

Wetland isolation facilitates larval mosquito density through the reduction of predators

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Abstract. 1. Wetlands harbour high biodiversity and offer important ecosystem services, but they are also a habitat for mosquito larvae (Diptera: Culicidae), which are important disease vectors.

2. Isolation among remnant, or newly created wetlands and ponds, and their consequent density in the landscape, is a key factor that can influence a variety of food web processes, including effects on mosquitoes which are important prey to many predators.

3. We assess the impact of habitat isolation on the density of pond-breeding mosquitoes (several *Anopheles* and *Culex* species) both directly and indirectly through the food web.

4. Results from structural equation modelling of survey data shows that larval mosquitoes are denser in ponds that are more isolated from one another, and that this result was primarily driven indirectly by a reduction of larval mosquito predators (e.g. predaceous insects and amphibians). Furthermore, results from a long-term mesocosm experiment factorially manipulating isolation and predator reduction show that the effect of isolation on mosquito density was eliminated when predators were experimentally reduced.

5. It is concluded that metacommunity processes, both directly and indirectly mediated through predators, can play an important role in the local abundance of wetland breeding mosquitoes and possibly the diseases they spread.

Key words. Food webs, habitat isolation, metacommunities, mosquitoes, predation, wetlands.

Introduction

More than one-half of the United State's original 220 million acres of wetlands have been drained and converted to other uses, and many other countries have lost a similar or greater fraction of wetlands (Millennium Ecosystem Assessment, 2005). An important consequence is that not only is there less area of wetland habitat remaining, but at the same time, those wetlands that do remain are more isolated from one another than they were historically (e.g. Gibbs, 2000). While both habitat area and habitat isolation are well known to reduce the diversity and abundance of many species, predators are often disproportionately reduced relative to prey in smaller and/or more isolated habitats, because predators often have larger

ranges and smaller populations, and thus require more area to maintain viable populations (reviewed in Holt & Hoopes, 2005). As such, in addition to altering the diversity and abundance of wetland organisms, reductions in habitat area and increases in isolation can alter the structure of wetland food webs (e.g. Spencer *et al.*, 1999; Shulman & Chase, 2007).

Many mosquito species (Diptera: Culicidae), either obligately or facultatively, use wetlands for their larval development. As mosquitoes are often important vectors of pathogens, there is considerable applied interest in the factors that influence their abundances. Historically, wetland destruction was justified as a means to reduce larval mosquito habitat, and thus associated disease risk (Mitsch *et al.*, 1994; Perry & Vanderklein, 1996). However, in addition to the reduction in the area of available mosquito habitat, wetland draining has an additional spatial consequence: isolation of, and density among, remnant habitat.

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Total wetland area and their isolation in the landscape can directly influence the local density of wetland breeding mosquitoes by influencing the mosquitoes' ability to reach those habitats, or by altering the availability of resources (e.g. hosts). Additionally, isolation could indirectly influence larval mosquito densities through interactions in the food web. As wetland breeding mosquitoes are often highly vulnerable to both vertebrate and invertebrate predators (reviewed in Kumar & Hwang, 2006), any factor that reduces the abundance of predators is likely to indirectly increase the density of larval mosquitoes. Since the abundances of many species of larval mosquito predators have been shown to decline with wetland isolation (e.g. notonectids and dytiscids: Wilcox, 2001; odonates: McCauley, 2006), we hypothesise that this decrease could translate into a concomitant increase in the density of larval mosquitoes in more isolated wetlands.

Habitat isolation is interrelated with a variety of other metacommunity variables, most notably the total amount of habitat in a given area. Indeed, habitat isolation and the total amount of available habitat usually have similar effects on community patterns, including the structure of, and interactions within, food webs (Holt & Hoopes, 2005). In ponds and wetlands, for example, when habitats are more isolated from one another, this will simultaneously mean that there is less available habitat for wetland living organisms in a given area than when habitats are less isolated from one another. Thus, while area and isolation are often treated as separate facets of metacommunities, they are generally intertwined when viewed at a scale appropriate to the metacommunity. As a result, herein, although we refer to variation in habitat isolation as a driver in pond community structure, it is the interaction between isolation and the concomitant changes in total habitat volume per area (density) that most likely influence the patterns observed.

We examined the direct and indirect effects of variation in habitat isolation on the abundance of mosquito larvae in several natural areas surrounding St. Louis, Missouri (U.S.A.) using a two-pronged approach. First, we surveyed larval mosquito and predator abundances in small ponds that varied in their degree of isolation, and thus density within a given area. Second, we established an experiment in artificial ponds manipulating the isolation/density of habitats and the presence of predators, to disentangle the direct effects of isolation on mosquito populations versus the indirect effects mediated via predators.

Materials and methods

Defining habitat isolation for mosquito larvae and mosquito predators

Mosquito species encountered in the ponds studied here included *Anopheles* (largely *An. quadrimaculatus*, but also *An. punctipennis* and *An. walkeri*) and *Culex* (*Cx. restuans*, *Cx. salinarius*, *Cx. tarsalis*, *Cx. territans*, and *Cx. erraticus*). As reliable identifications often require individuals to be in the fourth instar, species within genera were not distinguished. Other species (*Aedes* spp. and *Culiseta* spp.) made up less

than 1% of individuals encountered, and are not considered further. The *Anopheles* species encountered here are almost exclusively pond/wetland breeders, whereas some *Culex* also breed in smaller water bodies (e.g. containers, puddles). We only consider ponds and experimental mesocosms in our metrics of habitat isolation, and not these smaller water bodies. However, this will not likely alter the qualitative conclusions of our study, because (i) these field sites tended to have few such small water bodies, and (ii) the years in which this study took place (2006, 2007) were exceptionally dry at our field site. Nevertheless, even if such habitats were important, this would reduce any potential impact of our isolation metric on mosquito dynamics, and thus our tests would be conservative.

Surveys of existing ponds

The density of larval mosquitoes and their predators were surveyed in 16 small fishless ponds that had similar environmental conditions (e.g. chemistry, surface area), but varied in their isolation from other ponds. Fishless ponds were used because these are important components of many freshwater landscapes, and they contribute disproportionately to wetland breeding mosquito populations, as mosquito larvae are rarely encountered in ponds with fish except in vegetated margins that fish cannot access. Pond isolation was calculated by taking the mean distance to the five nearest fishless ponds using the ruler tool in Google Earth Pro® (results are qualitatively similar when this metric is varied from 1–10 nearest ponds). Pond surveys were conducted between 14 May and 11 June 2006. Again, note that this metric of isolation is correlated with the area of habitat available to wetland living organisms, and thus it is not possible to disentangle isolation from total area of habitat available.

Pond surveys were conducted between 14 May and 11 June 2006. Mosquito predators were surveyed by sweeping a 0.1-m wide D-net with 1-mm mesh 1 m along the bottom. This was repeated in 10 haphazard locations stratified across each pond in order to account for heterogeneity within ponds. Invertebrates were preserved in 70% ethanol for later identification and enumeration, and amphibians were counted and released at their site of capture. Individuals of mosquito predators, primarily insects in the orders Hemiptera, Odonata, and Coleoptera (Merritt & Cummins, 1996), as well as salamanders and newts (*Ambystoma* spp. and *Notophthalmus viridescens*), were converted to dry-weight biomass using species-specific conversions. Mosquito larvae were surveyed inside of a 0.5 m diameter × 0.5 m tall cylinder which was pushed into the sediment at 10 haphazard locations along the circumference of the pond edge (mosquito larvae are almost never observed in open water). Once the cylinder was secured in the substrate and the water was allowed to settle for ~60 s, mosquito larvae and pupae were identified to genus and enumerated as they came to the surface.

Structural equation modelling (SEM; reviewed in Grace, 2006) was used to examine the direct and indirect effects of habitat isolation on mosquito larvae. Although the low sample size ($n = 16$) warrants caution when interpreting results, this

statistical method allows the assessment of any discrepancies between our expectations and observational data. Mosquito densities were log transformed [$\log(x + 1)$] to meet the assumptions of multivariate normality. Pearson's correlation coefficients were used to examine the bivariate relationships between variables, and the adequacy of model fit was evaluated using χ^2 [results were consistent with other indices (AIC and GFI)]. Path coefficients were evaluated using z-tests and by testing the consequences of omitting paths for the χ^2 test. Bivariate relationships were analysed using JMP 7.0.1 (2007) and SEM was conducted in AMOS 5.0.1 (Arbuckle, 2003).

Experimental mesocosms

A 2-year experiment was established in the spring of 2005 using mesocosms to simulate pond ecosystems (1130-l *stock tanks*) at Washington University's Tyson Research Center near Saint Louis, Missouri (U.S.A.). Each mesocosm was filled with 4–6 cm of topsoil and ~900 l of well water. To inoculate less mobile organisms, we: (i) added 1 l of pooled water from 20 local ponds to introduce plankton; (ii) introduced ~500 g of several species of locally occurring macrophytes and macroalgae; and (iii) introduced small numbers of several species of less mobile invertebrates (e.g. snails and amphipods). A wide variety of species, including mosquitoes and their insect predators, readily colonised the mesocosms on their own.

Mesocosms were established in a 2×2 experimental design manipulating the degree of isolation (isolated, connected) and predator reduction (yes, no). Each treatment was replicated four times. Isolated mesocosms were placed a minimum of 500 m from any other experimental or natural water body where wetland breeding mosquitoes live. Connected mesocosms were placed at the corner of a square arrangement; sides of the square (distance of separation) were 5 m. This square array of four mesocosms was also at least 500 m away from other water bodies. We factored the isolation treatment with a predator reduction treatment to discern any indirect effects of isolation on mosquitoes mediated through predation. The predator reduction treatment was implemented weekly during the growing season (April to September) by scanning designated mesocosms for 30 min and removing mosquito predators gently with a hand net when they came into view. As this process had a small influence on the mesocosms relative to the normal course of activities by organisms, we did not initiate a *sham* treatment where predator communities were left intact.

Mosquito larvae were surveyed monthly from May to August in 2006–2007. During each census, mosquito larvae were surveyed visually in the entire mesocosm and identified to genus as above. Due to the somewhat destructive nature of sampling larger organisms such as insect predators, mesocosms were only sampled once per year in August 2006–2007. To sample predators (categorized as above), a 36 cm diameter cylinder was thrust into the soil (*stovepipe* sampling; e.g. Turner & Trexler, 1997), and we collected all organisms within the cylinder with 1-mm mesh nets. Sweeping continued until no individuals were found in five consecutive sweeps. This

process was repeated three times in each mesocosm, and all invertebrates were preserved in 70% ethanol for identification and enumeration; amphibians were identified, counted, and released.

Predator biomass was analysed using repeated-measures ANOVA examining the main and interactive effects of isolation and predator reduction. Numbers of *Anopheles* and *Culex* larvae/pupae were analysed using repeated-measures MANOVA with the same design. Post-hoc tests for multiple comparisons used the sequential Bonferroni method. Mosquito abundances were log transformed [$\log(x + 1)$] prior to analyses using JMP 7.0.1 (2007).

Results

Surveys of existing ponds

With increasing isolation, predator biomass decreased ($r = -0.65$, $P = 0.005$, Fig. 1A), whereas *Anopheles* ($r = 0.55$, $P = 0.030$, Fig. 1B) and *Culex* ($r = 0.72$, $P = 0.002$, Fig. 1C) density increased. Both *Anopheles* ($r = -0.91$; $P < 0.001$, Fig. 1D) and *Culex* ($r = -0.82$, $P < 0.001$, Fig. 1E) declined with higher predator biomass. As the direct path from isolation to *Anopheles* density was non-significant, it was dropped from the analysis. The resulting model yielded $\chi^2 = 2.47$ [d.f. = 2, $P = 0.29$ (non-significant χ^2 indicates no departure of predictions from the data)] (Fig. 2). Because amphibian predators (salamander larvae) and invertebrate predators could potentially differ in their responses to isolation, we repeated these analyses without amphibians (which were a relatively small proportion of the total biomass of predators), but found qualitatively similar results (not presented here).

Experimental mesocosms

Repeated measures ANOVA on predator biomass showed that there was a significant effect of isolation ($F = 21.95$, $P < 0.001$), predator reduction ($F = 11.38$, $P = 0.006$), and their interaction ($F = 8.33$, $P = 0.014$), but no significant within subjects (time) effects ($P > 0.5$). Pairwise comparisons illustrate that predator reduction decreased predator biomass in connected mesocosms, but not in isolated mesocosms (Fig. 3). This lower proportional reduction led to an interaction between isolation and predator removal on predator biomass (Fig. 3). Although a significant interaction might imply that we were less effective at removing predators from isolated mesocosms, this was on a proportional, and not absolute basis (we reduced predators to a similar absolute level in each treatment), and thus does not influence our interpretations. Nevertheless, we have repeated analyses without this interaction term, and found qualitatively similar conclusions. There were also significant main and interactive effects of isolation and predator reduction on the density of mosquitoes (Table 1). When predator assemblages were intact, mosquito density was higher in isolated mesocosms, whereas when predators were reduced, mosquito densities were similar among the isolated and connected mesocosms (Fig. 4).

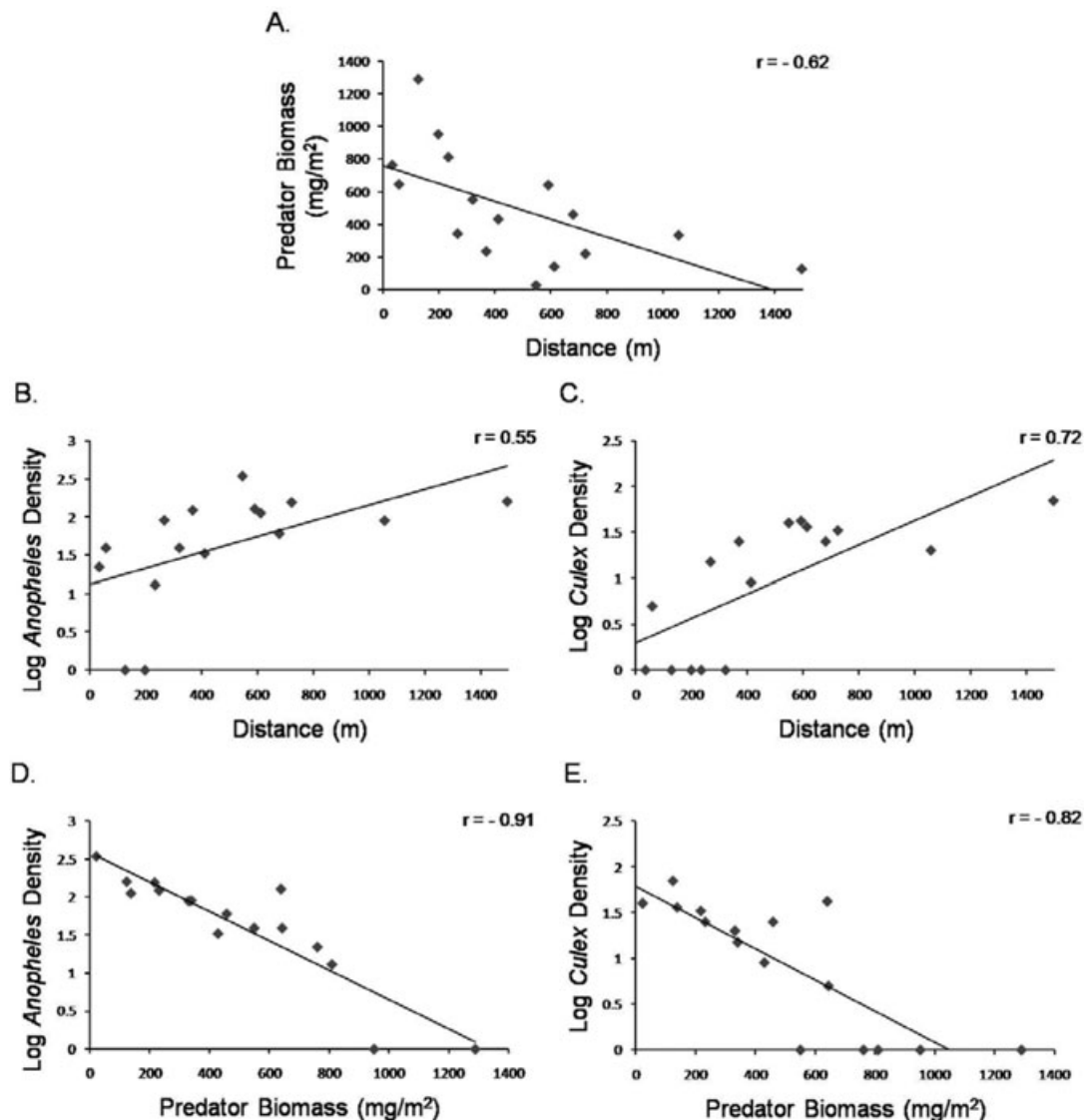


Fig. 1. Bivariate relationships from surveys of existing ponds between (A) isolation by distance (m) and predator biomass (mg/m²), (B) isolation by distance (m) and log transformed *Anopheles* density, (C) isolation by distance (m) and log transformed *Culex* density, (D) predator biomass (mg/m²) and log transformed *Anopheles* density, and (E) predator biomass (mg/m²) and log transformed *Culex* density.

Discussion

Overall, the results from the SEM analyses of the pond surveys highlight how habitat isolation among ponds lead to higher mosquito densities, primarily as an indirect effect through isolation's direct effect on predator biomass (Figs 1 and 2). Experimental results confirmed this by showing that isolated mesocosms had lower predator (Fig. 3), but higher mosquito (Fig. 4) densities than more connected mesocosms when predator communities were left intact. However, when predators were experimentally reduced, the isolation treatment had no such influence on mosquito densities, suggesting that the effect of isolation on mosquitoes was primarily an indirect response to isolation's negative influence on predator abundance.

While these results cannot fully elucidate the mechanisms by which predator biomass declined with habitat isolation, this result likely occurred because predators typically have lower local densities and hence larger spatial demands (reviewed in Holt & Hoopes, 2005). These larger spatial demands can either manifest in the foraging/movements of individuals among patches within a single generation, or in the persistence of populations in a spatial context. For example, in these wetland communities, many types of predators, especially those that are particularly effective predators, such as notonectids, dystiscids (Wilcox, 2001), and larval odonates (McCauley, 2006) require large and/or more connected areas in order to maintain viable populations. This is likely because predators typically have smaller population sizes than prey, such that isolated habitats

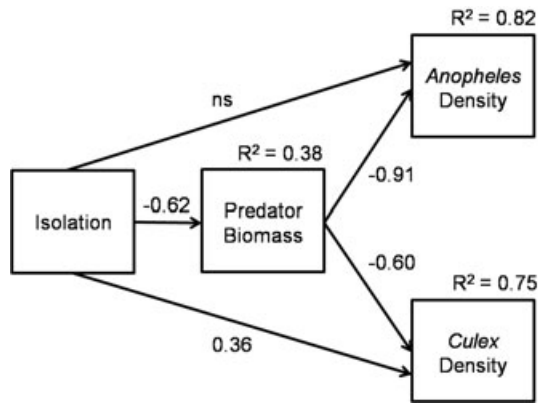


Fig. 2. Structural equation model from the surveys of existing ponds illustrating the direct and indirect effects of isolation on the densities of *Anopheles* and *Culex*. R^2 is the proportion of variance explained by upstream factors (predator biomass: $R^2 = 0.38$; *Anopheles* density: $R^2 = 0.82$; *Culex* density: $R^2 = 0.75$). Path coefficients presented are standardised values, and represent the change expected if a predictor is varied (in standard deviation units).

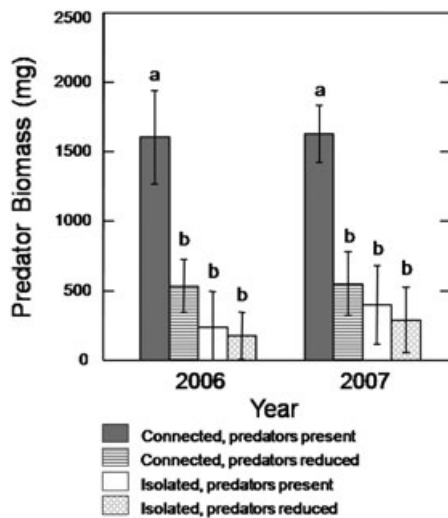


Fig. 3. Results from the experimental mesocosms showing the effects of isolation and predator reduction on the means of predator biomass (\pm SE) in 2006 and 2007. Letters correspond to pairwise comparisons between treatments.

would receive fewer predator colonists/propagules. Indeed, in our surveys, although we only presented data of the total biomass of predators, several of the larger and more efficient predators (e.g. aeshnid dragonflies) were notably absent in the more isolated ponds and mesocosms.

Many of the predators that live in these ponds exhibit complex life-cycles and are only present in the ponds during a portion of their life-cycle (e.g. amphibians and odonates). However, because their local population densities appear to be strongly influenced by the isolation and/or density among ponds, this suggests that the configuration of ponds in the landscape influences their overall metapopulation abundances despite their use of both habitat types.

Isolation's effect on mosquito abundances could also be influenced indirectly by effects on mosquito food densities (e.g. algae). While algal abundance was not measured in the surveys, they might be expected to be higher in more isolated habitats as a result of a different, but related mechanism. If predator effects cascade through the food web, lower densities of predators in more isolated habitats could lead to lower algal abundances via a trophic cascade through herbivores. In fact, this was indeed the case in the mesocosm experiment as estimated by fluorescence (a measure of Chlorophyll-a in the phytoplankton). Phytoplankton biomass was more than fourfold higher in connected than in isolated mesocosms, but this effect was eliminated when predators were removed (results will be published as part of a separate paper on the entire food web; J. M. Chase *et al.*, unpubl. manuscript). Importantly, however, if mosquito densities were limited by resources such as algal abundance, this would have led to a pattern opposite to the one we observed. Thus, even if increased algal abundances may have influenced mosquito densities in this experiment, it appears as if those effects were substantially overridden by predator effects (see also Chase & Knight, 2003).

Although the overall SEM results showed a strong indirect effect of isolation on mosquito densities through predator biomass, the two groups of mosquito species differed somewhat in their responses. Specifically, *Anopheles* was influenced only by the indirect effect mediated through predators, whereas *Culex* was both directly and indirectly affected by isolation (Fig. 2). One possible reason for this difference between the groups of species may be the divergent life history traits of species in the two genera. For instance, if *Anopheles* are better dispersers, this would allow *Anopheles* to more readily colonise isolated habitats, and thus be less influenced by the direct effects of habitat isolation than *Culex*. Additionally, it is possible that *Culex* adults could be responding directly to isolation as a result of possible higher concentrations of preferred hosts around these isolated ponds. However, there appeared to be no such evidence for direct effects of isolation in the mesocosm experiment where predators were experimentally reduced. A likely reason for this is that this experiment was at a much smaller spatial scale than the pond surveys.

In the mesocosm experiment, mosquito densities varied through time, but there was no time-by-treatment interaction. As mosquito abundances were not sampled until the second year of the experiment, we interpret these results as implying that there were no further temporal divergences through time among the treatments, and that all of the main experimental results were in place at the start of the sampling. Larval mosquito abundances peaked in May and June and declined as the growing season progressed. One possibility for this seasonal dynamic may be due to variation in predators, whereby after the winter, predators are rare, and they rebuild their populations over the course of the summer (e.g. Warren, 1989). However, as there was no time by predator removal interaction, if variation in predator abundance is a mechanism underlying the temporal dynamics of mosquitoes, this could only be the case because our predator reduction treatment was not completely successful. An alternative explanation could simply be because of phenological variation in the flight and

Table 1. Summary of repeated-measures MANOVAs for mosquito abundance (*Culex* and *Anopheles* species) in the experimental mesocosms.

Source of variation	d.f.	Wilks' λ	F	P
Between subjects				
Isolation (I)	1	—	74.122	<0.001
Predators reduced (PR)	1	—	20.894	0.001
I \times PR	1	—	22.166	0.001
Within subjects				
Time	7, 6	0.036	23.287	0.001
Time \times I	7, 6	0.301	1.990	0.210
Time \times PR	7, 6	0.687	0.391	0.877
Time \times I \times PR	7, 6	0.498	0.895	0.562
Time \times Species (S)	7, 6	0.159	4.518	0.043
Time \times S \times I	7, 6	0.696	0.374	0.888
Time \times S \times PR	7, 6	0.679	0.405	0.869
Time \times S \times I \times PR	7, 6	0.665	0.433	0.851

egg laying season of the mosquitoes themselves, or in their success at obtaining blood meals.

There is a long history of draining wetlands for reduction of mosquito nuisance and disease transmission (Mitsch *et al.*, 1994; Perry & Vanderklein, 1996), dating at least as far back as ancient Rome (Russell, 1955). This rationale has persevered, and increased disease risk associated with higher mosquito abundance is often considered to be a cost of wetland restoration and mitigation (e.g. Knight *et al.*, 2003; Willott, 2004). It is certainly true that the draining of wetlands reduces the total habitat available for larval mosquito development, and that this has likely led to large decreases in the number of mosquitoes in large areas. However, wetland draining also necessarily increases the isolation among, and reduces the density of the remaining habitats. We find that this can increase the local densities of mosquitoes by decreasing the local abundance of mosquito predators.

The absolute effect of habitat loss on the total abundance of mosquitoes will depend on the balance between the direct

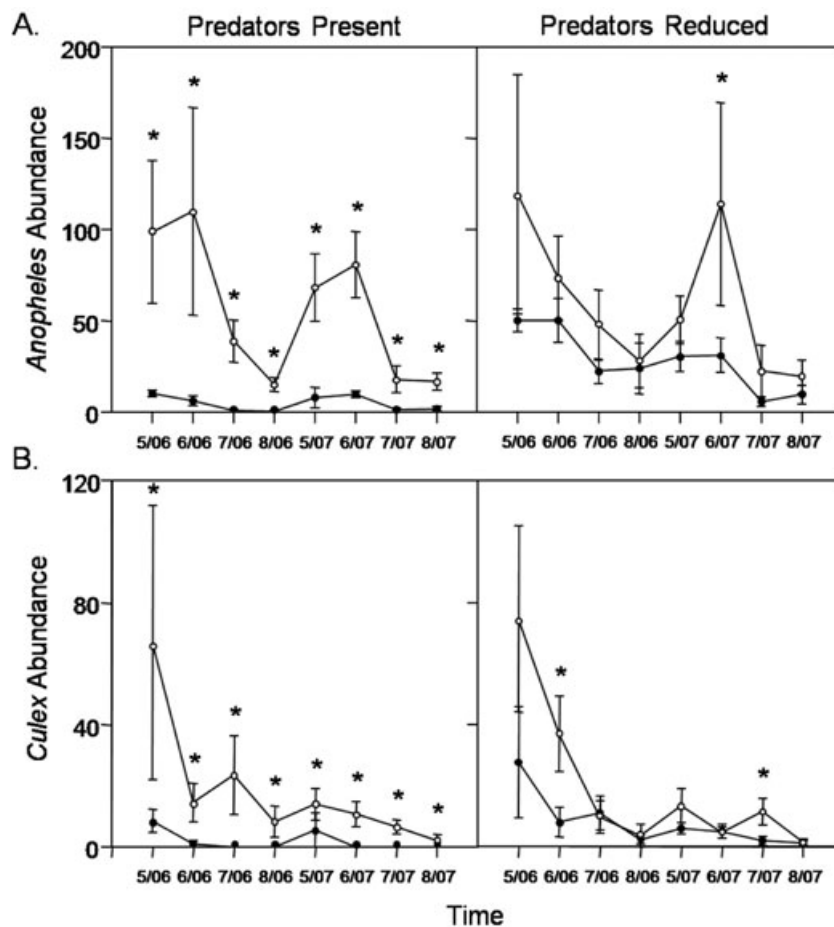


Fig. 4. Temporal changes in the means (\pm SE) of *Anopheles* (A) and *Culex* abundance (B) in mesocosms where predators were reduced vs those where predator communities were left intact. Open points are isolated mesocosms and closed points are connected mesocosms. Stars refer to significant differences among isolation treatments at individual sampling dates.

negative effects of habitat loss relative to the positive effects of habitat isolation/density mediated indirectly through predators. Even if loss of total habitat area reduces mosquito production overall, mosquitoes may be denser in the remaining small, isolated wetlands, which could have serious consequences for nuisance and disease risks. To mollify the increase in local mosquito density with increasing isolation, we suggest that management activities could enhance the local abundance of predatory species, through, for example, enhancing dispersal rates and/or adult habitat quality for predatory species with complex life cycles.

These results also have implications for construction of wetlands (e.g. for wildlife habitat or waste water management). For instance, in our Missouri study area, wildlife watering holes are typically constructed a minimum distance from each other (typically >500 m) to maximize their distribution. However, this spatial distribution might also act to maximize mosquito abundances. Less evenly (clumped) distributions of ponds in a landscape could then lead to higher predator biomass per pond, and thus lower mosquito abundances.

Several landscape factors have been suggested to influence mosquito abundances, including climatic and land use variables (Schäfer *et al.*, 2006; DeGroot *et al.*, 2007; Reiter & LaPointe, 2007; Vanwambeke *et al.*, 2007). However, ours is among the first to discuss the role of spatial processes *per se* on mosquitoes through their direct and indirect responses to isolation. By considering larval mosquitoes in a metacommunity context, we suggest that a more predictive framework can be developed for understanding variation in mosquito abundances through space and time (see also Chase & Knight, 2003; Blaustein & Chase, 2007).

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