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## Spatial heterogeneity of daphniid parasitism within lakes

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**Abstract** Spatially explicit models show that local interactions of hosts and parasites can strongly influence invasion and persistence of parasites and can create lasting spatial patchiness of parasite distributions. These predictions have been supported by experiments conducted in two-dimensional landscapes. Yet, three-dimensional systems, such as lakes, ponds, and oceans, have received comparatively little attention from epidemiologists. Freshwater zooplankton hosts often aggregate horizontally and vertically in lakes, potentially leading to local host–parasite interactions in one-, two-, or three-dimensions. To evaluate the potential spatial component of daphniid parasitism driven by these local interactions (patchiness), we surveyed vertical and horizontal heterogeneity of pelagic *Daphnia* infected with multiple microparasites in several north temperate lakes. These surveys uncovered little evidence for persistent vertical patchiness of parasitism, since the prevalence of two parasites showed little consistent trend with depth in four lakes (but more heterogeneity during day than at night). On a horizontal scale of tens of meters, we found little systematic evidence of strong aggregation and spatial patterning of daphniid hosts and parasites. Yet, we observed broad-scale, basin-wide patterns of parasite prevalence. These patterns suggest that nearshore off-

shore gradients, rather than local-scale interactions, could play a role in governing epidemiology of this open water host–parasite system.

**Keywords** Aquatic · Autocorrelation · *Daphnia* · Deep trouble hypothesis · Parasites · Patchiness

### Introduction

New diseases continue to erupt in natural plant and wildlife populations. Therefore, it is important to study the factors which regulate the successful invasion and spread of parasites into natural host populations. Most approaches to tackling them focus on the net reproductive rate of the parasite,  $R_0$  (Keeling and Grenfell 2000). As a result, traits of hosts and parasites (e.g., transmission rate, virulence, threshold densities of susceptible hosts) draw most attention since these traits determine whether a parasite can ( $R_0 > 1$ ) or cannot ( $R_0 < 1$ ) invade and persist in a host population (Anderson and May 1991). When epidemiologists determine  $R_0$ , they typically assume that hosts and parasites interact in homogeneously mixed environments (Anderson and May 1991; Keeling and Grenfell 2000). However, natural host–parasite systems often flout these assumptions (Real and McElhany 1996; Holmes 1997), as populations frequently aggregate and interact locally (Tilman and Kareiva 1997). Thus, factors regulating disease spread in natural populations could depend explicitly upon spatial arrangements of interactions between hosts and parasites.

Moreover, spatially explicit epidemiological theory reveals that local interactions between hosts and parasites can inhibit invasion and create spatial patchiness of parasitism. Such predictions arise from lattice (cellular-automata) and moment-closure models. These models represent discrete individuals which interact locally within fairly small, two-dimensional “neighborhoods”. Local “neighborhood” interactions occur when hosts

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move over short distances or interact only with other proximate hosts. As a result, parasites can locally exhaust their resource (susceptible hosts), and this resource depletion inhibits transmission of disease between neighborhoods (Holmes 1997; Keeling 1999; Keeling and Grenfell 2000). When a parasite can persist in a host system, these local interactions also produce heterogeneous, persistent spatial patterns of disease incidence (Bolker and Pacala 1997; Holmes 1997; Keeling et al. 1997). Thus, approaches to epidemiology which ignore space are likely to miss key aspects of host–parasite systems when inherently local, spatial interactions prevail.

Experience in several empirical systems has testified to the importance of local interactions and distributions of parasites and hosts. They include plant–pathogen systems (Burdon et al. 1995; Thrall and Antonovics 1995; Alexander et al. 1996; Real and McElhany 1996; Ericson et al. 1999), and human diseases such as foot-and-mouth disease (Keeling et al. 2001), influenza (Bonabeau et al. 1998), and measles (Grenfell et al. 2001). In these cases, hosts are distributed patchily and parasites disperse over relatively short distances over two-dimensional landscapes. Yet, these systems provide some of the few cases which can meet the huge demands of spatially explicit models for spatio-temporal data. These large data demands create a logistical challenge for empiricists, and this problem could be especially pronounced in three-dimensional habitats. These habitats, such as ponds, lakes, and oceans, certainly occur frequently in nature. Despite their commonness, they have not received full treatment from a spatially explicit, epidemiological perspective. However, before testing spatially explicit models in such habitats, it seems prudent to first quantify spatial patchiness of disease. Can one break the three-dimensional spatial problem into smaller, more tractable components?

In lake ecosystems, three-dimensional habitat can be readily separated into vertical and horizontal components to study parasitism of daphniid zooplankton. Several different bacterial and fungal parasites infect species of *Daphnia* (Green 1974; Stürnadel and Ebert 1997; Ebert et al. 2001). However, until now, the role of space in daphniid parasitism within a system (as opposed to among systems, e.g., rock pools in Ebert et al. 2001) remains unexplored. Yet this role is potentially promising, as plankton exhibit patchiness in both vertical and horizontal dimensions. In the vertical dimension, migrating populations avoid fish predation during daytime by uniformly moving to deeper regions, yet they enjoy higher growth rates by moving to shallower, warmer waters of the water column at night (Lampert 1989). Additionally, plankton aggregate horizontally at various spatial scales driven by biological and physical factors, but horizontal patchiness is unrelated to vertical structure (Pinel-Alloul et al. 1988; Pinel-Alloul 1995; Folt and Burns 1999).

One hypothesis already predicts that localized, vertical interactions should impact incidence of parasitism in

freshwater zooplankton, but it has not yet been tested in natural systems. The “deep trouble” hypothesis predicts, all else being equal, higher rates of parasitism of *Daphnia* in deeper sections than in shallower sections of a water column (Decaestecker et al. 2002). Such vertically segregated incidence of parasitism could occur because some clones of *Daphnia* may avoid fish predation by preferentially remaining close to sediments (in ponds) or colder, deeper waters (near the thermocline in lakes). According to the hypothesis, spores settle on sediments in ponds or aggregate along the thermocline of stratified lakes, where the strong density gradient retards sinking of spores out of the water column (Decaestecker et al. 2002). Because they have higher exposure to parasites, these clones inhabiting deeper waters should experience higher rates of infection, all else being equal among deeper and shallower clones.

Horizontal aggregation of zooplankton presents a more obvious match to the two-dimensional geometry of generic spatial models. Horizontal patchiness in lakes occurs on a variety of spatial scales, driven by physical factors, such as currents, eddies, and downwellings, and biological factors, such as attraction to food and predator avoidance (Jakobsen and Johnsen 1987; Pinel-Alloul 1995; Folt and Burns 1999; Kratz et al. 2005). Such patchiness implies that zooplankton populations interact locally, at least to some degree. However, it remains unknown whether this horizontal aggregation significantly influences host–parasite interactions. If it does, theory predicts that lasting spatial patterns of parasite prevalence should emerge in lakes experiencing epidemics (Bolker and Pacala 1997; Holmes 1997; Keeling et al. 1997). Therefore, patchy horizontal distributions of infected and uninfected hosts should provide a signature of the presence of strong, local interactions.

Thus, space plays an uncertain but potentially important role in planktonic host–parasite interactions. To explore this role, we surveyed four lakes for vertical patchiness and three lakes for horizontal patchiness of daphniid hosts and several parasites in search of spatial heterogeneities of infection. If pronounced in these surveys, strong spatial heterogeneities would demand a quantitative approach to future work in daphniid epidemiology that explicitly incorporated space in one-, two-, or three-dimensions (Real and McElhany 1996). Alternatively, lack of or only weak evidence of spatial effects in these surveys would suggest that non-spatial models could still provide meaningful insights into host–parasite interactions in this particular three dimensional system.

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## Materials and methods

### Field sampling and laboratory analysis

We surveyed spatial heterogeneity of parasite prevalence in three lakes in Barry County, southwest Michigan, but limited our analyses to parasites which were sufficiently

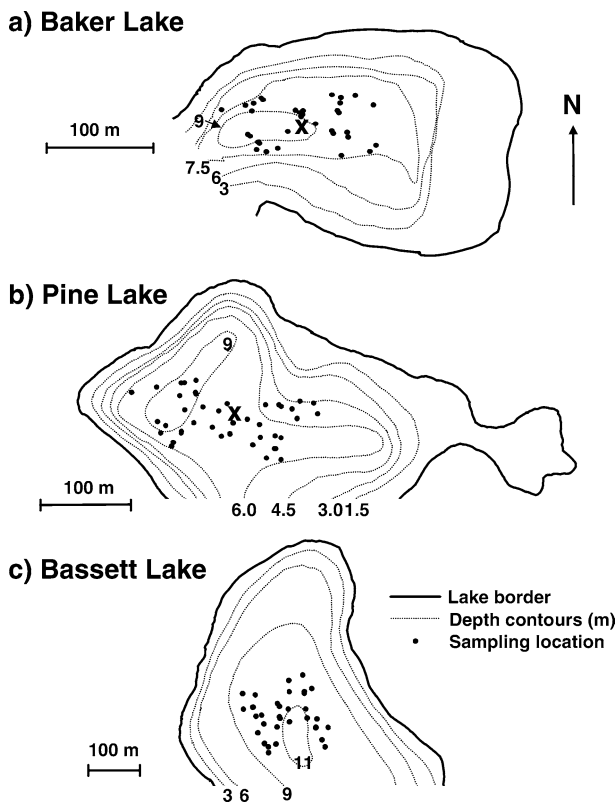
prevalent to quantify reliably. Baker Lake (maximum depth=9.1 m, mean depth=4.7 m, surface area=19.3 ha) is eutrophic, and the fungus *Metschnikowia bicuspidata* was the most commonly observed parasite of *Daphnia dentifera* (the only daphniid host in this lake). This parasite is horizontally transmitted as the daphniid host consumes fungal spores (Condreanu and Condreanu-Balcescu 1981), is highly virulent, and depresses host reproduction by 25–50% (Ebert et al. 2000; A. Tessier and M. Duffy, unpublished data). Bassett Lake (maximum depth=9.8 m, mean depth=6.7 m, surface area=18.8 ha) is mesotrophic. The two main parasites observed in horizontal sampling (October) were *Metschnikowia* and fungal brood parasites (of unknown species identity and unknown transmission mechanism). This latter parasite destroys the eggs of its host. In vertical sampling (August), the main parasite was the bacterium *Spirobacillus cienkowskii*. This parasite is likely to spread via horizontal transmission, but it greatly reduces reproduction of its host and is the most virulent of the three parasites. Pine Lake (maximum depth=8.5 m, mean depth=4.2 m, surface area=27.6 ha) is oligotrophic and had epidemics of brood parasites in *D. dentifera* and epidemics of *Spiro-*

*bacillus* in *D. dentifera* and *Daphnia retrocurva*. Mesotrophic Three Lakes II (maximum depth=10 m, surface area 15 ha) was surveyed for vertical heterogeneity of *Spirobacillus* only. In all of these lakes, the bluegill sunfish *Lepomis macrochirus* is the main planktivore (Hall and Werner 1977; Tessier and Woodruff 2002).

To estimate vertical variability of *D. dentifera* and their parasites, we visited Baker Lake on 24–25 September 2002 during a *Metschnikowia* epidemic, Three Lakes II on 30 September 2003 to 1 October 2003 during a *Spirobacillus* epidemic, Pine Lake on 23–24 September 2003 during a *Spirobacillus* epidemic, Bassett Lake on 4–5 August 2003, also during a *Spirobacillus* epidemic. In a single, central, deep location of each lake, we collected animals at 1- or 2-m intervals using a 20 l, plexiglass Schindler trap with an 80- $\mu$ m mesh. Since *D. dentifera* typically migrates to shallower depths of a water column during night and deeper depths during day, we sampled during both times. Using simple linear regression, we examined parametrically derived confidence intervals (CIs) around the slope coefficient relating sampling depth to prevalence of infection (% of animals infected). A priori, we expected the 95% CIs of the slope coefficient to overlap with zero if there was no pronounced trend with depth. In contrast, a positive slope would indicate increasing prevalence with depth (as predicted by the deep trouble hypothesis).

We also surveyed three of these lakes to quantify horizontal variability of these infections. We visited each lake once (Baker on 17 September, Pine on 8 October, Bassett on 15 October 2003) on sunny, calm days. In these conditions, patchiness should be most pronounced (Folt et al. 1993). At each lake, we erected an approximately rectangular grid, 50–100 m per side, with stations at each corner and with one central station (Fig. 1). This grid helped to ensure coverage of a reasonably large (>5,000 m<sup>2</sup>) area of the target basins. Between 1030 hours and 1230 hours, we collected 34–45 samples, depending on the lake (Fig. 1, each dot is a site). In each of five areas of the grid (the four corners and the center), we first collected two replicate samples at an initial site using bottom-to-surface tows of a Wisconsin net (153- $\mu$ m mesh). Then, we collected single samples at five to six additional sites before moving to the next area. These sites radiated along random trajectories from the initial site and were located approximately 10 or 20 m away (chosen randomly). A Trimble GPS (TSCE running Pathfinder 2.90.2.55) provided coordinates for each site (<0.7 m). Samples remained on ice until we returned to the lab.

In the lab, we kept animals at 4°C until we could visually diagnose the entire samples for parasites at 25–50X, using a dissecting microscope. Visual diagnosis without dissection was possible because infection by each parasite was obvious in transparent *Daphnia* bodies (Ebert et al. 2000). We categorized *D. dentifera* and *D. retrocurva* (in Pine Lake) based on stage (juveniles or adults) and on infection status (uninfected or infected).



**Fig. 1** Maps of sampling locations in each of the three study lakes **a** Baker Lake,  $n=34$  samples, **b** Pine Lake ( $n=45$  samples), and **c** Bassett Lake ( $n=35$  samples) during a survey of horizontal heterogeneity, September–October 2003. The “X” marks the center of the sampling grid for comparison to the large-scale spatial patterns (see Fig. 4)

## Statistical analysis of horizontal heterogeneity

In the spatial analyses, we looked for convergent descriptions of aggregation from two different statistical methods (Real and McElhany 1996). We first characterized spatial patterns in horizontal datasets using a relatively simple global index of patchiness,  $\hat{I}_P$  (Lloyd 1967)

$$\hat{I}_P = 1 + \frac{s^2}{\bar{X}^2} - \frac{1}{\bar{X}} \quad (1)$$

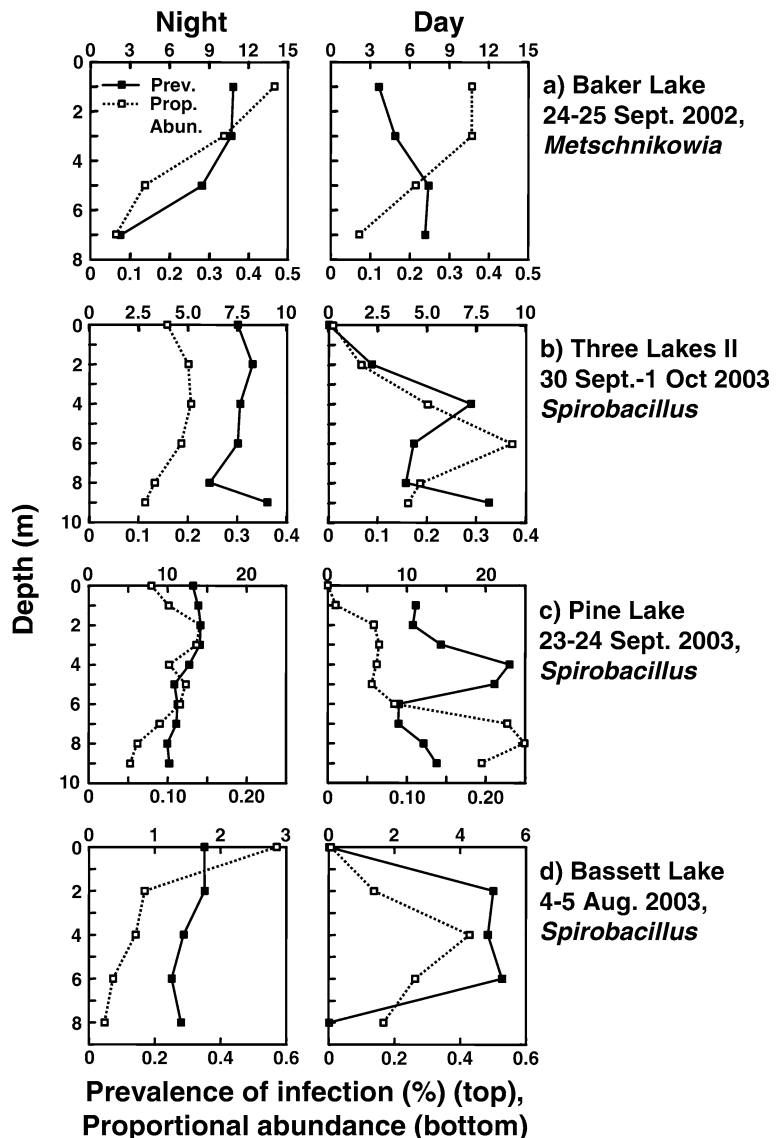
where  $s^2$  is the least-squares estimate of variance among samples, and  $\bar{X}$  is the estimated mean density among samples. Previous planktonic data collected using  $\hat{I}_P$  provide a benchmark for comparison (Folt et al. 1993). The  $\hat{I}_P$  index characterizes aggregation of individuals in a neighborhood (sample) relative to the mean number of individuals present in the sampled

neighborhoods. Here, it is a within-infection category measure (i.e., among uninfected adults, or among *Spirobacillus*-infected animals). As  $\hat{I}_P$  increases past unity, the population becomes overdispersed as compared to a Poisson distribution. Thus, if  $\hat{I}_P$  equaled 1.5, an individual in a neighborhood experiences 50% higher density of others in its category than expected if the population was distributed “randomly”. We also calculated a cross-infection category index,  $\hat{I}_{P,XY}$  (Lloyd 1967; Folt et al. 1993):

$$\hat{I}_{P,XY} = \frac{\sum_{i=1}^n X_i Y_i}{\bar{X} \sum_{i=1}^n Y_i} \quad (2)$$

where  $X_i$  and  $Y_i$  are densities of two categories in sample  $i$  (where  $X$  might be uninfected animals and  $Y$  might be *Metschnikowia*-infected animals), and where  $n$  is the number of samples. This cross-category index works symmetrically, i.e., it does not change if  $X$  and  $Y$

**Fig. 2** Prevalence of infection (percent of *Daphnia dentifera* infected; black squares with solid lines) and abundance of daphniid hosts (as proportion of total; white squares with dotted lines) as a function of lake depth at night and day vertical sampling in **a** Baker, **b** Three Lakes II, **c** Pine, and **d** Bassett Lakes. Slopes of regressions relating prevalence to depth are presented elsewhere (Appendix Table A1)



are reversed. A positive cross-category index indicates that high-density patches of uninfected animals overlapped with high-density patches of infected animals. For both indices, we created non-parametric, bias-corrected, bootstrapped CIs using 10,000 bootstraps in Matlab (Mathworks, Inc. 1999), following Dixon (2001).

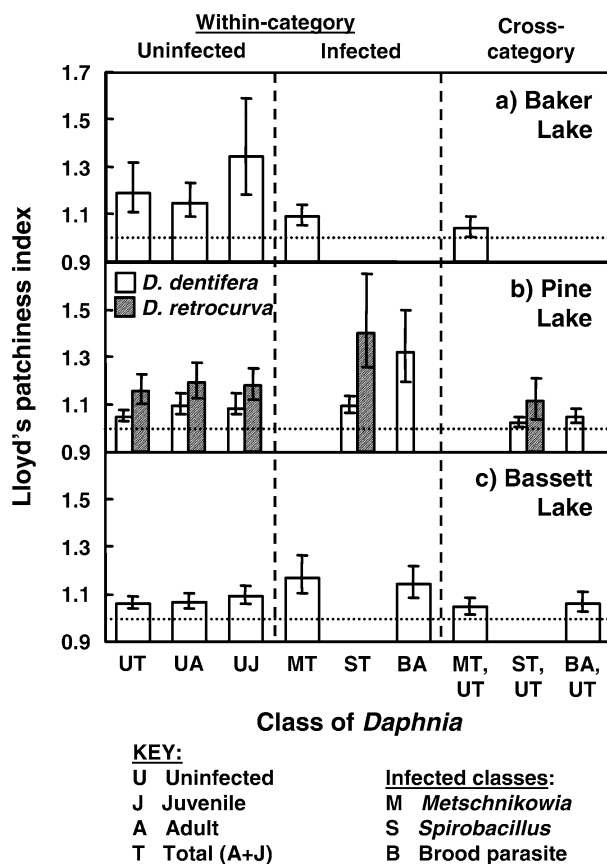
We also characterized spatial structure of susceptible and infected *Daphnia* by first estimating large-scale spatial patterns and then calculating spatial correlograms. Correlograms plot distance classes among sites versus spatial dependency (autocorrelation), and they provide potential data–theory bridges to spatially explicit models (Bolker and Pacala 1997). However, correlograms become biased by large-scale trends. Thus, to characterize and then remove any large-scale spatial tendencies, we first detrended our data using a best-fitting model using ordinary least squares (Legendre and Legendre 1998, p. 739). To complete this step, we fit regression models which expressed a response variable  $y$  as a polynomial function of longitude ( $X$ ) and latitude ( $Y$ ) with cross-product terms. Of the possible models with various combinations of linear and quadratic terms, we selected the best fitting one using the small-sample version of Akaike’s Information Criterion, AIC<sub>c</sub>. This method is based on maximum likelihood (Burnham and Anderson 2002). When the  $r^2$  of this best-fitting broad-scale model was higher than an arbitrarily chosen 10%, we used its residuals to estimate small-scale spatial dependency.

To measure autocorrelation on these smaller spatial scales, we calculated Moran’s  $I$  following Legendre and Legendre (1998). This metric partitions spatial dependency among distance classes. It is similar to Pearson’s correlation coefficient, where zero indicates no spatial autocorrelation between points within a given distance class, while values approaching (and possibly exceeding) negative (positive) 1 indicates strong negative (positive) autocorrelation. Like Pearson’s  $r$ , it permits meaningful comparison of spatial patterns between variables with different means and variances. We used a standard procedure (Sturge’s rule) to optimally determine the number of distance classes to be used in the correlograms (Legendre and Legendre 1998, p. 717). With either detrended or raw data, we then calculated Moran’s  $I$  for each of the distance classes and estimated its associated statistical significance using 9,999 randomizations and the progressive Bonferroni correction method to correct for multiple comparisons (p. 671, 721). The progressive Bonferroni method assumes that statistically significant autocorrelation arises at smallest distance classes; thus, significant autocorrelation becomes increasingly difficult to detect as distance increased. Given our small sample sizes, we were not able to test for directionality of the correlograms (anisotropy). In the correlograms, distances between highly positive or highly negative autocorrelation values indicates the distance between similar points at neighboring peaks (or troughs) in space.

## Results

### Vertical sampling

In each of the three study lakes, we found little evidence for persistent vertical segregation of uninfected and infected animals (Fig. 2, Appendix Table A1). In three of the four lakes (Three Lakes II, Pine, and Bassett), *D. dentifera* migrated from deeper depths during day to shallower depths at night, while in Baker Lake the animals remained in shallow waters during the day. At night, prevalence of infection either remained relatively constant or decreased with depth (Fig. 2). Thus, we did not observe higher prevalence of infections in deeper waters when animals migrated upwards at night, as predicted by the “deep trouble” hypothesis. During day, the prevalence of infection changed more complexly with depth (Fig. 2). However, we found no consistent,



**Fig. 3** Horizontal patchiness of uninfected and infected *Daphnia* in a Baker, b Pine, and c Bassett Lakes surveyed September–October 2003, based on Lloyd’s indices (1967). Values greater than 1 (dashed horizontal lines) indicated that individuals experienced higher densities (on a proportional basis) of animals in the same class (within-category), or different classes (cross-category) than expected by chance, assuming that animals are randomly (Poisson) distributed. Error bars are 95%, bias-corrected confidence intervals obtained from 10,000 bootstraps. Note: Baker Lake experienced only one parasite. Hence, only one column appears in the “infected” and “cross-category” sections

clear relationship relating prevalence with depth among lakes (and each of the slope coefficients derived for day sampling all overlapped with zero; Appendix Table A2). In two of the lakes (Three Lakes II and Pine), prevalence of infection during daylight seemed to peak at depths more shallow than the population as a whole (Fig. 2).

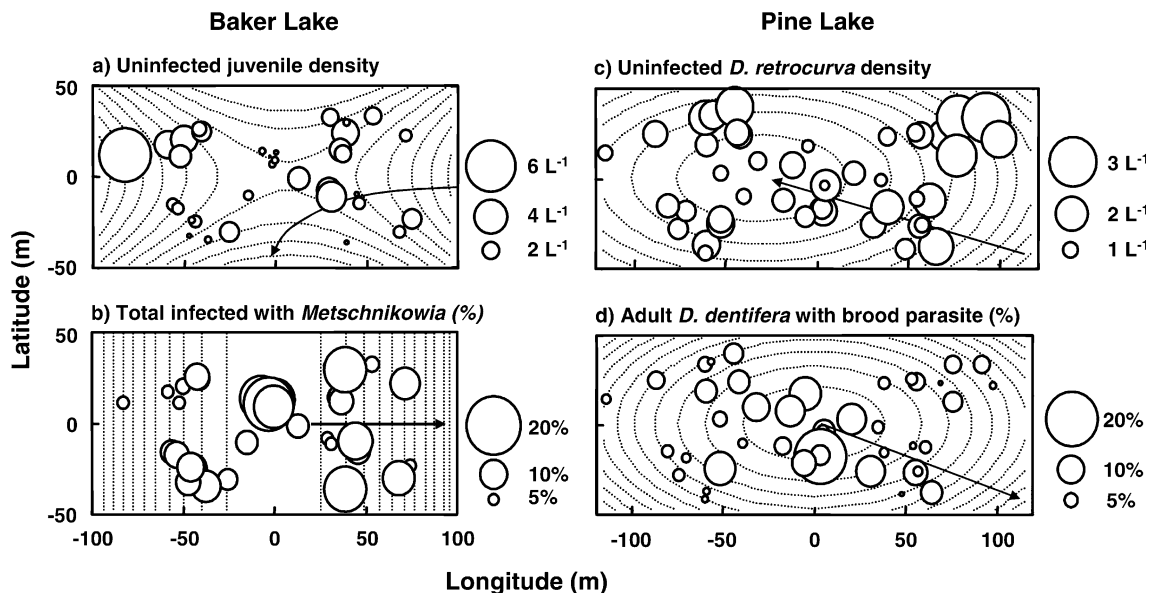
### Horizontal sampling

In all three lakes, we observed some degree of horizontal patchiness within all uninfected and infected classes (Fig. 3). In each lake, the 95% CIs around the patchiness indices did not overlap with unity for any category, revealing statistically significant departure from Poisson randomness (assuming  $\alpha=0.05$ ; Fig. 3). These indices ranged from 1.02 to 1.40, meaning that individuals crowded at 2% to 40% higher density than anticipated from Poisson randomness, respectively (Fig. 3). The patchiness index showed relatively patchy distributions of uninfected juveniles in Baker Lake, brood parasites in Pine Lake, and infections with *Spirobacillus* in the *D. retrocurva* population of Pine Lake. Notably, the cross-category indices of patchiness indicated that infected animals were distributed only slightly patchily (<10%) among uninfected hosts. Stated alternatively, patches with higher density of uninfected animals contained <10% higher density of infected animals than would be expected at random.

This global index combines but cannot differentiate between large-scale and smaller-scale spatial patterns in lakes. To characterize the large-scale spatial patterns, we

detrended the spatial data using polynomial regressions. From these regression, two sets of large-scale trends emerged in Baker and Pine Lakes that corresponded to basin shape (Fig. 1, 4; see Appendix Table A2 for trend surface equations). In Baker Lake, uninfected juveniles were more abundant on shallower edges of the sampling area, while the incidence of infection with *Metschnikowia* was highest in the central region of the sampling area (Figs. 1, 4a,b). Thus, infection prevalence was higher in regions with lower densities of uninfected juveniles. In Pine Lake, density of uninfected *D. retrocurva* was higher in the shallower edges of the sampling array (Figs. 1, 4c). Meanwhile the prevalence of brood parasites among adult *D. dentifera* was highest in the deeper, central part of the lake basin where *D. retrocurva* were less abundant (Figs. 1, 4d). We also removed east–west trends in data from Bassett Lake. These directional trends did not correspond to a nearshore–offshore gradient, as in the other two lakes, because our sampling grid only occupied the central, deep portion of the lake basin without bordering shallower, nearshore areas.

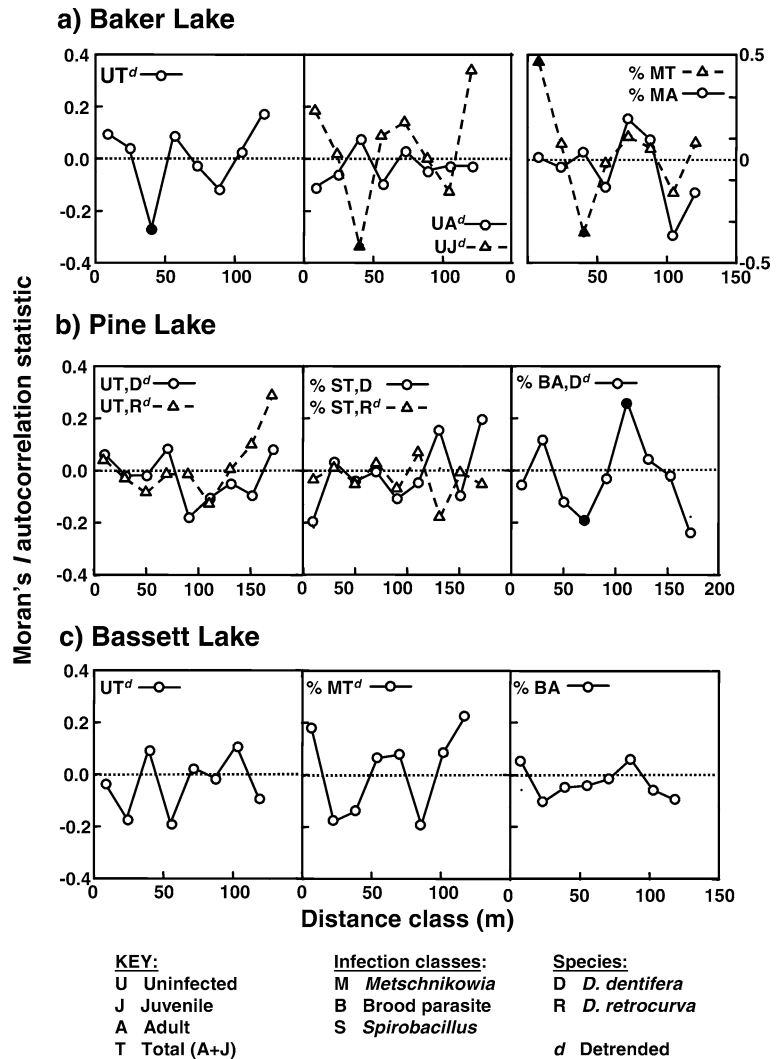
After removing these large-scale spatial trends from the horizontal datasets, we found few pronounced instances of smaller-scale spatial patterning using autocorrelation analysis (Fig. 5). Thus, the heterogeneity detected by the global patchiness indices was largely driven by broad spatial trends and variation that lacked small-scale spatial patterning. This lack of systematic patterning was most obvious in Bassett Lake. There, coefficients of the autocorrelation functions for prevalence of two parasites and densities of total uninfected animals (and juveniles and adults, not shown) were all



**Fig. 4** Four examples showing prominent spatial trends in **a** density of uninfected juveniles and **b** prevalence of *Metschnikowia* in Baker Lake, and **c** density of uninfected *D. retrocurva* and **d** prevalence of brood parasites in *D. dentifera* found in horizontal surveys, September– October 2003. The *arrows* point from higher

to lower density or prevalence of parasites (scaled as percentage of animals infected) along ten evenly spaced contours from best-fit polynomial regression models (see Appendix Table A2 for equations). Density and prevalence scale with the width of the *circles*

**Fig. 5** Estimated autocorrelation functions describing smaller-scale, horizontal spatial patterns of absolute abundance or prevalence of infection of *Daphnia* in **a** Baker, **b** Pine, and **c** Bassett Lakes. Prevalence of parasite is scaled as percentage of animals infected (%). *Black symbols* indicate statistical significance after a progressive Bonferroni correction, as determined by 9,999 randomizations. Note the different scale used in the top right panel



small (absolute values were close to zero) and statistically insignificant. In Baker Lake, density of total and adult uninfected animals also showed little spatial patterning. However, the sinusoidal correlogram for uninfected juveniles indicated that they occurred patchily in “bumps” larger than the first distance class (i.e., > 16 m; Fig. 5). Additionally, these bumps were spread roughly 60-m apart (based on the distances between positive peaks of the correlogram). The autocorrelation function for prevalence of infection by *Metschnikowia* largely mirrored this spatial pattern for juveniles. Indeed, the juvenile pattern likely drove it, since the autocorrelation pattern for prevalence of infection among adults did not show similar spatial patchiness. It is not surprising that sampling locations with high juvenile abundance had low prevalence of infection. Adults experienced more infection than juveniles in Baker Lake ( $22.8 \pm 1.18$  vs.  $0.44 \pm 0.10\%$  infected, mean  $\pm$  1 SE, respectively). In Pine Lake, autocorrelation analysis revealed little patterning in density of uninfected *Daphnia* of two species and prevalence of infection by *Spirobacillus* among them. Thus, the high degree of patchiness of *D. retro-*

*curva* infected with *Spirobacillus* found using the global index (Fig. 3) did not occur in a strongly repeating, small-scale spatial pattern (Fig. 5). Patchiness of the prevalence of brood parasites of *D. dentifera* in Pine Lake provided the only strong evidence of spatial patterning of parasites in our study lakes (Fig. 5). In this case, the autocorrelrogram showed evidence of peaks of prevalence separated by roughly 80 m.

## Discussion

Spatially explicit epidemiological models increasingly illustrate how spatial heterogeneity in two-dimensions can determine the invasion success, persistence, and distribution of parasites in host populations when hosts interact locally and parasites only disperse over short distances (Holmes 1997; Bolker and Pacala 1997; Brown and Bolker 2004). While space can mediate host–parasite interactions, a spatial approach to epidemiology demands a huge amount of data and rigorous sampling. This data-demand problem could be severe in host–

parasite systems that occupy three-dimensional habitats, such as plankton of lakes. However, our surveys of vertical and horizontal heterogeneity of daphniid parasitism in several north temperate lakes do not show systematic evidence for small-scale vertical or horizontal patchiness or patterning of three different parasites in two host species.

A mechanistic driver of vertical heterogeneity of parasitism has been explored in the laboratory, but we did not find evidence that it drove vertical heterogeneity in our lakes. Decaestecker et al. (2002) have hypothesized that, in order to avoid fish predation, some clones of *Daphnia* might experience higher parasitism rates by preferentially remaining close to sediments in the deeper, darker regions of ponds. Such habitat choice exposes these clones to higher densities of parasite spores, which settle on the sediments. When extended to lakes with vertically migrating daphniids, this hypothesis predicts, all else being equal, that higher density of infected animals and prevalence of infection should occur in deeper portions of the water column because sinking spores should aggregate along the density gradient of the thermocline (Decaestecker et al. 2002). Prevalence of infection should be greatest in deeper waters at night, when migrating clones move upwards in the water column. We did not find evidence of this signature of the “deep trouble” hypothesis in our survey. In all the four study lakes, we found very close correlation between densities of infected and susceptible hosts along depth gradients and little support for higher prevalence of infection in deeper waters, despite that daphniids aggregated vertically. Certainly, a thorough test of the “deep trouble” hypothesis itself warrants more detailed empirical and theoretical development than the vertical sampling reported here. However, the germane point is that the “deep trouble” mechanism, if operating, did not produce consistent, pronounced, obvious gradients of infection with depth in these lakes

We did uncover a small degree of horizontal patchiness in uninfected and infected host populations using two global indices. These global patchiness indices, which combined broad-scale and small-scale spatial heterogeneities, indicated a range of aggregation from 2% to 40% higher than would be expected from a random distribution. This degree of patchiness occurred within the range reported elsewhere for zooplankton (Folt et al. 1993). Notably, the cross-category patchiness indices were all 10% or smaller, indicating that denser patches of infected hosts only overlapped slightly with denser patches of uninfected hosts. However, it is hard to tell whether this degree of patchiness among and between uninfected and infected hosts is important for daphniid epidemiology. As far as we know, no extant theory links these global patchiness indices to invasion, persistence, and distribution of parasites in host populations. Ives developed inter-specific competition theory with these two patchiness indices that, when combined with experiments, showed that spatial heterogeneity promoted coexistence of insects (Ives 1991). Yet, theo-

retical epidemiologists have favored more complicated models, which demand extensive spatio-temporal datasets. Such datasets are fairly difficult to collect for non-human systems. In the short-term, then, host–parasite models built around patchiness indices, or perhaps pair approximations (Filipe et al. 2004), might facilitate closer links between epidemiological theory and extant data.

Autocorrelation functions provide a potential statistical link to predictions of spatially explicit disease models. However, our estimated autocorrelation functions uncovered little systematic spatial patterning in three of the study lakes. Indeed, we found only one case (brood parasites in one lake, but not another) revealing true, small-scale patterning of parasite incidence. The other instance of strong, small-scale spatial patterning was driven by abundance of uninfected juveniles, not prevalence of infection among adults (Real and McElhany 1996). Both of these examples involved parasites which mainly or only infected adults. Otherwise, this lack of systematic patterning emerged after sampling in ideal weather conditions to detect small-scale patterns (Folt et al. 1993). Such findings suggest that a key mechanism in generic, spatially explicit epidemiological models (highly localized interaction of hosts and parasites) lacked sufficient power to produce persistent horizontal heterogeneities in these daphniid–parasite systems (Holmes 1997). Perhaps physical mixing mechanisms disrupted biologically driven spatial patterning by dispersing spores of parasites throughout the lakes. Such wide-spread dispersal should reduce the chance that parasites locally exhaust their resources (Holmes 1997; Brown and Bolker 2004). Alternatively, these findings indicated that our spatio-temporal surveying regime would have to be greatly (impractically?) extended to detect stronger evidence for systematic, small-scale spatial patterning. However, our results do not encourage us to greatly expand our small-scale sampling, at least for these two host species.

Instead of emphasizing small-scale spatial heterogeneities in this system, one might focus on basin-wide horizontal heterogeneity of daphniid parasitism in future research. Our surveys were designed to detect smaller-scale heterogeneities that could be qualitatively linked to predictions of generic spatial models. However, larger-scale spatial patterns of uninfected host density and parasite prevalence still arose in two lakes because the sampling grids overlapped to some degree with a nearshore–offshore (depth) gradient. We found smaller-sized daphniids closer to shore (i.e., juvenile *D. dentifera* in Baker, *D. retrocurva* in Pine) but higher prevalence of parasites that predominately infected the adults in deeper, mid-basin waters (i.e., *Metschnikowia* in Baker, brood parasites in Pine). One explanation for this large-scale pattern could involve spatially heterogeneous predation pressure, since planktivorous fish frequently congregate closer to shore (Jakobsen and Johnsen 1987; González and Tessier 1997; Tessier and Woodruff 2002). In our lakes this planktivore is the bluegill sunfish



(Tessier and Woodruff 2002), which selectively preys upon parasitized daphniids (Duffy et al. 2005). Theory predicts that parasite prevalence should increase with decreases in selective predation from bluegill offshore (Packer et al. 2003; Duffy et al. 2005; Hall et al. 2005). While such a nearshore/offshore-predation hypothesis remains speculative, our observations suggest that future spatial studies should investigate causes and consequences of heterogeneous parasitism at the basin-wide scale.

Theoretical studies of spatially explicit epidemiology warn that local interactions between discrete hosts can impact invasion of parasites, spread of epidemics, and spatial distribution of infected hosts. This suite of theoretical results has yielded great insight into dynamics of plant–pathogen and human diseases (Burdon et al. 1995; Alexander et al. 1996; Bonabeau et al. 1998; Grenfell et al. 2001; Keeling et al. 2001). However, such predictions may pertain more to sessile or locally interacting organisms, such as plants, rather than to more continuously distributed and mobile populations such as zooplankton in lakes. Given a reasonable amount of effort expended in surveys of several lakes, we did not find strong evidence for small-scale vertical and horizontal heterogeneity of infection by three parasites in two host species. This conclusion should be confirmed by future studies in lake ecosystems, particularly if the host species is known a priori to patchily aggregate more than the two *Daphnia* species studied here. In the meantime, our findings suggest that larger-scale mechanisms more likely govern transmission dynamics in these systems. Consequently, these large-scale results reveal a potentially promising avenue for future, spatially explicit research in this system.

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