Title: Top predator introduction changes the effects of spatial isolation on freshwater community structure

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Abstract

Spatial isolation can differentially affect the distribution of predators and thus affect lower trophic levels by resulting in trophic cascades. Similarly, the introduction of top predators into isolated ecosystems can cause the same cascading effects because they mostly prey upon larger frequently predatory taxa, indirectly benefiting consumers. Here we experimentally tested whether spatial isolation can affect the outcome and strength of the cascading effects caused by fish on macroinvertebrate community structure. We found that fish did reduce the abundance of predators but had no effect on consumers. Spatial isolation, however, did cause trophic cascades, but only in the absence of fish. We believe this happened because fish also preyed upon consumers when they increase in abundance. Additionally, and in contrast with simple theoretical expectations for metacommunities, we found that the difference between ponds with and without fish increased with isolation, probably because fish dampened the cascading effects of spatial isolation.

Introduction

Dispersal is a key driver of community and metacommunity structure (Mouquet & Loreau 2003; Leibold *et al.* 2004; Vellend 2010; Leibold & Chase 2018). Classic metacommunity theory recognizes that the frequency and intensity of dispersal can determine the relative importance of stochasticity and niche selection in structuring metacommunities (Leibold *et al.* 2004). When mean dispersal rate among species is too low or patches are very isolated, stochastic events are likely to cause communities to drift towards multiple different states that aren't necessarily related to local environmental conditions because of the contingency of colonization history (Leibold & Chase 2018). In contrast, if dispersal rate is too high or patches are extremely highly connected, the constant arrival of migrants should override the effects of local drift or niche selection making communities more similar to each other irrespective of local environmental variation (Mouquet & Loreau 2003; Leibold & Chase 2018). Thus, niche selection processes are more likely to shape community structure in intermediate levels of dispersal rate or intermediate levels of connectivity. However, it is also important to incorporate interspecific variation in dispersal rates and not only mean dispersal rates (Levins & Culver 1971; Finlay *et al.* 2002; McCann *et al.* 2005; Shurin *et al.* 2009; Astorga *et al.* 2012; Vellend *et al.* 2014; Guzman *et al.* 2019). Vellend *et al.* (2014), for example, suggested that variation in dispersal rates can reduce the effect of stochasticity because species with higher dispersal rates would always colonize a patch first, leaving less room for the establishment of different community structures. They also suggested that the consequences of niche selection on patterns of community structure would be stronger if traits that confer higher fitness within a set of local environmental conditions are positively correlated with dispersal rate.

Freshwater pond species can vary substantially in dispersal and colonization rates (Bilton *et al.* 2001; Shulman & Chase 2007; Chase & Shulman 2009; Shurin *et al.* 2009; Guzman *et al.* 2019) and such rates can vary with trophic level among invertebrates. For example, predatory insects tend to have larger body sizes than consumers, and thus higher locomotory ability (McCann *et al.* 2005). However, predatory insects also tend to have smaller population sizes (Cohen *et al.* 2003) and longer generation times than their prey, possibly making colonization events in spatially isolated ponds rarer (Chase & Shulman 2009). Predators can also be indirectly disfavored by habitat isolation if their prey is dispersal-limited or unable to reach high population sizes (Hein & Gillooly 2011). The smaller body sizes of non-predatory insects, by contrast, may greatly expand their dispersal range by wind transport (Muehlbauer *et al.* 2014). An important outcome of this negative correlation between dispersal rate and trophic level is that spatial isolation can lead to trophic cascades, causing herbivores and detritivores to be more abundant in more isolated habitats (Shulman & Chase 2007; Chase & Shulman 2009).

Freshwater community structure is also strongly influenced by the presence of predatory fish (Wellborn *et al.* 1996; Howeth & Leibold 2008; Pope & Hannelly 2013). In the absence of fish, predatory invertebrates such as aquatic beetles and dragonfly larvae are often the top predators. Compared to fish they are usually less efficient, gape-limited sit-and-wait predators that consume smaller prey (Wellborn *et al.* 1996). However, when present fish, which are usually large visually oriented predators, tend to preferentially consume large prey, which frequently happens to be predatory insects (Wellborn *et al.* 1996; McCauley 2008). This can also lead to trophic cascades, causing the abundance of small herbivore and detritivore species to increase (Diehl 1992; Goyke & Hershey 1992). Humans frequently purposefully introduce fish for aquaculture to habitats that greatly vary in its degree of isolation and there is thus a possible interaction between isolation and fish distributions that may determine the degree to which trophic cascades are found in a landscape of ponds.

Our study aimed at experimentally assessing whether and how spatial isolation can change the effects of the introduction of a generalized fish predator on freshwater community structure. We hypothesized that the presence of predatory fish would (1) promote trophic cascades because it should preferentially prey upon larger predatory insects, increasing the abundance of consumers (*i.e.* herbivores and detritivores). Additionally, (2.1) if spatial isolation promotes a similar cascade effect, by reducing the abundance of predatory insects, which frequently have lower dispersal rates, increasing isolation should intensify the effect of fish on community structure because the ecological traits that promote trophic cascades in both cases are positively correlated (*i.e.* body size and dispersal rate, respectively). (2.2) Alternatively, if spatial isolation represents a similar limitation to both predators and consumers, the effect of fish predation on community structure should be stronger at intermediate spatial isolation where dispersal rate is neither too high nor too low to override the consequences of any niche selection process, as predicted by classic metacommunity models.

Methods

We conducted a field experiment at the Estação Ecológica de Santa Bárbara (EESB) in Águas de Santa Bárbara, São Paulo, Brazil (22º48’59” S, 49º14’12” W). The EESB is a 2,712-ha protected area predominantly covered with open savanna phytophysiognomies, with smaller portions of seasonal semideciduous forests, *Pinus* sp. and *Eucalyptus* sp plantations (Melo & Durigan 2011). Soils are sandy, and climate is Koeppen´s Cwa, i.e., warm temperate with dry winters and hot summers (CEPAGRI 2018). Mean annual rainfall is ~1350mm with a distinct rainy season from October to March (January being the wettest month with ~200mm rainfall) and a dry season from April to September (July being the driest month with ~40mm rainfall; (CEPAGRI 2018). In the EESB the experiment was implemented in an area covered by second growth cerrado *sensu stricto*, a moderately dense, open-canopy savanna phytophysiognomy (Melo & Durigan 2011).

Experimental units consisted of ~1,200L artificial ponds dug into the ground and lined with a 0.5 mm thick, high-density polyethylene geomembrane to retain water. Each pond was 4 m long, 1m wide and 40 cm deep. Walls were vertical along the length of the pond; 1 m-long ramps terminating at ground level at each short side of the pond provided shallow microhabitats for freshwater organisms and escape for terrestrial fauna that eventually fell into the water. Two roof tiles were placed at the waterline in each of the short sides to provide shelter and/or oviposition habitat. Three 30 cm-long, 10 cm-wide PVC pipes were placed in the water to provide shelter for fishes.

Experimental design

The experiment followed a fully factorial design crossing fish presence (presence/absence) with spatial isolation (three levels of isolation). The isolation treatment was achieved by establishing 8 artificial ponds along each of three parallel transects 30m, 120m and 480m from a source wetland consisting of a stream (Riacho Passarinho) and its floodplain (Fig. 1). Within each transect, the distance between adjacent artificial ponds was 30 m. The well-drained sandy soils ensured that no other ponds and puddles formed during the rainy season at our study site, which could confound our manipulation of isolation distances. Each fish-by-distance treatment was replicated four times for a total of 24 artificial ponds.

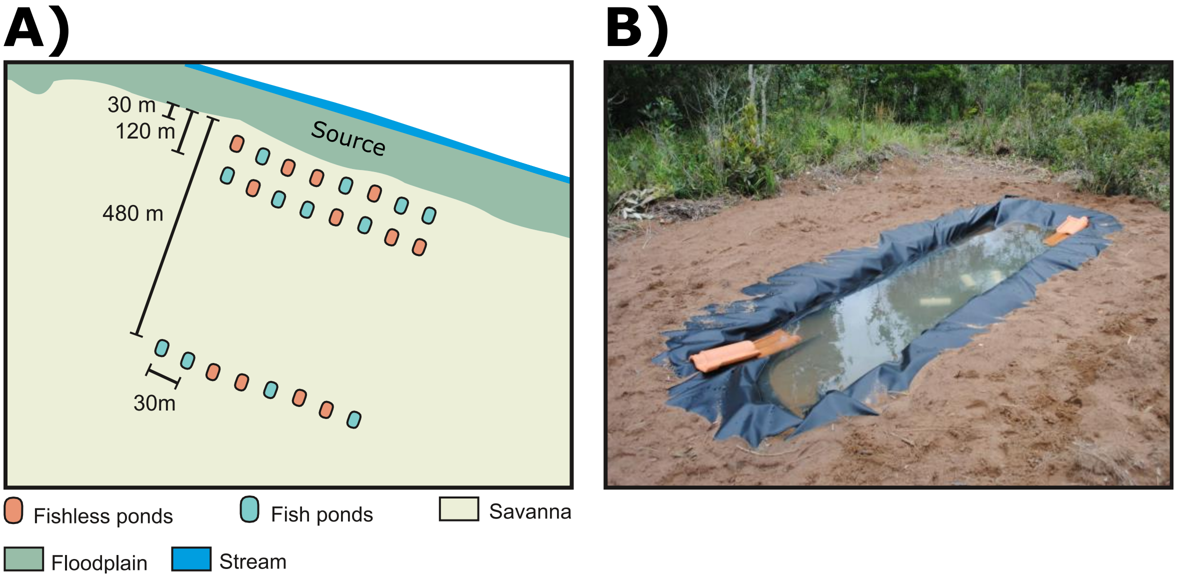


Figure. 1. A. Experimental setup. B. One of the ponds during the experiment.

The experiment ran from 18-Jan-2017 to 24-Apr-2017. Between 18 and 25-Jan-2017 mesocosms were filled with well water. On 28-Jan-2017 we added to each mesocosm 1000g (wet mass) of leaf litter composed of equal amounts of grass and tree leaf litter to provide structural complexity for benthic organisms. On 29-Jan-2017 we added to each mesocosm 15g of dog chow to provide an initial pulse of nutrients. The same day we added one Redbreast Tilapia (*Coptodon rendalli*, standard length 99.2 mm ± 5.9 mm, wet mass 40.2 g ± 8.8 g, mean ± SD, N=12) per predator treatment pond, collected in a small reservoir outside the EESB.

The reasons for manipulating Redbreast Tilapias are twofold. First, Tilapias are hardy generalized predators (confirmed in a pilot lab experiment, see appendix 2), capable of surviving in a wide range of environmental conditions including low oxygen levels and a broad range of temperatures (Caulton 1977; Tran-Duy *et al.* 2008), conditions likely to be found in our shallow artificial ponds. Second, the Redbreast Tilapia is, along with the Nile Tilapia (*Oreochromis niloticus*), one of the most widely introduced fishes in the world for aquaculture and recreational fisheries (Britton & Orsi 2012). These African species represented ~11% (6.1 million tons) of the entire freshwater fish production in the world and ~40% (0.6 million tons) in the Americas in 2017 (FAO 2019). In Brazil, Redbreast and Nile Tilapias are found in reservoirs and lakes in most river basins, and their spread to new river basins may be a matter of time considering that their stocking is still encouraged by public policies (Zambrano *et al.* 2006; Britton & Orsi 2012; Pelicice *et al.* 2014; Daga *et al.* 2016). Indeed, a very common land management practice in rural Brazil is the construction of dugout or impounded lakes, where the Tilapia is usually the first choice of fish species for stocking.

Freshwater community sampling surveys

To assess the influence of fish presence, spatial isolation, and their interaction on community assembly we conducted three sampling surveys of freshwater communities after ~3 weeks (18 to 23-Feb-2017), ~8 weeks (23 to 27-Mar-2017) and ~12 weeks (20 to 24-Abr-2017) of experiment. Freshwater communities were dominated by insects, which were sampled by sweeping half of the pond twice, including both pelagic and benthic habitats, with a hand net (mesh size 1.5 mm). Samples were cleaned of debris and stored in 70% ethanol. We identified and counted all aquatic macroinvertebrates to the lowest reliable taxonomical level using taxonomic keys for South American freshwater insects (Costa *et al.* 2004; Pereira *et al.* 2007; Segura *et al.* 2011; Hamada *et al.* 2014).

Over the course of the experiment we monitored ponds for fish survival; dead fish were replaced as soon as noticed. In the day following fish addition four fishes had died, possibly due to handling stress. One week later one more fish had died. In the following weeks, mesocosms water became turbid and it was not always possible to assess fish presence without netting.

Because netting could represent a considerable disturbance to freshwater communities, we waited until the end of each sampling survey to seine the ponds and thereby assess fish presence in treatment ponds. Two fishes were found to be missing by the end of the first sampling survey, two by the end of the second sampling survey and none by the end of the third sampling survey. Also, we had to exclude four ponds from the analysis in the last sampling survey because of sampling issues (see appendix 3).

Data analysis

To test the hypothesis that community structure is influenced by fish presence, distance to the source, and their interaction, we used a model-based approach for multivariate data where the matrix of site-by-raw species abundance data represents community structures (Warton *et al.* 2015a). The main advantages of model-based approaches are the possibility of accounting for the mean-variance relationship of abundance data, and the better interpretability of data. Specifically, we can assess which and how species in a community are being influenced by treatments and test for the effect of traits in their responses (Warton *et al.* 2015b). Prior to this analysis, singletons and doubletons were removed both because they are uninformative to general community patterns and because they complicate model parameter estimation (Warton *et al.* 2015a).

Because abundance data are counts, both Poisson and Negative Binomial distributions were considered. We chose the Negative Binomial distribution after concluding that there was overdispersion in our abundance data by inspecting the mean-variance relationship (appendix 4). We also tested for differences in the effect of treatments across different sampling surveys. To do that, we performed likelihood ratio tests to test if the progressive addition of terms to the model provided a statistically better fit based on values of deviance. First, we tested if we had any effect of time in species abundances, then we tested if progressively adding the effect of fish, isolation, and their interaction provided a significant better fit to the data. Those tests were always assessed by comparing the best model so far against the next more complex model. After that, we tested if the effect of treatments were different across different sampling surveys by adding an interaction between the effect of time and the interaction between fish and isolation treatments. To account for correlations in species abundances when computing p-values we shuffled entire rows of the incidence matrix (ponds), keeping species abundances in the same ponds always together. To account for lack of independence between the same ponds sampled across time, ponds were considered blocks, so in each permutation step we shuffled ponds freely within blocks (*i.e.* only across time), then we shuffled the entire bocks freely (*i.e.* across fish and isolation treatments). P-values were computed using the PIT-trap bootstrap resample procedure, which operates on probability integral transform residuals (Warton *et al.* 2017). P values were from 10,000 bootstrap resamples. Because we found significantly different effects of fish and isolation treatments across different sampling surveys, we repeated the analysis in each sampling surveys separately. Those analyses were implemented using functions *manyglm()* and *anova.manyglm()* from package ‘mvabund’ version 4.0.1 (Wang *et al.* 2012, 2019). To see how individual taxa, respond to the different treatments we looked at 95% confidence intervals of estimated parameters for each taxon in each treatment. To see if the trophic level (*i.e.* strict predator VS consumer) is a good predictor of the changes in community structure, we further tested if the inclusion of the categorical trait predator/consumer increases the fit of the models also performing likelihood ratio tests. We considered strict predators only the predators that were prone to prey upon other sampled macroinvertebrates. Insects that are not predators at all, that prey mostly upon zooplankton, or that have only a small portion of their diet based on predation were considered consumers (see appendix 5). This trait-based approach is called the model-based fourth corner solution (Brown *et al.* 2014) and was also implemented using functions *traitglm()* and *anova.traitglm()* from package ‘mvabund’ version 4.0.1 (Wang *et al.* 2019).

A significant interaction between fish and isolation means that there is either or both a difference in direction or magnitude of the effect of fish in different isolation treatments (*i.e.* positive or negative effect). To specifically test for differences in the size of the effect of fish, regardless of direction, we performed a model-based unconstrained ordination via generalized linear latent variable models (GLLVM; Niku *et al.* 2017) with a negative binomial distribution using two latent variables for each of the sampling surveys (Hui *et al.* 2015). The latent variables were estimated via variational approximation (Hui *et al.* 2016). After performing the ordination, we computed the centroids of each treatment group, and the distance between the centroids of fish and fishless treatments in each isolation treatment as a measure of the size of the effect of fish. Then we tested whether this distance is significantly different across all the isolation treatments. To test for that we designed a permutation procedure to only permute ponds across isolation treatments, keeping the fish treatment constant. This represented a null scenario where the effect of fish is the same in all isolation treatments. We corrected p-values for multiple comparisons using the false discovery rate (FDR). We also used those ordinations to visualize the effect of treatments on community structure. These analyses were implemented using the function *gllvm()* from package ‘gllvm’ version 1.1.7 (Niku *et al.* 2019). All analyses were implemented in software R version 3.6.1 (R Core Team 2019). Code and data to perform reproduce the analysis and figures are available on github in the following repository: RodolfoPelinson/Pelinson_et_al_2019_Top_predator_isolation.

Results

Mesocosms were colonized by aquatic and semiaquatic insects comprising five orders and 17 families (Odonata: Libellulidae, Coenagrionidae; Coleoptera: Dytiscidae, Hydrophilidae, Noteridae; Diptera: Ceratopogonidae, Chaoboridae, Chironomidae, Culicidae; Ephemeroptera: Baetidae, Caenidae, Polymitarcyidae; Hemiptera: Corixidae, Gerridae, Naucoridae, Notonectidae, Veliidae). The most abundant families, with more than 200 individuals sampled in the entire experiment, were chironomids (total abundance of 11,558 individuals), veliids (1,097 individuals), libellulid dragonflies (901 individuals) and culicid mosquitoes (707 individuals). The rarest families, with less than 10 individuals sampled in the entire experiment, were Gerridae, Naucoridae, Corixidae, Coenagrionidae, Ceratopogonidae and Hydrophilidae. More detailed information is available in appendix 5.

We analyzed each sampling survey separately because the effect of treatments on community structure changed and became clearer from the first to the last survey (Fig. 2; Table 1). Therefore, here we focused on the results for the last survey. We found that there was generally a significant effect of fish, isolation and their interaction on community structure for the last surveys, and those effects were mediated by trophic level (Table 1). The presence of fish had a negative impact on the abundance of predators only at 120 and 480 m (Fig. 3 A). At 30 m *Pantala* dragonflies suffered no effect of fish and *Orthemis* dragonflies were strongly positively affected (Fig. 4 A). Consumers were not positively affected by fish by the end of the experiment (Fig. 3 A). Spatial isolation negatively affected predators from 30 to 120 and 480 m of isolation, but only in ponds with fish (Fig. 3 C and B). However, the same two predators, *Pantala* and *Orthemis* dragonflies, were actually positively affected by isolation in fishless ponds (Fig. 4 A).

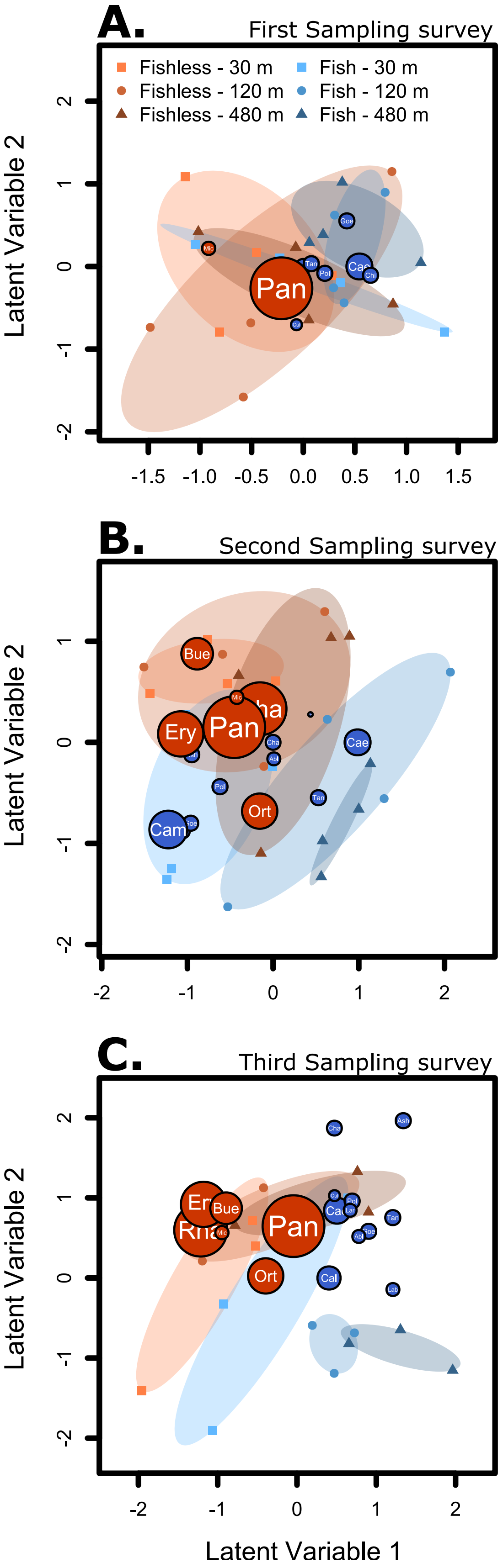
Also, consumers were positively affected by isolation from 30 m to 120 m and 480 m of isolation, but only in fishless ponds (Fig. 3 C).

Table 1. Summary of likelihood ratio tests of models explaining community structure. All values of deviance within each sampling survey or all sampling surveys together are relative to the simpler model immediately above it. Bold values represent a significative improvement in model fit ($p < 0.05$).

	Diff. of Degrees of Freedom	**Deviance**	**p**
All Sampling Surveys			
1- Time (Compared to no effects of time or treatments)	**2**	**392.8**	**<0.001**
2 - Time + Fish (Compared to model 1)	**1**	**89.2**	**<0.001**
3 - Time + Fish + Isolation (Compared to model 2)	**2**	**109.2**	**0.001**
4 - Time + (Fish * Isolation) - Compared to model 3	**2**	**120.3**	**<0.001**
5 - Time * (Fish * Isolation) (Compared to model 4)	**10**	**210.6**	**0.034**
1st Sampling Survey			
6 – Fish (Compared to no effect of treatments)	1	19.01	0.104
7 – Isolation (Compared to no effect of treatments)	2	22.45	0.497
8 – Fish * Isolation (Compared to no effect of treatments)	5	85.99	0.053
2nd Sampling Survey			
9 - Fish (Compared to no effect of treatments)	**1**	**62.28**	**0.002**

10 - Fish + Isolation (Compared model 9)	**2**	**71.81**	**0.021**
11 - Fish * Isolation (Compared model 10)	**2**	**72.15**	**0.016**
12 – (Fish * Isolation):Trophic Level (Compared model 11)	**5**	**33.74**	**0.002**
3rd Sampling Survey			
13 – Fish (Compared to no effect of treatments)	**1**	**49.09**	**0.018**
14 -Fish + Isolation (Compared to model 13)	2	72.96	0.056
15 - Fish * Isolation (Compared model 13)	**4**	**164.1**	**0.009**
16 - (Fish * Isolation):Trophic Level (Compared to model 15)	**5**	**33.71**	**0.028**

Figure 2. Model-based unconstrained ordinations showing pond communities (symbols) and species (bubbles) in each of the three sampling surveys. Red bubbles are predatory-insects and blue bubbles are consumers. Size of bubbles are proportional to body size of each taxa (the volume of the largest individual of each species in a log-scale). A – First sampling survey; B – Second sampling survey; C – Third sampling Survey. Abbreviations of names of taxa provided in appendix 5.



When we tested for differences in the size of the effect of fish on community structure, we found that the effect of fish at 480 m was bigger than at 30 m (Dif: 1.63; adj. p: 0.038). The size of effect of fish at 120 m was not significantly different from 30 m (Dif: 1.25; adj. p: 0.098) or 480 m (Dif: 0.38; adj. p: 0.601).

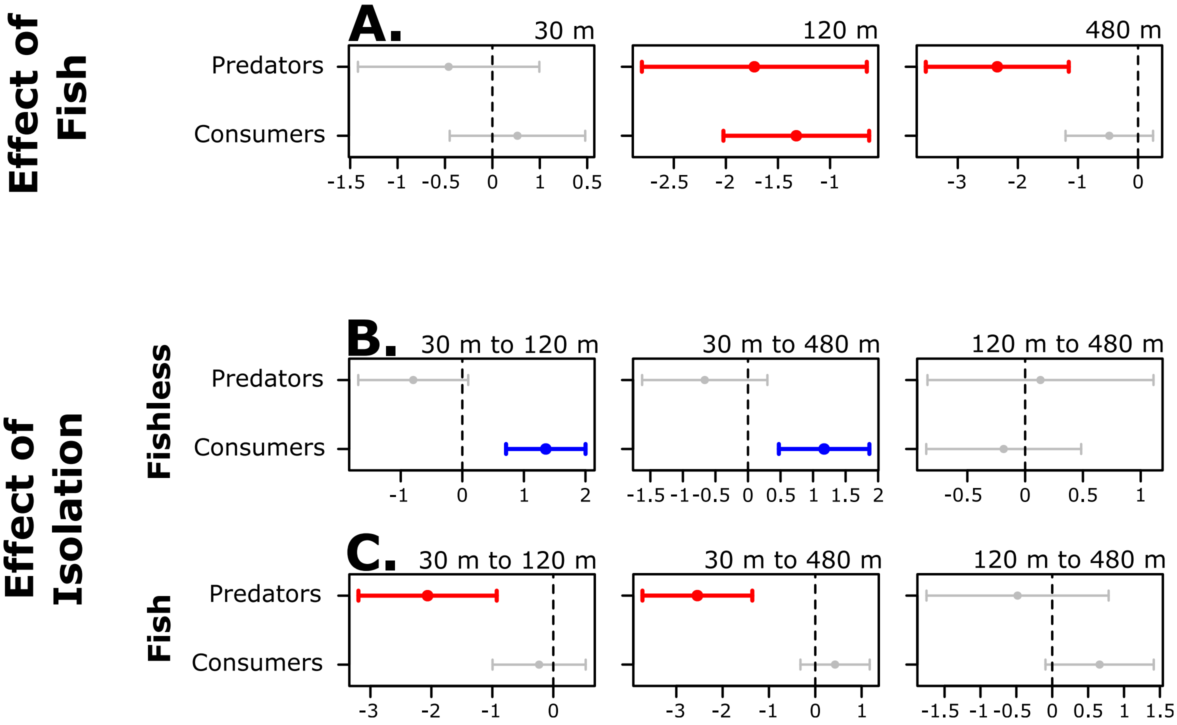


Figure 3. 95% Confidence intervals for the maximum likelihood estimates (MLE) of the effect of fish and isolation on abundance of predators and consumers when comparing pairs of treatments for the last survey. Confidence intervals not crossing the zero hatched line were considered significant effects and colored; blue bars represent an increase and red bars a decrease in abundance from the reference treatment. A are effects of the presence of fish in each isolation treatment. B are effects of isolation in fishless ponds and C in ponds with fish. In C and B we show effects of increasing isolation from 30 to 120 m, from 30 to 480 m, and from 120 m to 480 m. Similar results for the second sampling survey are available in appendix 10.

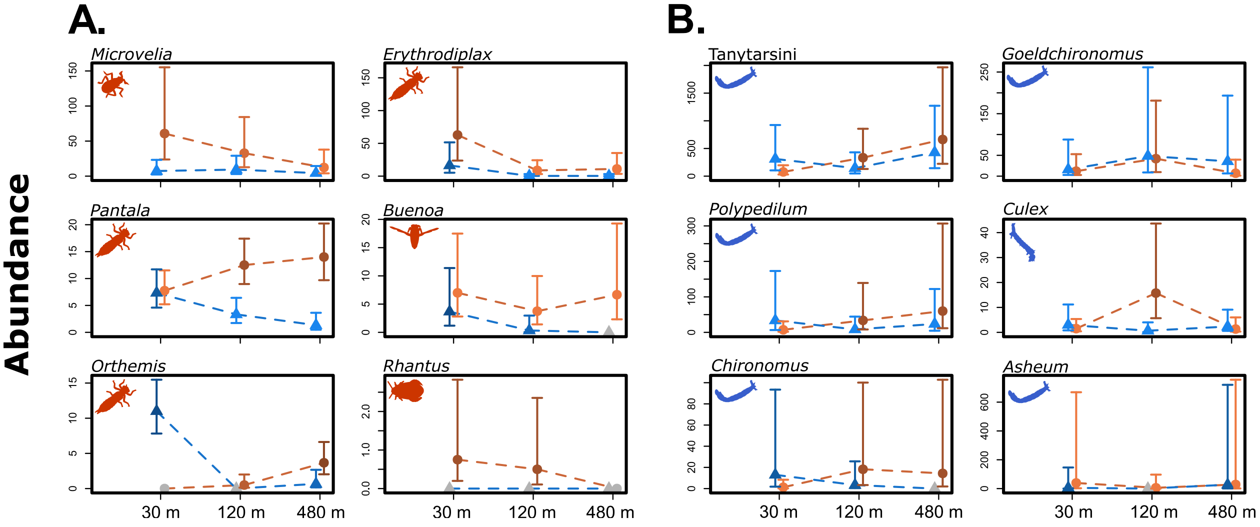


Figure 4. Maximum likelihood estimates (MLE) of abundance and their 95% confidence interval (for Model 16 in Table 1) of abundance for the six most abundant predatory taxa (A), and six most abundant consumer taxa (B), in the last sampling survey. Gray symbols indicate absolute absence (zero abundance) of a taxon in a treatment. Blue triangles are MLEs for fish treatments and orange balls are MLEs for fishless treatments. MLEs that are not contained inside the 95% confidence interval of other estimates were considered to be different. The actual estimated differences are provided in appendix 8. MLE of abundance for all species in the second and third sampling surveys are provided in appendix 6.

Discussion

Generally, both the presence of fish and spatial isolation had important effects on freshwater community structure. Specifically, responses to treatments were different for different trophic levels, as we expected, but we also found that some species are interesting exceptions for the predicted patterns. More importantly, the effects of fish and isolation are highly dependent on each other in ways that differ from those expected by classic metacommunity models (*e.g.* Mouquet & Loreau 2003; Leibold *et al.* 2004; Leibold & Chase 2018), but similar to what would be expected for multitrophic metacommunities with variable dispersal rates among species (Vellend *et al.* 2014; Guzman *et al.* 2019).

We initially hypothesized that the presence of fish would change community structure by preferentially preying on more conspicuous predators, therefore increasing invertebrate consumer abundance through trophic cascades. We found that the presence of fish indeed shifts species composition through a reduction of predatory insects. The most negatively affected taxa were dytiscids beetles, notonectids and dragonfly larvae, all of them relatively large taxa (see appendix 5 and 7). We also observed an increase in the abundance of consumers, but only for the second sampling survey (see appendix 10). However, contrary to our expectations and previous works, this effect disappeared by the end of the experiment. Goyke & Hershey (1992) found that chironomid density was higher in lakes with trout because they were preying upon an important chironomid predator, the slimy sculpin fish. However, different from trout, tilapias are generalist omnivores and might prey not only upon most conspicuous prey, but also any available prey, including consumers. In fact, in a pilot experiment (appendix 2) Tilapias readily ate not only large conspicuous prey such as *Scinax* tadpoles, small fishes (*Phalloceros* sp.), belostomatids, nepids and dragonflies, but also much smaller ones, such as damselflies and mosquito larvae, the latter having about 5 orders of magnitude less wet mass than the manipulated Tilapias. For instance, (Diehl 1992) found that the presence of juvenile perch, a generalist benthic predator, caused biomass of invertebrate predators to decrease, but had no effect on the biomass of consumers, possibly because it was also preying upon consumers. The same process might have occurred in our experiment where consumers might suffer an effect that is a balance between direct negative effects of predation by fish, and indirect positive effects caused by the reduction of predatory insects also by fish (*i.e.* trophic cascade).

Most of the substantial changes in community structure that we observed because of spatial isolation were from low (30 m) to higher levels of isolation (120 m and 480 m). We hypothesized that a gradient in community structure should develop from low to high levels of habitat isolation, also due to trophic cascades. Specifically, the abundance of dispersal-limited predatory insects should decrease as spatial isolation increases, and the abundance of consumers should increase in response, as a cascade effect. We partially found support for this hypothesis. The indirect positive effect of isolation on consumers was only observed in fishless ponds, while the direct negative effect on predatory insects was only observed in ponds with fish. We believe that the lack of this effect in ponds with fish is also due to the fact that fish also prey upon consumers, especially in higher isolation treatments where the abundance of predators decreases as a consequence of spatial isolation. Indeed, if we consider predatory insect biomass as an estimate of food availability for fishes, we observe that average insect biomass per fishless pond decreases about 17% from low to intermediate and high isolation (19.2 g in low isolation to 15.9 g in intermediate and high isolation). The lack of a negative effect of isolation on predatory insects in fishless ponds, however, might be explained by two clear exceptions to the expected patterns for predatory insects: *Pantala* and *Orthemis* dragonflies. Dragonflies are known to be good dispersers. Therefore, it is not surprising that at the scale of our experiment they would suffer small negative effects or no effect at all of spatial isolation (McCauley 2006). However, both of these dragonflies were positively affected by isolation in fishless ponds, while other predators, such as the *Erythrodiplax* dragonflies, diving beetles (*i.e. Rhantus*) and water striders (*i.e. Microvelia*) exhibited a strong decrease in abundance with spatial isolation. Therefore, we hypothesize that the absence of other predatory insects in more isolated ponds might have either released those dragonflies from competition or made these ponds more attractive for adults to lay their eggs.

Orthemis dragonflies were also an exception for the expected negative effects of fish on predatory insects. They consistently had higher abundance in ponds with fish, but only in low isolation treatments. Some dragonfly species are known to exhibit different vulnerability to predation depending on body size and flexible antipredatory behavior, allowing them to coexist with fish (Johnson 1991; McPeek 1998; Johansson 2000; Hopper 2001; McCauley 2008). Indeed, *Orthemis* larvae was one of the smallest benthic predators we had in our experiment (around 20 times smaller than *Pantala*; see appendix 5) and it has been reported to occur in high abundance in aquaculture ponds with no vegetation (Marco *et al.* 1999). It is thus possible that higher availability of other suitable prey (*i.e.* other predatory insects) in low isolation decreased predation rate on *Orthemis*, allowing it to have a greater abundance in ponds with fish.

Classic metacommunity models predict that the effect of environmental filtering and local interactions on community structure should be stronger at intermediate levels of isolation since the flux of individuals would not be high enough to homogenize community structure (i.e. mass effects), nor low enough to allow communities to drift to different structures due to stochastic events. However, in our experiment, we observed an increase in the difference between ponds with and without fish with the increase of spatial isolation. Most common metacommunity models have two important assumptions that are often not true: First, species have the same dispersal rates; second, species are from the same trophic level, that is, competition would be much more important than predation in shaping community structure (Mouquet & Loreau 2003; Leibold *et al.* 2004). Our results are more aligned with multi-trophic metacommunities that consider interspecific variation in dispersal rates (*i.e.* Vellend *et al.* 2014; Guzman *et al.* 2019).

Because the species in our communities have different dispersal rates, isolation was not only a process that increases stochasticity in the frequency of species arrival (*i.e.* mean dispersal), but also an environmental filter that selects which taxa arrive first and more frequently. However, different from what we expected, the effects of fish and isolation were not fully correlated, and the observed increase in the difference between ponds with and without fish is not due to an increase in the consequences of local niche selection. Instead, isolation had different effects on ponds with and without fish. Specifically, fish prevented both consumers and dragonflies to increase in abundance with increasing isolation in ponds with fish.

Here we show that the Redbreast Tilapia, as any generalized fish predator, have direct negative effects on both predatory insects and consumers, dampening indirect positive effects of isolation on less dispersal limited taxa. Additionally, we show that considering multitrophic communities and variation in dispersal rates is important if we seek to understand patterns of biodiversity at regional scales. Of course, our experimental mesocosms consisted of simple habitats and previous work has shown that structural complexity can mitigate the effects of generalist fish predators (Diehl 1992). Although we did not explore this issue here, an important concern about the introduction of Tilapias is that large-bodied individuals are known to reduce macrophyte coverage in lakes (Rao *et al.* 2015). Thus, the results we observed here might be true even in more structurally complex habitats. It is also important to acknowledge that our results could be due to either consumptive (*i.e.* direct predation upon available prey) or non-consumptive effects of fish and other predatory insects (*i.e.* avoidance of ponds with fish or high density of predatory insects through habitat selection; see Binckley & Resetarits 2005; Blaustein *et al.* 2005; Resetarits 2005), or both. Although our experimental design does not allow us to tease apart those two specific processes, we nevertheless show how the presence of fish and isolation changes community structure in a realistic and well controlled scenario. Finally, in accordance with other recent work (*e.g.* de Meutter *et al.* 2007; Vellend *et al.* 2014; Hill *et al.* 2017; Guzman *et al.* 2019) our work provides strong evidence of how variation in dispersal rates can change the effects of isolation on community structure, especially when we consider multi-trophic metacommunities.

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