park complex codes are listed with each pond and are as follows: BTG: Blue Oaks Ranch Reserve, Rancho Canada del Pala, and Joseph Grant County Park, EB: Briones Reservoir Watershed, San Pablo Reservoir Watershed and Briones Regional Park, LMM: Los Vaqueros Watershed, Morgan Territories Regional Park, and Mt Diablo State Park, PGV: Pleasanton Ridge Regional Park, Garin/Dry Creek Pioneer Regional Park, and Vargas Plateau Regional Preserve, and SOD: Sunol Regional Park, Ohlone Regional Wilderness, and Del Valle Regional Park.

parasite richness were within 4 AIC<sub>c</sub>; thus, we used model averaging to integrate information among models in assessing variable importance (Table 2, 3). pH was the only significant factor in the model averaging, but pond surface area was marginally significant ( $p = 0.003$ ,  $p = 0.079$  respectively, Table 3). pH correlated negatively while surface area correlated positively with parasite richness (Table 3). The best fitting GLMM models for metacommunity structure, which was represented by the reciprocal averaging ordination scores, were the biotic and the abiotic + biotic

models. Total dissolved nitrogen, non-host mollusk density, and *H. trivolvis* biomass were significant factors in the model averaging (Table 3). Total dissolved nitrogen and non-host mollusk density correlated positively while *H. trivolvis* biomass correlated negatively with the ordination scores (Table 3). Additionally, total dissolved nitrogen correlated positively with *H. trivolvis* biomass (unstandardized coefficient = 0.075) and non-host mollusk densities (unstandardized coefficient = 0.013). However, non-host mollusk density correlated negatively with *H. trivolvis* biomass (unstandardized coefficient = -0.225), suggesting competitive effects within the mollusk community. In sum, metacommunity structure was affected by local abiotic (total dissolved nitrogen) and biotic (*H. trivolvis* biomass and non-host mollusk density) factors, with no obvious signature of regional factors.

## Discussion

While generally omitted from metacommunity research, parasite communities can be effective models for testing such theory. Our results indicated that the parasite metacommunity was a non-random assemblage that appeared to be influenced primarily by local factors. Within ponds, larger hosts were more likely to be infected, particularly in ponds with a high overall level of infection. Among ponds, the trematode metacommunity exhibited a quasi-nested structure with stochastic species loss, which suggests that individual species have different tolerances along the ordinated gradient. Abiotic and biotic variables that likely affected the availability of mollusk hosts were most important in influencing metacommunity structure (e.g. *H. trivolvis* biomass, non-host mollusk density, and total N). Parasite species richness was related (weakly) to local abiotic variables that represent the quality and size of the wetland (e.g. pH and wetland surface area). Taken together, these results suggest that this larval trematode metacommunity fits the species sorting or mass effects paradigm of metacommunities, due to the importance of local, niche factors.

At the scale of individual hosts, host size and overall pond prevalence positively predicted trematode infection, indicating that larger snails and snails in ponds with higher prevalence were more likely to be infected. Host size has been shown to positively correlate with infection (Kuris 1990, Faltynkova et al. 2008) and has multiple possible mechanisms. Larger hosts can provide a bigger target for parasites, which contributes to higher infection rates. Older hosts, which are usually larger, are also exposed to parasites for a longer period of time, thereby increasing their risk of infection (Sousa 1992); this relationship is also known as the age-prevalence curve in epidemiology and is supported in the closely related *Biomphalaria–Schistosoma* system (Anderson and Crombie 1984). Finally, some trematodes cause ‘gigantism’, or increased growth rate in the host snail. This occurs because the host snail is castrated by the infection and reallocates resources from reproduction to growth (Mouritsen and Jensen 1994). Interestingly, our results also revealed variation in the coefficient for host size between the six parasite groups, with the only parasite

Table 2. The results of the generalized linear mixed model (GLMM) selection using AIC<sub>c</sub> and negative log likelihood for parasite species richness (n = 96) and parasite community structure (reciprocal averaging ordination scores, n = 69) among ponds in the San Francisco Bay area, California. The models are ordered from best to least fitting models according to their AIC<sub>c</sub> values. Models within 4 AIC<sub>c</sub> are considered equally good fitting models and are indicated by gray shading.

Models	No. parameters	Model comparisons					
		AIC <sub>c</sub>	Δ AIC <sub>c</sub>	AIC <sub>c</sub> weight	Cumulative weight	Negative Log likelihood	Deviance
<b>Parasite species richness</b>							
abiotic	8	138.81	0	0.3	0.3	-60.57	121.2
abiotic + biotic	10	139.71	0.89	0.19	0.5	-58.57	117.1
intercept	2	140.55	1.74	0.13	0.62	-68.21	136.5
dispersal	5	140.7	1.88	0.12	0.74	-65.01	130.2
abiotic + dispersal	11	141.3	2.49	0.09	0.83	-58.06	116.1
biotic	4	141.73	2.92	0.07	0.9	-66.64	133.3
global	13	142.22	3.41	0.06	0.95	-55.86	111.7
biotic + dispersal	7	142.56	3.74	0.05	1	-63.64	127.3
<b>Community structure (ordination scores)</b>							
biotic	5	213.82	0	0.54	0.54	-101.43	203
abiotic + biotic	11	214.73	0.91	0.34	0.89	-94.05	188.1
biotic + dispersal	8	218.46	4.64	0.05	0.94	-100.03	200.1
abiotic	9	219.7	5.88	0.03	0.97	-99.32	198.6
intercept	3	220.73	6.9	0.02	0.99	-107.18	214.4
global	14	221.59	7.77	0.01	1	-92.91	185.8
dispersal	6	224.74	10.92	0	1	-105.69	211.4
abiotic + dispersal	12	226.49	12.67	0	1	-98.46	196.9

group (*Alaria*) that has a larval stage within the snails with no mouth or gut (sporocysts) having the smallest coefficient with host size. This stage is thought to be a weaker competitor than the alternative form with a mouth and gut (rediae) and that they thus regularly inhabit smaller snails due to competitive exclusion from larger snails (Kuris 1990). The relationship between infection, larger size, and overall pond prevalence was moderated by parasite identity.

Among ponds, the larval trematode metacommunity exhibited quasi-nested structure with stochastic species loss (Presley et al. 2010), in which the most common species range enveloped all other species ranges. The metacommunity showed quasi-nested rather than fully nested structure because a few species with more restricted ranges (e.g. *Ribeiroia* and *Halipegus*) sometimes occurred without more common species (e.g. *Alaria*). Within the trematode

Table 3. Model averaging parameter estimates from the 8 models of local and regional factors affecting larval trematode species richness and metacommunity structure, represented by the reciprocal averaging ordination scores, for 96 ponds (species richness) or 69 ponds (metacommunity structure) across 5 park complexes in San Francisco Bay area, California. The significant factors are in bold.

Variables	Estimate	Standard error	Z value	p value
<b>Model averaging parameter estimates – species richness</b>				
Log surface area	0.11	0.06	1.76	0.0787
Log percentage vegetation	-0.04	0.06	0.75	0.4524
Log number of trees within 15 m	-0.06	0.06	1.05	0.2943
Log conductivity	-0.02	0.06	0.35	0.7296
pH	<b>-0.16</b>	<b>0.05</b>	<b>3.00</b>	<b>0.0027</b>
Log total N	0.07	0.05	1.32	0.1863
Square root non-host mollusk density	0.07	0.05	1.41	0.1598
Log <i>H. trivolvis</i> biomass	0.05	0.05	0.99	0.3223
Pond density	-0.08	0.07	1.08	0.2797
Log distance to nearest <i>H. trivolvis</i> positive site	-0.07	0.05	1.24	0.2162
Log distance to nearest lake >10 000 m <sup>2</sup>	0.15	0.09	1.57	0.1173
<b>Model averaging parameter estimates – ordination scores</b>				
Log surface area	0.05	0.11	0.40	0.6865
Log percentage vegetation	-0.10	0.10	1.01	0.3143
Log number of trees within 15 m	-0.09	0.10	0.94	0.3459
Log conductivity	0.07	0.11	0.60	0.5500
pH	-0.07	0.10	0.71	0.4798
Log total N	<b>0.34</b>	<b>0.10</b>	<b>3.37</b>	<b>0.0008</b>
Square root non-host mollusk density	<b>0.29</b>	<b>0.11</b>	<b>2.78</b>	<b>0.0054</b>
Log <i>H. trivolvis</i> biomass	<b>-0.27</b>	<b>0.11</b>	<b>2.48</b>	<b>0.0131</b>
Pond density	-0.10	0.13	0.72	0.4734
Log distance to nearest <i>H. trivolvis</i> positive site	0.10	0.11	0.94	0.3493
Log distance to nearest lake >10 000 m <sup>2</sup>	0.07	0.14	0.51	0.6125

metacommunity, the most widely distributed species such as *Echinostoma* and *Alaria* tended to have low host specificities and often use highly mobile vertebrate hosts (i.e. mammals or birds, Schell 1985, Fried and Graczyk 2004), which may facilitate dispersal. Similarly, Poulin et al. (2012) found that generalist parasites had larger geographic ranges relative to specialist parasites in a fish-parasite system.

The observation of stochastic species loss in the metacommunity suggests that each parasite species responds uniquely to the range of environmental conditions encountered in this region. Given that many of the parasites included in this study (e.g. *Alaria*, *Echinostoma*, *Ribeiroia*, *Cephalogonimus*, *Halipegus*) require three or more hosts to complete their life cycle, it is possible that host availability was a factor underlying the pattern of stochastic species loss. Unfortunately, the complete host range for many of these parasites is incomplete, but future efforts aimed at comparing the relative influence of host requirements, host dispersal ability, and additional environmental factors in driving parasite metacommunity structure should be a priority.

The major gradient supported by the reciprocal averaging analysis correlated with factors associated with the mollusk host (i.e. total N, non-host mollusk density, and *H. trivolvis* biomass). Non-host mollusk density represents two potential mechanisms for interactions with parasite species. Non-host mollusks compete for resources with the host species, thus impacting host species dynamics (Brown 1982). They can also act as 'decoy hosts', meaning parasite species unsuccessfully try to infect an unsuitable mollusk species leading to reduced infection levels in the target host (Johnson and Thielges 2010). Total dissolved nitrogen interacts with parasite species by increasing pond productivity; increased productivity can increase *H. trivolvis* biomass and the availability of hosts for parasites (Johnson et al. 2007). There was no signal from dispersal processes, as indicated by the lack of support for the dispersal variables in the GLMM. Thus, the availability of mollusk hosts (a local process) is likely the main structuring factor for this larval trematode metacommunity.

Local factors were also most important for species richness of larval trematode communities. Although none of the models were selected as the best fit, the strongest predictor of parasite species richness was local abiotic characteristics (pH and surface area). pH may be important to multi-host parasites through several direct and indirect mechanisms (Anderson and Sukhdeo 2010). Larval trematodes have several free-living stages where they are exposed to the environment, and these stages are sometimes sensitive to water quality (Karvonen et al. 2003). Additionally, pH could affect parasite species richness indirectly because it is correlated with many water quality measurements such as dissolved oxygen, water temperature, and productivity. Though the negative correlation with pH was surprising, Soldanova et al. (2010) also found a negative effect of pH on parasite species richness in a study of freshwater snails and their parasites in Germany. Indeed, complex life cycle parasites are not the only groups to be affected by pH; microbial communities in both streams and soils are most similar to each other when the environmental pH is similar

(Fierer et al. 2007, Lauber et al. 2009). Future experiments are needed to further evaluate the relationship between pH and trematode parasite richness. Species richness also correlated weakly with surface area, consistent with well-established work on the species-area relationship for free-living communities (Connor and McCoy 1979). Surface area has also been shown to be positively associated with parasite community structure and species richness in a fish-parasite system (Zelmer and Campbell 2011).

The larval trematode community can be classified as 'interactive' on the competition continuum. Interactive communities demonstrate non-random patterns of community assembly and positive or negative species associations (e.g. due to species interactions) (Holmes and Price 1986). Positive associations are inferred in our study system because two parasite groups, *Ribeiroia* and *Allasostomoides* never occurred within single species communities (Fig. 2). Although we did not test for species associations directly, previous studies have also found positive associations between particular trematode species across wetlands (Urabe and Hinoue 2004), presumably because trematode species share vertebrate hosts and are thus dispersed together. Thus, there are two possible mechanisms for the positive associations in our metacommunity, direct interactions such as competition, or indirect interactions through shared dispersal mechanisms.

Taken together, our results suggest that this larval trematode metacommunity in *H. trivolvis* most closely resembles the species sorting or mass effects paradigms of metacommunities. According to the meta-analysis by Cottenie (2005), most systems follow species sorting or mass effects, especially when they have passive dispersal in aquatic systems. Species sorting emphasizes that differences in habitat patches, such as environmental gradients, determine species composition and structure, while dispersal allows for communities to track environmental changes (Leibold et al. 2004, Holyoak et al. 2005). Mass effects is similar to species sorting, but allows for greater dispersal such that species can be rescued from local extinction (Leibold et al. 2004, Holyoak et al. 2005). Logue et al. (2011) argued that mass effects and species sorting cannot be distinguished without direct measurement of dispersal. Thus, we do not discern between these overlapping metacommunity paradigms. In our study system, we found evidence that local niche factors were most important in determining metacommunity structure and species richness. We acknowledge, however, that such results may vary as a function of spatial extent and the particular parasite assemblage under study, emphasizing the importance of more comparative studies that incorporate metacommunity tools to study parasites. Future studies should also try to incorporate multiple community levels or a nested design of metacommunities to evaluate how hierarchical organization affects the role of local and regional processes in metacommunity structure and species richness.

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Supplementary material (Appendix E7868 at <[www.oikosoffice.lu.se/appendix](http://www.oikosoffice.lu.se/appendix)>). Appendix 1.