Rodolfo Mei Pelinson

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Effects of local processes and spatial isolation on freshwater community assembly: a simulation of land-use intensification

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Índice

Resumo

Introdução: Ambientes de água doce estão entre os mais afetados por alterações no uso da terra, particularmente pela introdução de peixes exóticos para aquacultura e contaminação por agroquímicos como fertilizantes e pesticidas. No entanto, ainda não sabemos como e se estas alterações no uso da terra interagem com processos espaciais afetando comunidades locais, e portanto, a biodiversidade. Nosso objetivo nesta Tese foi entender, de forma experimental e usando o arcabouço teórico da ecologia de metacomunidades, como a introdução de peixes predadores exóticos, usados em aquacultura, e a contaminação por agrotóxicos podem influenciar a estrutura de comunidades de macroinvertebrados e anfíbios em diferentes contextos espaciais.

Métodos: Nós construímos poças artificiais de 1200-L (24 em um primeiro experimento e 45 em um segundo) em uma paisagem de savana, a três diferentes distâncias de uma área úmida, que serviu como fonte de colonizadores (30 m, 120 m, e 480 m). As poças foram espontaneamente colonizadas por insetos semiaquáticos e anfíbios que se dispersavam pela paisagem terrestre. No primeiro experimento nós manipulamos a presença e ausência de um peixe predador onívoro, a Tilapia. No segundo experimento, nós manipulamos a intensificação no uso de agroquímicos, simulando a conversão de ambientes de savana em pastos manejados (poças tratadas com fertilizante) e plantações de cana-de-açúcar (poças tratadas com fertilizante a um único pulso do inseticida Fipronil e um do herbicida 2,4-D, seguindo um regime de aplicação e doses realistas para esta cultura).

Principais resultados: Nós vimos que, em geral, o isolamento espacial pode reduzir a abundância de insetos predadores, pois possuem menor capacidade de dispersão, com raras exceções (*i.e.* libélulas do gênero *Pantala* e *Orthemis*). Isso aumenta aumenta o tamanho das comunidades (*i.e.*, abundancia total de indivíduos) por favorecer consumidores, que por sua vez, possuem maiores taxas de dispersão. Também vimos que o isolamento espacial pode aumentar a variabilidade na estrutura das comunidades (*i.e.*, diversidade beta em cada tratamento) por aumentar o efeito de contingenciamento histórico em espécies com taxas de dispersão similares. No entanto, estes efeitos podem mudar drasticamente quando peixes predadores estão presentes. A estocagem de peixes pode ter efeito negativo tanto sobre insetos predadores com grande tamanho corpóreo, quanto sobre insetos consumidores, o que enfraquece os efeitos positivos indiretos do isolamento espacial sobre consumidores. A presença de peixes também parece enfraquecer os efeitos do contingenciamento histórico, fazendo com que a variabilidade entre comunidades diminua em consequência do aumento do tamanho das comunidades. Mudando para agroquímicos, a fertilização em poças de ‘pastagem’ causou aumento na abundância de alguns poucos insetos predadores via efeitos *bottom-up*. Já o pulso de inseticida no tratamento que simulava poças em canaviais causou uma forte, porém temporária, redução nas populações de insetos. Efeito que foi seguido de aumento na abundância de larvas de anfíbios generalistas. Diferente dos efeitos da presença de peixes, os efeitos da fertilização, e os pulsos de pesticidas, não mudaram com o isolamento espacial, provavelmente porque eles tiveram efeitos agudos semelhantes os insetos ao longo de todo o gradiente de isolamento, e os efeitos indiretos foram, em sua maioria, sobre táxons que não sofreram com limitação de dispersão (*i.e.* libélulas e anfíbios). Portanto, nesta Tese nós mostramos que o potencial de filtros ambientais locais relacionados ao uso da terra de interagir com o isolamento espacial depende muito do tipo de uso da terra em questão. Além disso, nós mostramos que a variação interespecífica nas taxas de dispersão das espécies e os múltiplos níveis tróficos em comunidades aquáticas precisam ser considerados se quisermos de fato entender como diferentes mudanças ambientais afetam a estrutura de comunidades.

Abstract

Introduction: Freshwater environments are among the most affected by land-use change, particularly by the introduction of exotic fish species for aquaculture and contamination by agrochemicals such as fertilizers and pesticides. However, we essentially ignore whether and how land-use change interacts with spatial processes to affect local communities, and, therefore, biodiversity. In this Thesis we aimed at experimentally understanding how the introduction of exotic predatory fish for aquaculture, and contamination by agrochemicals, can affect macroinvertebrate and amphibian community structure in different spatial contexts using an explicit metacommunity framework.

Methods: We constructed 1,200-L artificial ponds (24 in the first experiment and 45 in the second) in a savanna landscape at three different distances from a source wetland (30 m, 120 m, and 480 m). Ponds were spontaneously colonized by semiaquatic insects and amphibians dispersing in the terrestrial landscape. In the first experiment, we manipulated the presence and absence of the exotic omnivorous fish, Tilapia. In the second experiment, we manipulated agrochemical intensification simulating the conversion of savannas into managed pastures (ponds treated with fertilizers) and sugarcane fields (ponds treated with fertilizers and a single pulse of the insecticide fipronil and the herbicide 2,4-D following realistic dosages and application schedules).

Main Findings: We generally found that spatial isolation can reduce the abundance of dispersal-limited predatory insects, with few exceptions (*i.e.*, *Pantala* and *Orthemis* dragonflies), thus increasing community size (*i.e.*, total abundance of individuals) by favoring insect consumers, which have higher dispersal rates. Spatial isolation can also increase community-to-community variability (*i.e.*, beta-diversity within treatments), by increasing the effects of historical contingency on species with similar dispersal rates. However, these effects can drastically change when predatory fish is present. The stocking of fish can have a strong negative effect on large-bodied predatory insects, and a milder negative effect on insect consumers, dampening the indirect positive effects of isolation on most of them. Fish also appear to override the effects of historical contingency, making community variability decrease because of the increase in community size. Shifting to agrochemicals, fertilization in 'pasture' ponds caused a slight increase in the abundance of few predatory insects via bottom-up effects. The insecticide pulse in ‘sugarcane’ ponds caused a very strong but temporary negative effect on insect populations, followed by an increase in the abundance of generalist larval amphibians. Different from the effects of fish, the effects of fertilization and pesticide pulses do not change with spatial isolation, likely because they have equally acute effects on all invertebrate taxa across isolation treatments, and their indirect effects are mostly on non-dispersal-limited taxa (i.e., dragonflies and amphibians). Therefore, we show that the potential of local environmental processes to interact with the effects of spatial isolation is highly dependent on the type of land management. More importantly, we show that the interspecific variation in dispersal rates and the multi-trophic nature of freshwater communities must be considered if we seek to understand the consequences of environmental change on community structure.

General Introduction

Humans can be thought of as the greatest ecosystem engineers in nature (Jones *et al.* 1994), having caused massive intended and unintended alterations in the physical environment for as far as we have archeological records (*e.g.*, Levis *et al.* 2018). To date, humans have directly converted 37% of the total land area of the planet (excluding Antarctica) into agricultural lands (FAO 2020), and this number is expected to grow as the human population is predicted to reach 9.7 billion people by 2050 (HLEF, 2009). To meet the demands of a larger global population with increasing per capita rates of consumption, the 'High-Level Expert Forum on How to Feed the World in 2050' predicts the need for a 70% increase in food production (HLEF, 2009). Such an increase reveals a need for agricultural expansion or intensification of already existent croplands (Foley *et al.* 2011; Crist *et al.* 2017), meaning that agriculture will continue to be the main global source of environmental change in biological systems, transforming natural ecosystems into newer and simpler ones (Foley *et al.* 2005; Nagendra *et al.* 2013). However, even though such changes are recognized to be pervasive in nature, we still lack a proper understanding of how different aspects of biodiversity respond to those changes.

Freshwater environments are among the most affected by different land-use managements (Foley *et al.* 2005). Among them, the introduction of exotic species for aquaculture and fisheries, and water contamination by agrochemicals are of utmost importance. Freshwater fish aquaculture production has almost doubled in the last decade, reaching 42.9 million tons in 2015 (FAO 2020). Alongside this tremendous growth, aquaculture became a leading cause of biological invasions worldwide, since most of the cultivated fishes are exotic or translocated species (Vitule *et al.* 2009). In South America, scenarios of fish farming vary from large scale stocking of free-ranging or caged fish in hydroelectric dam reservoirs to small scale introductions in sometimes previously fishless, impounded, or dugout ponds that are widespread in virtually thousands of small rural properties (Britton & Orsi 2012). More importantly, such introductions are even encouraged by public policies (Pelicice *et al.* 2014). One of the causes of the success and popularity of this practice is the choice of hardy species tolerant to a wide range of environmental conditions and that exhibit broad generalized diets – irrespective of their original distributional ranges (Britton & Orsi 2012).

Besides direct fish introduction into aquatic habitats, land use management can also indirectly affect freshwater environments. Many freshwater habitats are embedded in rural agricultural landscapes, being therefore subject to contamination by different types of agrochemicals (Matson *et al.* 1997; Balinova & Mondesky 1999; Armas *et al.* 2005; Foley *et al.* 2005; Schiesari & Grillitsch 2011). Fertilizers are of special concern. For instance, the world's use of phosphate fertilizers grew from around 38 to 48 million tons (a 26% growth) from 2005 to 2016 (FAO 2020), with Brazil being responsible for 10% of this total (FAO 2020). Such an increase has led to worldwide contamination of both freshwater and marine ecosystems (Smith *et al.* 2006), causing increased primary productivity and eutrophication (Matson *et al.* 1997; Smith *et al.* 1999). Similarly, the world consumption of pesticides grew from 2.3 million tons to 4.1 million tons from 1990 to 2017 (a 78% growth; FAO 2020), with Brazil becoming the world's largest consumer (~540 thousand tons of active ingredients commercialized in 2017; IBAMA 2019). More importantly, even though water contamination is common when pesticides are used as intended by the manufacturer, overuse is common among landowners, especially in small properties, increasing the likelihood of contamination of many aquatic habitats (Schiesari *et al.* 2013).

Here we propose that to truly understand how land-use change can locally affect aquatic community structure, we must understand land management practices such as fish stocking and agrochemical management as simple niche selection processes (*e.g.*, environmental filtering) acting on local habitats that are embedded in a spatially structured world - thus explicitly placing land-use change into a metacommunity framework (Schiesari *et al.* 2019). One of the key recent advances in understanding how local environmental change affects ecological communities is the acknowledgment that local communities are connected, to a greater or lesser degree, through the dispersal of individuals forming metacommunities (Leibold *et al.* 2004; Leibold & Chase 2018). Thus, processes happening at the local and regional scales cannot be interpreted in isolation. More recently, Vellend (2010) united the metacommunity and other frameworks into the four fundamental processes shaping ecological communities: niche selection, ecological drift, dispersal, and speciation (Vellend 2010, 2016). Because speciation is only relevant in broader temporal (*i.e.*, evolutionary scale) and spatial scales (*i.e.*, biogeographic scale), here we focus on how niche selection, drift, and dispersal can shape communities in altered landscapes.

Both niche selection and drift operate at a local scale, that is, at the community scale. Niche selection corresponds to deterministic processes favoring or disfavoring species with specific traits under different environmental pressures, such as the presence of a local abiotic filter, a better competitor, or a predator (Vellend 2016). The ultimate consequence of niche selection is the increase or decrease in the abundance of species with attributes that confer them an advantage or disadvantage in the local habitat. Ecological drift, by contrast, are stochastic demographic processes of birth and death (Vellend 2016). For instance, if a community is completely isolated from others and species have the same niche requirements and fitness, drift will be the only process driving community structure, ultimately leading all species, but one, to local extinction (Hubbell 2001; Vellend 2016).

The intensities of both drift and niche selection can be regulated by dispersal (Leibold & Chase 2018). If all species in a community have high dispersal abilities, populations of species with locally negative growth rates can be supplemented by dispersal, thus overriding the effects of both selection and drift (Pulliam 1988; Leibold *et al.* 2004; Leibold & Chase 2018). However, if species dispersal rates are uniformly low, even communities that undergo similar niche selection pressures are predicted to have different structures due to ecological drift (Vellend 2016; Leibold & Chase 2018) and possibly historical contingency (*i.e.*, priority effects; Fukami 2015). Recently, interspecific variation in dispersal rates has been incorporated in predictions of metacommunity dynamics (Vellend *et al.* 2014; Guzman *et al.* 2019). For instance, if species within a metacommunity have high variation in dispersal rates, community structure might be determined neither by drift nor local niche selection. Rather, it will be deterministically determined by dispersal abilities, where species with higher dispersal rates dominate communities (Vellend *et al.* 2014). However, this is also dependent on possible correlations between niche selection and dispersal. If species with higher dispersal rates are also favored by local niche selection, spatial isolation will potentialize the consequences of selection (Vellend *et al.* 2014). But, if niche selection is negatively correlated to species dispersal rates, such as in competition-colonization trade-offs (Yu & Wilson 2001; Leibold *et al.* 2004), niche selection might compensate the deterministic effects of different dispersal rates (Vellend *et al.* 2014).

In the context of land-use change, the above framework would mean that the same local environmental change can have different consequences in communities that are inserted in different spatial contexts. It could also mean that different spatial contexts might affect communities differently, depending on the different environmental pressures that they are subjected to. Therefore, in this Thesis, we aimed at understanding how the introduction of exotic predatory fish for aquaculture, and contamination by agrochemicals, can affect macroinvertebrate and amphibian community structure in different spatial contexts.

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Chapter 1

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

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.

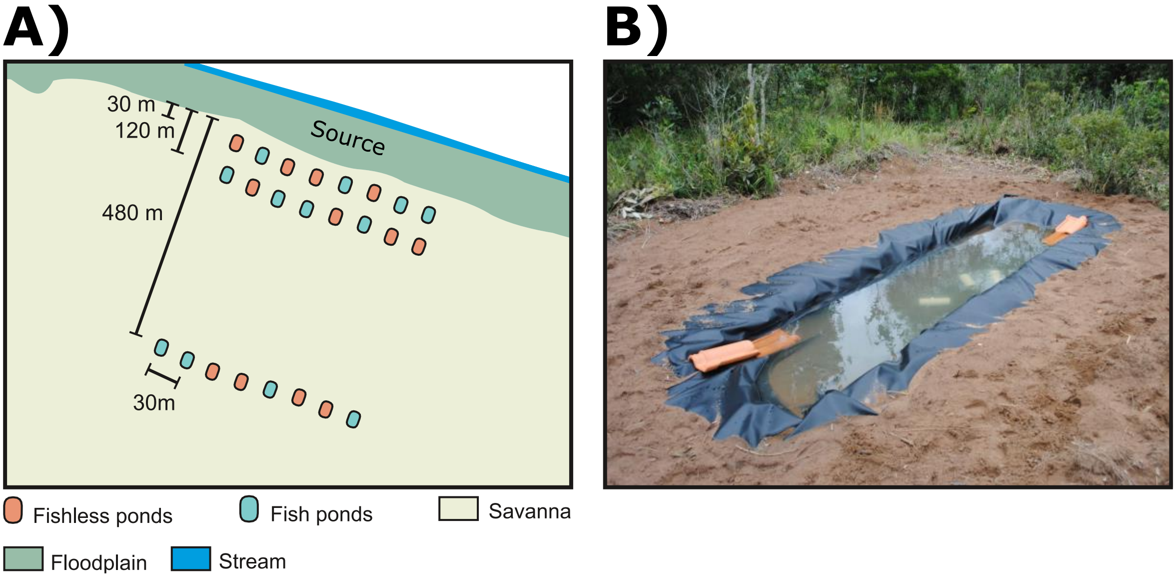


Figure. 1. A. Experimental setup. B. One of the ponds during the experiment.

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

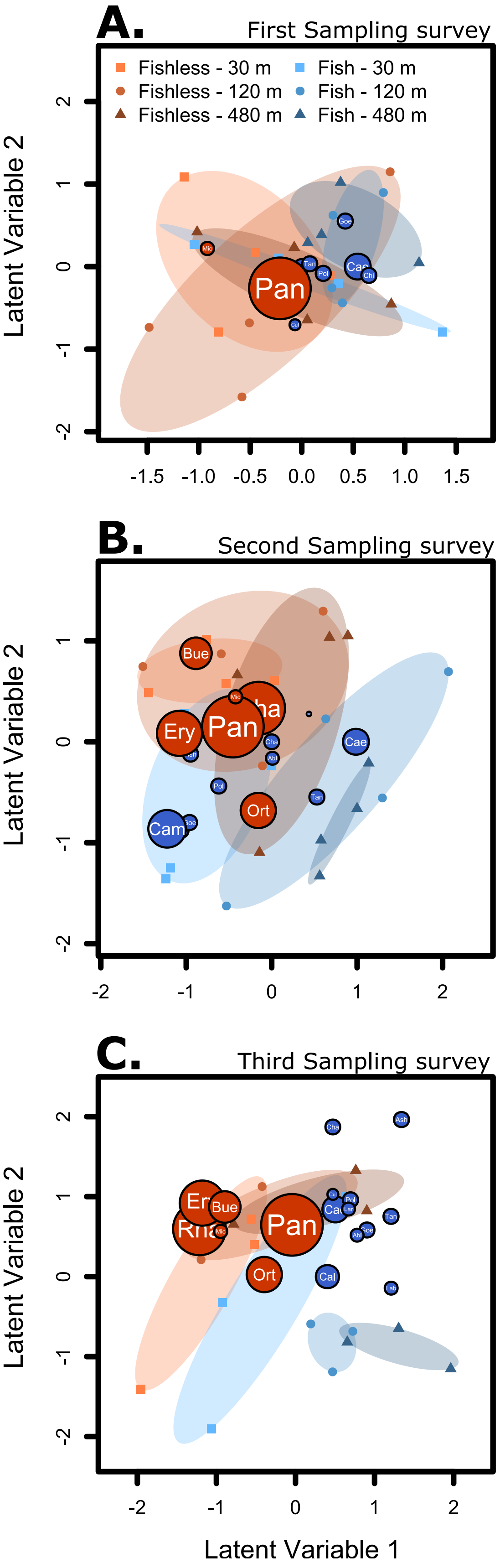
Mesocosms were colonized by aquatic and semiaquatic insects comprising five orders and 17 families (Odonata: Libellulidae, Coenagrionidae; Coleoptera: Dytiscidae, Hydrophilidae, Noteridae; Diptera: Ceratopogonidae, Chaoboridae, Chironomidae, Culicidae; Ephemeroptera: Baetidae, Caenidae, Polymitarcyidae; 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).

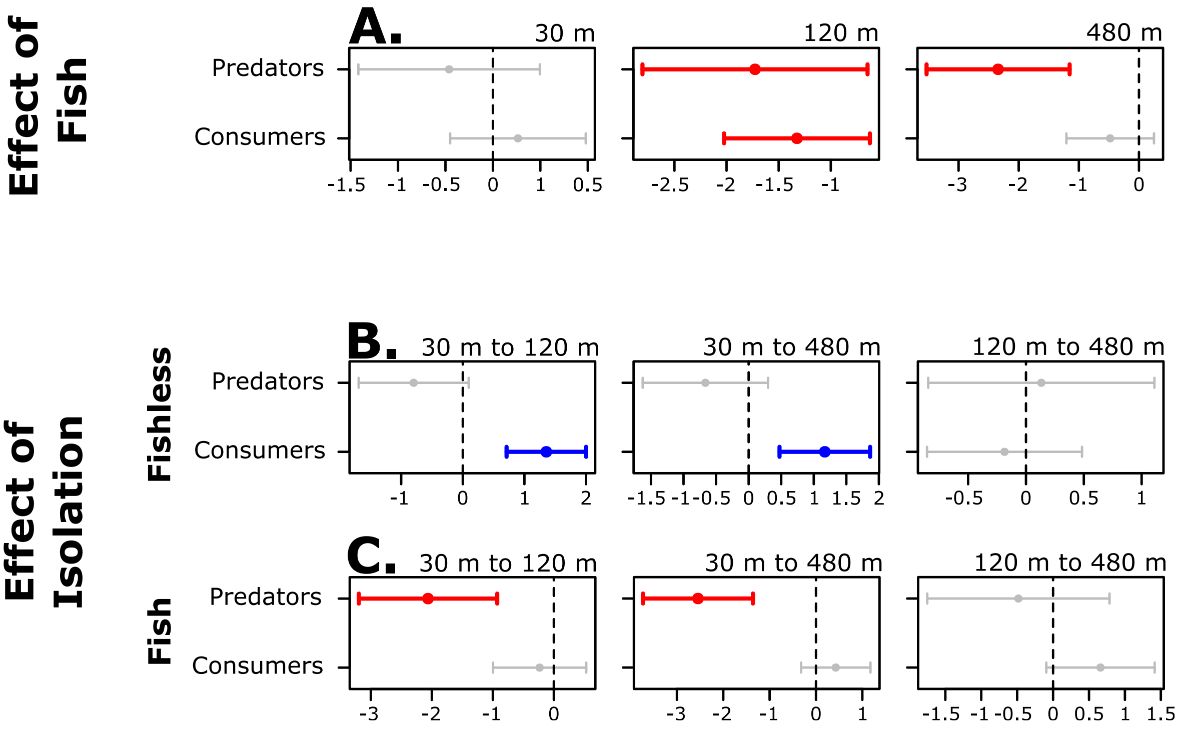


Figure 3. 95% Confidence intervals for the maximum likelihood estimates (MLE) of the effect of fish and isolation on abundance of predators and consumers when comparing pairs of treatments for the last survey. Confidence intervals not crossing the zero hatched line were considered significant effects 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.

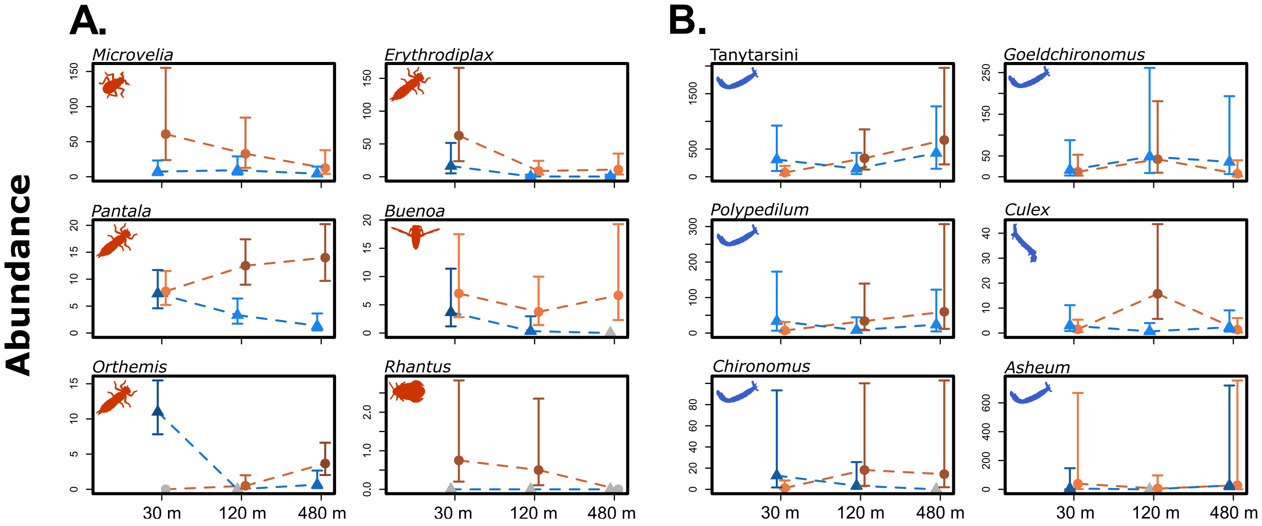


Figure 4. Maximum likelihood estimates (MLE) of abundance and their 95% confidence interval (for Model 16 in Table 1) of abundance for the six most abundant predatory taxa (A), and six most abundant consumer taxa (B), in the last sampling survey. Gray symbols indicate absolute absence (zero abundance) 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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Appendix - Chapter 1

SUPPLEMENT 1

Table S1.1. Identity code treatments assigned to each pond of the experiment.

Pond ID	Fish Treatment	Isolation Treatment
A1	present	30 m
A2	present	30 m
A3	absent	30 m
A4	present	30 m
A5	absent	30 m
A6	absent	30 m
A7	present	30 m
A8	absent	30 m
B1	absent	120 m
B2	absent	120 m
B3	present	120 m
B4	present	120 m
B5	absent	120 m
B6	present	120 m
B7	absent	120 m
B8	present	120 m
C1	present	480 m
C2	absent	480 m
C3	absent	480 m
C4	present	480 m
C5	absent	480 m
C6	absent	480 m
C7	present	480 m
C8	present	480 m

SUPPLEMENT 2

Table S2.1. Pilot laboratory experiment in which we offered four individuals of a variety of vertebrate and invertebrate prey common at our study site to eight different individuals of Redbreast Tilapia.

Date	**Average room temperature**	**Fish ID**	**Taxa**	**Number of individuals left after:**					
				0 (min)	**30 (min)**	**60 (min)**	**120 (min)**	**240 (min)**	**1440 (min)**
09/jan/2017	26.7	1	*Phalloceros* sp.	4	4	4	4	4	4
10/jan/2017	27.4	1	Zigoptera	4	2	0	0	0	0
11/jan/2017	29.2	1	Nepidae	4	3	2 and 1*	2 and 1*	2 and 1*	2 and 1*
09/jan/2017	26.7	2	*Scinax* sp.	4	0	0	0	0	0
10/jan/2017	27.4	2	Anisoptera	4	0	0	0	0	0
11/jan/2017	29.2	2	*Phalloceros* sp.	4	4	4	3	3	0
12/jan/2017	29.2	2	Nepidae (Large)	4	4	4	4	4	4
13/jan/2017	-	2	*Aedes*	4	0	0	0	0	0
09/jan/2017	26.7	3	Anisoptera	4	4	4	0	0	0
10/jan/2017	27.4	3	Nepidae (Large)	4	3	2 and 1*	2 and 1*	2 and 1*	1
11/jan/2017	29.2	3	*Phalloceros* sp.	4	4	4	4	4	0
12/jan/2017	29.2	3	*Scinax* sp.	4	0	0	0	0	0
13/jan/2017	-	3	Belostomatidae	4	0	0	0	0	0
09/jan/2017	26.7	4	*Scinax* sp.	4	0	0	0	0	0
10/jan/2017	27.4	4	*Phalloceros* sp.	4	0	0	0	0	0
11/jan/2017	29.2	4	Anisoptera	4	0	0	0	0	0
12/jan/2017	29.2	4	Zigoptera	4	0	0	0	0	0
09/jan/2017	26.7	5	Nepidae (Small)	4	0	0	0	0	0
10/jan/2017	27.4	5	Beetle	4	4	4	4	4	4
11/jan/2017	29.2	5	*Scinax* sp.	4	0	0	0	0	0

12/jan/2017	29.2	5	Anisoptera	4	0	0	0	0	0
13/jan/2017	-	5	*Phalloceros* sp.	4	4	4	4	0	0
09/jan/2017	26.7	6	Zigoptera	4	0	0	0	0	0
10/jan/2017	27.4	6	*Phalloceros* sp.	4	4	4	4	4	0
11/jan/2017	29.2	6	Anisoptera	4	0	0	0	0	0
09/jan/2017	26.7	7	*Phalloceros* sp.	4	4	4	4	4	0
10/jan/2017	27.4	7	Nepidae (Large)	4	4	4	4	4	3*
11/jan/2017	29.2	7	Anisoptera	4	0	0	0	0	0
09/jan/2017	26.7	8	Anisoptera	4	0	0	0	0	0
10/jan/2017	27.4	8	Nepidae (Small)	4	0	0	0	0	0
11/jan/2017	29.2	8	Zigoptera	4	0	0	0	0	0
12/jan/2017	29.2	8	*Phalloceros* sp.	4	3	3	2	0	0

*Numbers with an asterisk symbol represent half of an individual, meaning that the other half was eaten by the fish.

SUPPLEMENT 3

Unfortunately, we lost samples from ponds A4 (30m, fish present), B3 (120m, fish present), C3 (480m, fish absent) and C4 (480m, fish present) from the third sampling survey. Therefore, we had to exclude samples from the same ponds for the first and second sampling surveys when we analyzed all three surveys together (*i.e.* Models 1 to 5 from Table 1). This was necessary to achieve a balanced design among ponds for the permutation design (*i.e.* 3 replicates per block). Similarly, we had to randomly exclude two ponds (one fishless replicate from the 30 m isolation treatment, and one fishless pond from the 120 m isolation treatment) from the third sampling survey when we tested for differences in distances between centroids of fish and fishless treatments in different isolation treatments (Table 2). We did that, again, to achieve a balanced design, necessary to equally permute ponds among distances, keeping the fish treatment constant. In this case ponds A3 and B7 were randomly selected to be excluded from this specific analysis.

SUPPLEMENT 4

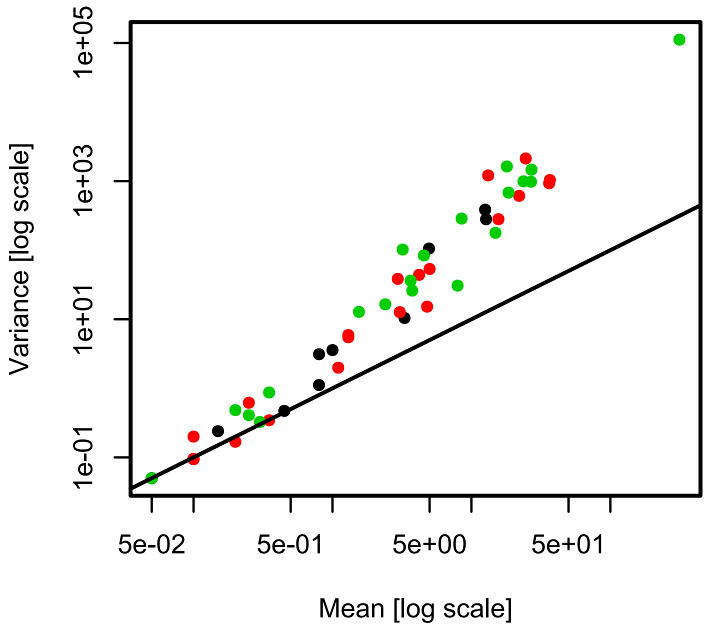

Figure S4.1. Mean-Variance relationship of our abundance data for the entire experiment. Each dot is one taxon in one sampling survey. Different colors represent different sampling surveys.

SUPPLEMENT 5

Table S5.1. Abundance and traits (trophic level, maximum recorded body volume in experiment) of taxa colonizing experimental ponds.

Order	Family	Subfamily	Taxa	Abbreviation	Volume of largest individual (mm^3)	Total Abundance	Trophic Level	Reference to Trophic Level
Coleoptera	Dytiscidae		*Rhantus*	Rha	387.52	14	Predator	Ramírez & Gutiérrez-Fonseca 2014
Coleoptera	Dytiscidae		*Thermonectus*	The	0.51	3	Predator	Ramírez & Gutiérrez-Fonseca 2014
Coleoptera	Dytiscidae		*Derovatellus*	Der	2.60	2	Predator	Ramírez & Gutiérrez-Fonseca 2014
Coleoptera	Dytiscidae		*Hydaticus*	Hda	210.15	1	Predator	Ramírez & Gutiérrez-Fonseca 2014
Coleoptera	Hydrophilidae		*Berosus*	Ber	5.02	4	Predator	Ramírez & Gutiérrez-Fonseca 2014
Coleoptera	Hydrophilidae		*Tropisternus*	Tro	231.38	2	Predator	Ramírez & Gutiérrez-Fonseca 2014
Coleoptera	Noteridae		*Hydrocanthus*	Hdr	3.14	1	Predator	Ramírez & Gutiérrez-Fonseca 2014
Diptera	Ceratopogonidae		Ceratopogonidae	Cer	0.65	6	Consumer**	Aussel & Linley 1994; Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chaoboridae		*Chaoborus*	Cha	4.29	68	Consumer**	ARCIFA 2000; Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Chironominae	Tanytarsini	Tan	4.44*	7214	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Chironominae	*Goeldchironomus*	Goe	4.44*	1455	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Chironominae	*Polypedilum*	Pol	4.44*	1436	Consumer	Ramírez & Gutiérrez-Fonseca 2014

Diptera	Chironomidae	Chironominae	*Chironomus*	Chi	4.44*	534	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Chironominae	*Asheum*	Ash	4.44*	399	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Chironominae	*Caladomyia*	Cla	4.44*	38	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Chironominae	*Apedilum*	Ape	4.44*	4	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Chironominae	*Beardius*	Bea	4.44*	3	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Chironominae	*Parachironomus*	Par	4.44*	3	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Tanytarsinae	*Ablabesmyia*	Abl	3.28	363	Consumer**	Henriques-Oliveira *et al.* 2003; Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Tanytarsinae	*Larsia*	Lar	3.28	100	Consumer**	Henriques-Oliveira *et al.* 2003; Ramírez & Gutiérrez-Fonseca 2014
Diptera	Chironomidae	Tanytarsinae	*Labrundinia*	Lab	3.28	9	Consumer**	Henriques-Oliveira *et al.* 2003; Ramírez & Gutiérrez-Fonseca 2014
Diptera	Culicidae		*Culex*	Cul	2.42	707	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Ephemeroptera	Baetidae		*Callibaetis*	Cal	12.56	14	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Ephemeroptera	Caenidae		*Caenis*	Cae	17.53	155	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Ephemeroptera	Polymitarcyidae		*Campsurus*	Cam	68.81	10	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Hemiptera	Corixidae		*Tenagobia*	Ten	1.72	3	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Hemiptera	Corixidae		*Heterocorixa*	Het	16.73	1	Consumer	Ramírez & Gutiérrez-Fonseca 2014
Hemiptera	Gerridae		*Rheumatobates*	Rhe	38.00	1	Predator	Ramírez & Gutiérrez-Fonseca 2014

Hemiptera	Naucoridae	*Ctenipocoris*	Cte	27.42	1	Predator	Ramírez & Gutiérrez-Fonseca 2014
Hemiptera	Notonectidae	*Buenoa*	Bue	34.43	139	Predator	Ramírez & Gutiérrez-Fonseca 2014
Hemiptera	Notonectidae	*Notonecta*	Not	118.11	6	Predator	Ramírez & Gutiérrez-Fonseca 2014
Hemiptera	Veliidae	*Microvelia*	Mic	3.44	1097	Predator	Ramírez & Gutiérrez-Fonseca 2014
Odonata	Coenagrionidae	*Oxyagrion*	Oxy	8.74	5	Predator	Ramírez & Gutiérrez-Fonseca 2014
Odonata	Libellulidae	*Erythrodiplax*	Ery	163.48	465	Predator	Ramírez & Gutiérrez-Fonseca 2014
Odonata	Libellulidae	*Pantala*	Pan	1054.38	349	Predator	Ramírez & Gutiérrez-Fonseca 2014
Odonata	Libellulidae	*Orthemis*	Ort	52.50	87	Predator	Ramírez & Gutiérrez-Fonseca 2014

*We used the same volume value for all Chironominae (4.44) and Tanytarsinae taxa (3.28).

**Insects that also have some predatory behavior but mostly consume plankton (either phytoplankton or zooplankton) or only small amounts or parts of the other organisms considered in our study.

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SUPPLEMENT 6

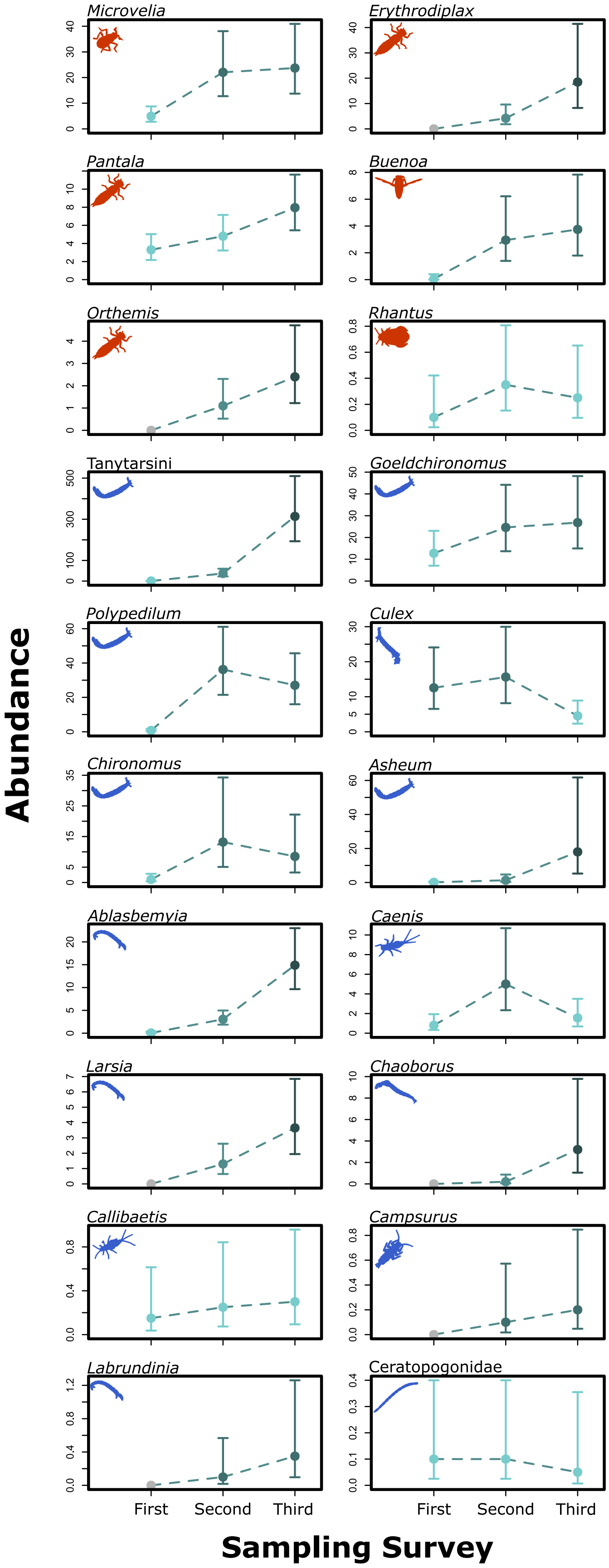

Figure S6.1. Effect of time on species abundance according to maximum likelihood estimates of abundance and their 95% confidence interval for Model 1 in Table 1. Grey symbols indicate absolute absence (zero abundance) of a taxon in a treatment. Maximum likelihood estimates (dots) that are not contained inside the 95% confidence interval of other estimates were considered to be different. More information about the estimated effects are provided in supplementary material 7.

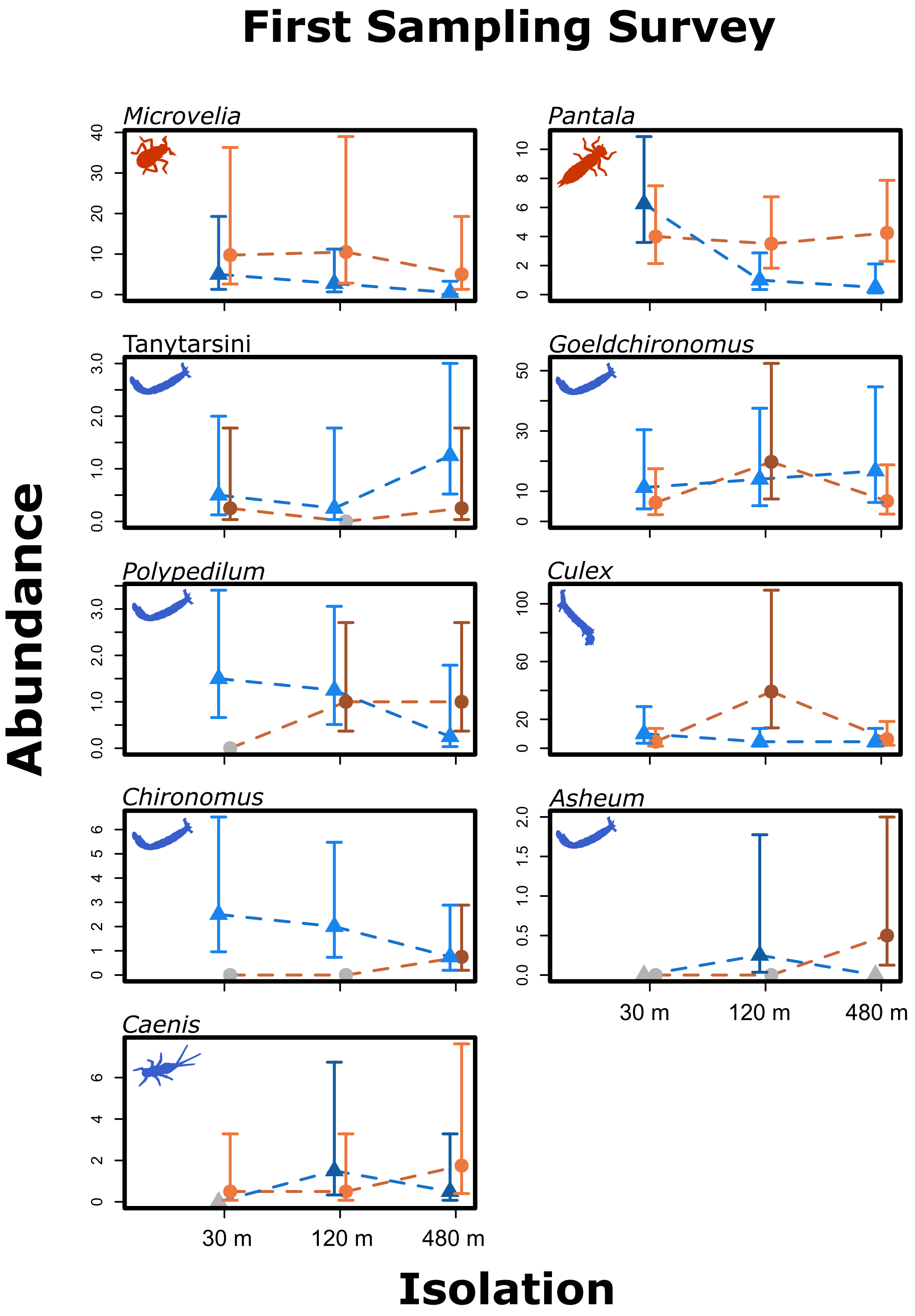

Figure S6.2. Effect of treatments on species abundance in the first sampling survey according to maximum likelihood estimates (MLE) of abundance and their 95% confidence interval for Model 8 in Table 1. Grey symbols represent absolute absence (zero abundance) of a taxon in a treatment. Blue triangles 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 Supplementary material 7.

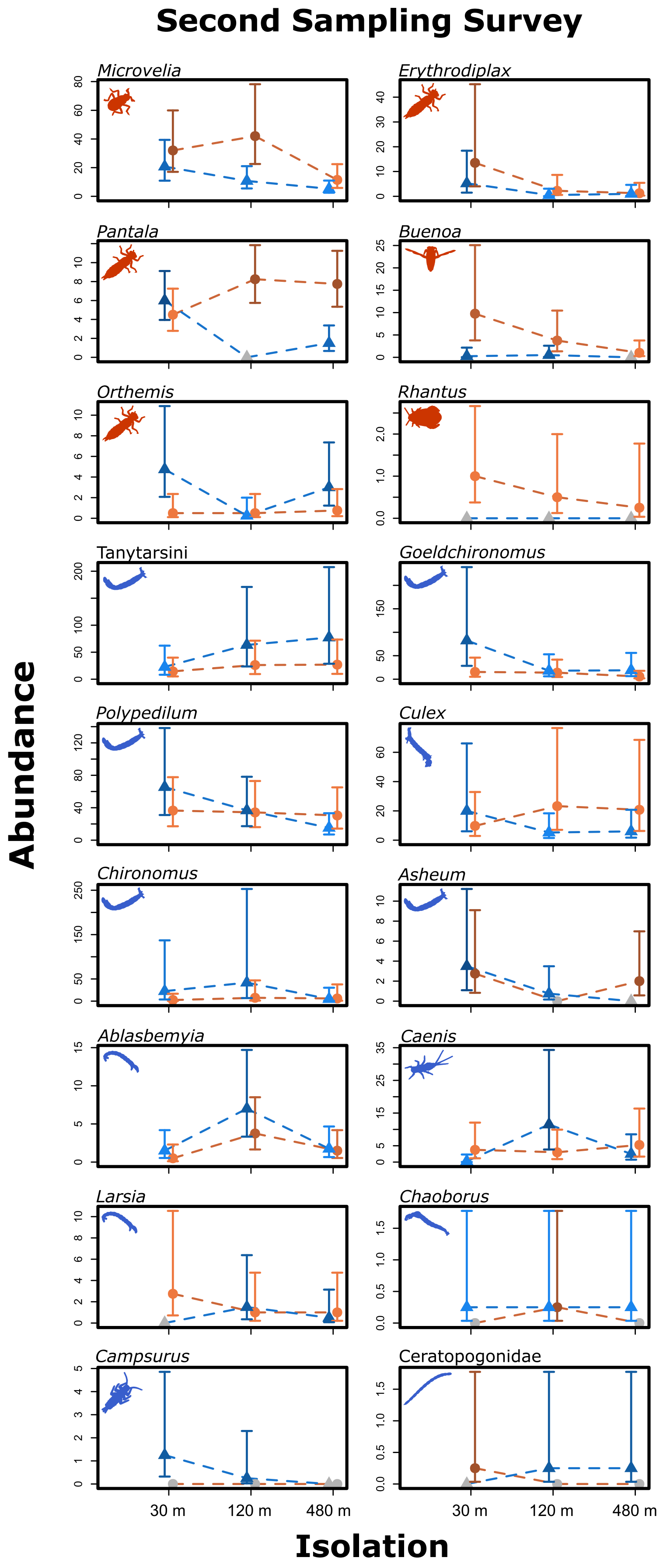

Figure S6.3. Effect of treatments on species abundance in the first sampling survey according to maximum likelihood estimates (MLE) of abundance and their 95% confidence interval for Model 11 in Table 1. Grey symbols represent absolute absence (zero abundance) of a taxon in a treatment. Blue triangles 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 Supplementary material 7.

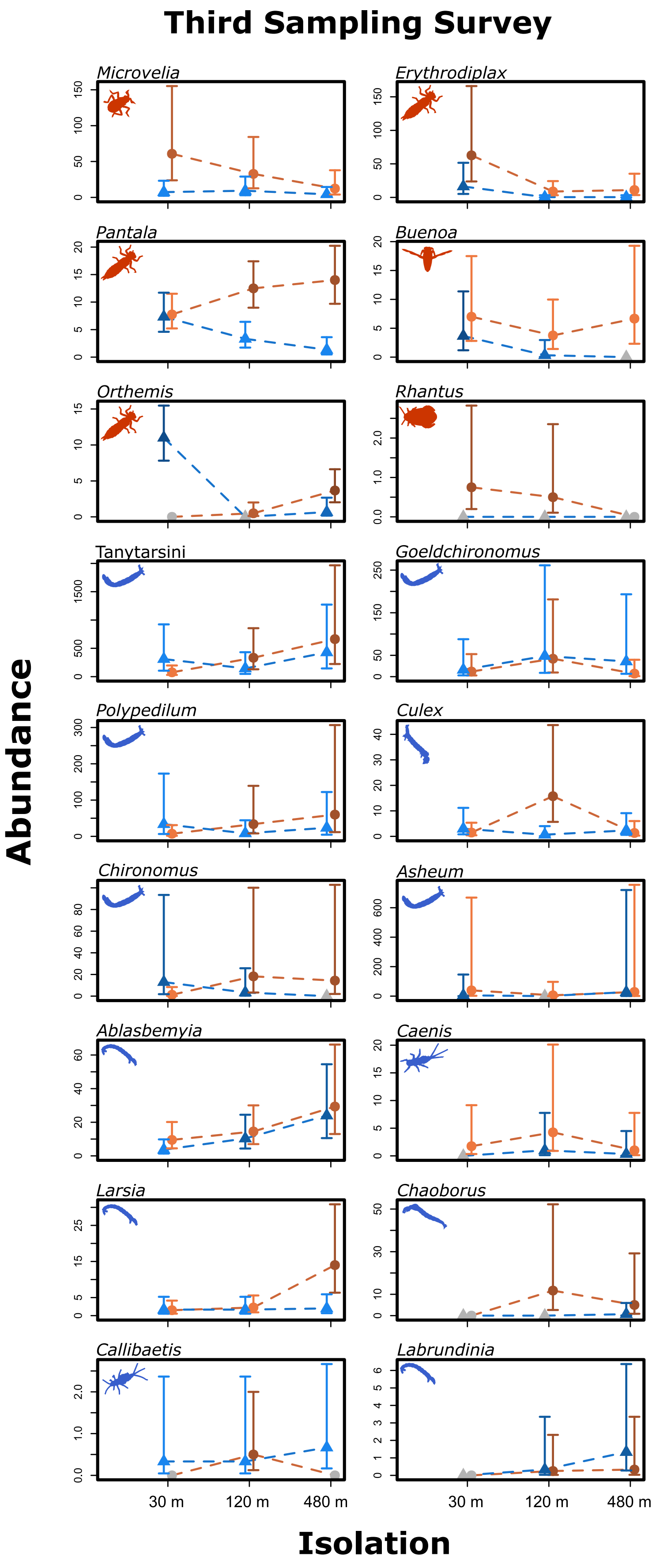

Figure S6.4. Effect of treatments on species abundance in the first sampling survey according to maximum likelihood estimates (MLE) of abundance and their 95% confidence interval for Model 15 in Table 1. Grey symbols represent absolute absence (zero abundance) of a taxon in a treatment. Blue triangles 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 Supplementary material 7.

SUPPLEMENT 7

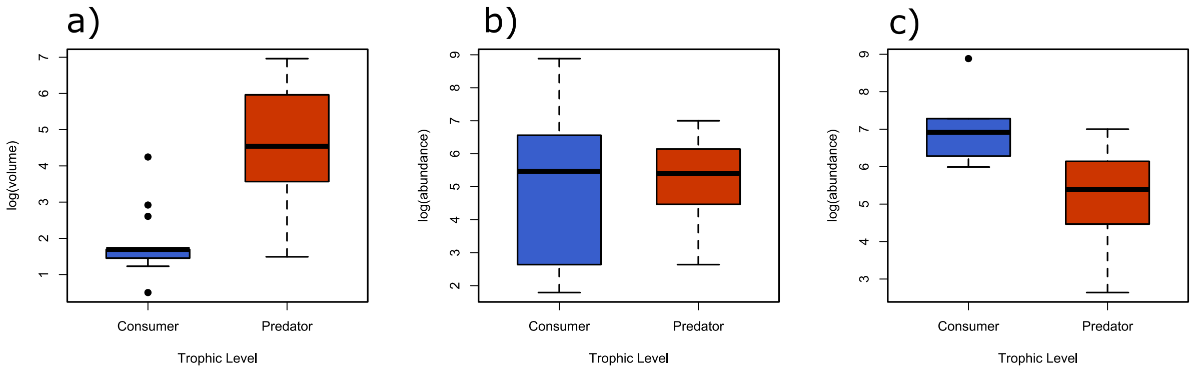

Figure S7.1. a) Volume of the largest individual sampled in the entire experiment by trophic level (permutational ANOVA, SS: 29.570, Df: 1; p < 0.001). b) Total abundance of each taxa by trophic level (permutational ANOVA, SS: 0.046, Df: 1; p: 1). c) Total abundance of the six most abundant taxa from each trophic level, by trophic level (permutational ANOVA, SS: 10.509, Df: 1; p: 0.005).

SUPPLEMENT 8

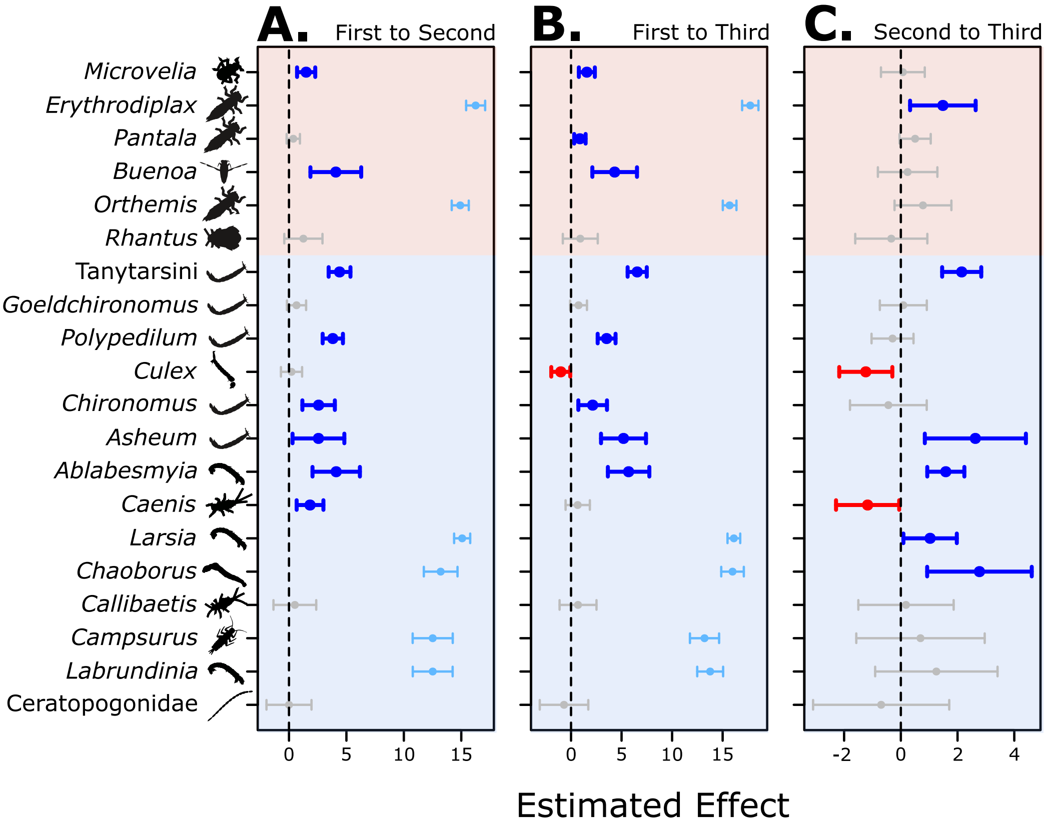

Figure S8.1. Confidence intervals for the effect of time on abundance for each taxon estimated from Model 1 in Table 1. Taxa are ordered from most (top) to less abundant (bottom) predators (red background) and most to less abundant consumer (blue background). Bars which the 95% confidence interval does not cross the zero-line are colored. Blue bars mean an increase in abundance from the reference treatment to the other. Red bars mean a decrease in abundance from the reference treatment to the other. Lighter blue bars mean that a taxon was absent from the reference treatment. A – Effect of moving from the first to the second sampling survey; B – Effect of moving from the first to the third sampling survey; C – Effect of moving from the second to the third sampling survey.

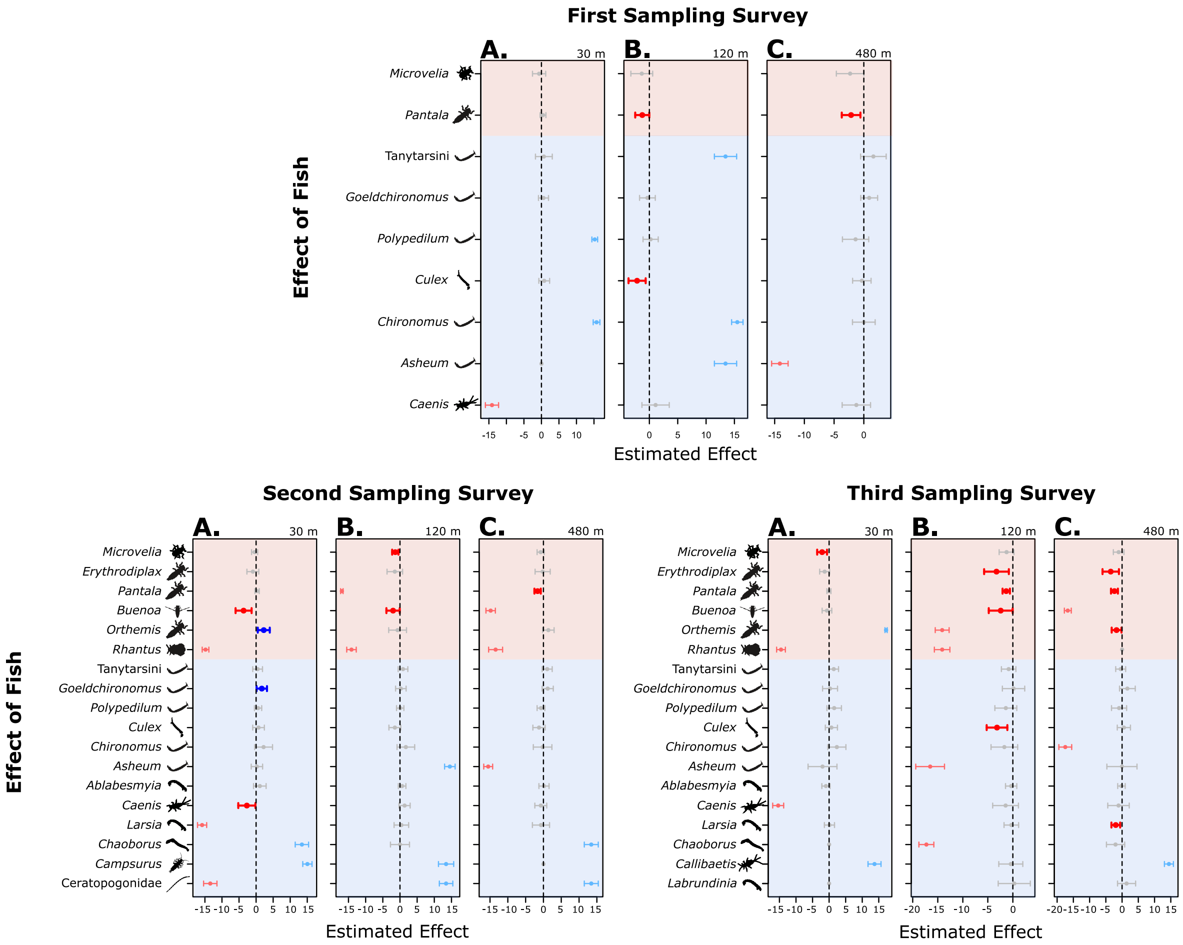

Figure S8.2. Confidence intervals for the effect of fish on abundance for each taxon, predators and consumers estimated from Models 8, 11 and 15 in Table 1. Taxa are ordered from most (top) to less abundant (bottom) predators (red background) and most to less abundant consumer (blue background). Bars which the 95% confidence interval does not cross the zero-line are colored. Blue bars mean an increase in abundance from the reference treatment to the other. Red bars mean a decrease in abundance from the reference treatment to the other. Lighter blue bars mean that a taxon was absent from the reference treatment. Lighter red bars mean that a taxon was absent from the treatment which the reference are being compared to. A,B and C are effects for the first sampling survey, D, E and F are for the second and G, H and I are for the third. A, D and G are effects of the presence of fish in low isolation; B, E and H are effects of the presence of fish in moderate isolation; C, F and I are effects of the presence of fish in high isolation.

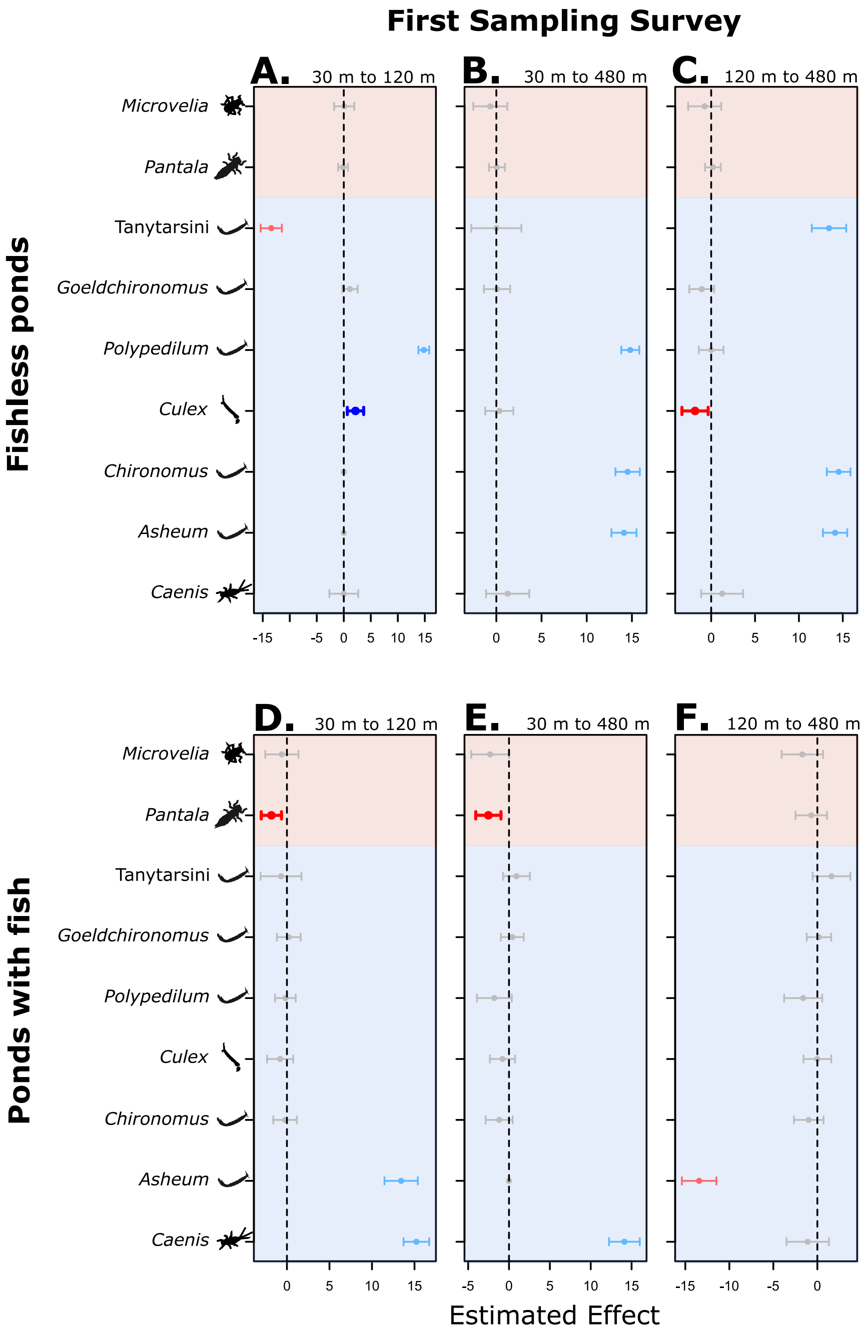

Figure S8.3. Confidence intervals for the effect of isolation on abundance for each taxon in the first sampling survey estimated from Model 8 in Table 1. Taxa are ordered from most (top) to less abundant (bottom) predators (red background) and most to less abundant consumer (blue background). Bars which the 95% confidence interval does not cross the zero-line are colored. Blue bars mean an increase in abundance from the reference treatment to the other. Red bars mean a decrease in abundance from the reference treatment to the other. Lighter blue bars mean that a taxon was absent from the reference treatment. Lighter red bars mean that a taxon was absent from the treatment which the reference is being compared to. A to C are effects for fishless ponds and D to F are for ponds with fish. A and D are effects of increasing isolation from 30 to 120 m; B and E are effects of increasing from 30 to 480 m. C and F are effects of increasing from 120 m to 480 m.

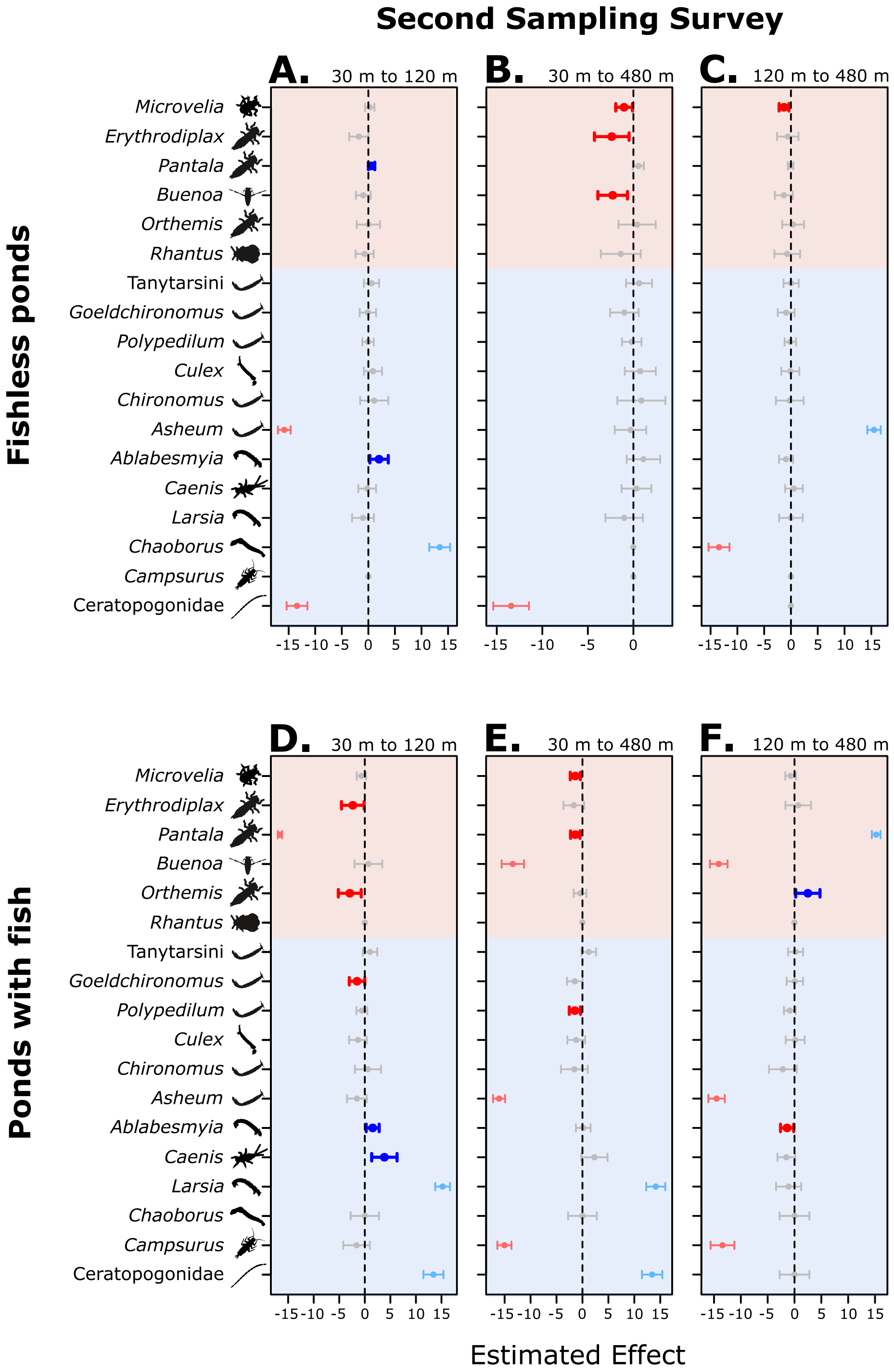

Figure S8.4. Confidence intervals for the effect of isolation on abundance for each taxon, predators and consumers in the second sampling survey estimated from Model 11 in Table 1. Taxa are ordered from most (top) to less abundant (bottom) predators (red background) and most to less abundant consumer (blue background). Bars which the 95% confidence interval does not cross the zero-line are colored. Blue bars mean an increase in abundance from the reference treatment to the other. Red bars mean a decrease in abundance from the reference treatment to the other. Lighter blue bars mean that a taxon was absent from the reference treatment. Lighter red bars mean that a taxon was absent from the treatment which the reference is being compared to. A to C are effects for fishless ponds and D to F are for ponds with fish. A and D are effects of increasing isolation from 30 to 120 m; B and E are effects of increasing from 30 to 480 m. C and F are effects of increasing from 120 m to 480 m.

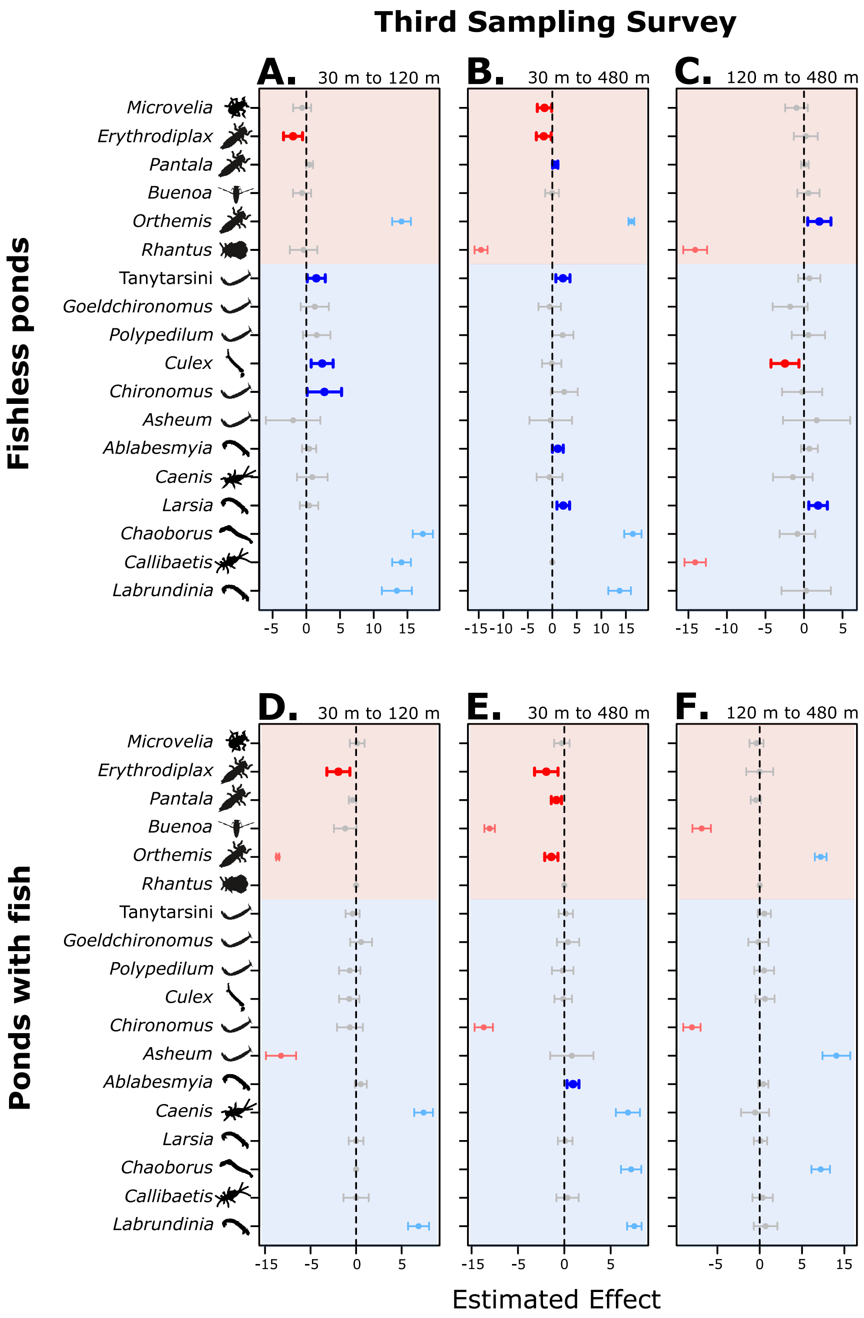

Figure S8.5. Confidence intervals for the effect of isolation on abundance for each taxon, predators and consumers in the second sampling survey estimated from Model 15 in Table 1. Taxa are ordered from most (top) to less abundant (bottom) predators (red background) and most to less abundant consumer (blue background). Bars which the 95% confidence interval does not cross the zero-line are colored. Blue bars mean an increase in abundance from the reference treatment to the other. Red bars mean a decrease in abundance from the reference treatment to the other. Lighter blue bars mean that a taxon was absent from the reference treatment. Lighter red bars mean that a taxon was absent from the treatment which the reference is being compared to. A to C are effects for fishless ponds and D to F are for ponds with fish. A and D are effects of increasing isolation from 30 to 120 m; B and E are effects of increasing from 30 to 480 m. C and F are effects of increasing from 120 m to 480 m.

SUPPLEMENT 9

Table S9.1. Increase (positive values) or decrease (negative values) in the effect of fish from one level of isolation to another measured by the difference in distance between the centroids of each treatment in a model-based unconstrained ordination. Bold lines represent significative increase or decrease in distance values. P values were adjusted for false discovery ratio.

	difference in effect of fish	**p value**	**adj. p value**
1st Sampling Survey			
30 m to 120 m	0.410588	0.5301	0.8565
30 m to 480 m	0.040839	0.949	0.949
120 m to 480 m	-0.369749	0.571	0.8565
2nd Sampling Survey			
30 m to 120 m	0.27635	0.6525	0.9798
30 m to 480 m	0.003335	0.996	0.996
120 m to 480 m	-0.273015	0.6532	0.9798
3rd Sampling Survey			
30 m to 120 m	1.251939	0.0654	0.0981
30 m to 480 m	**1.628767**	**0.0126**	**0.0378**
120 m to 480 m	0.376828	0.6011	0.6011

SUPPLEMENT 10

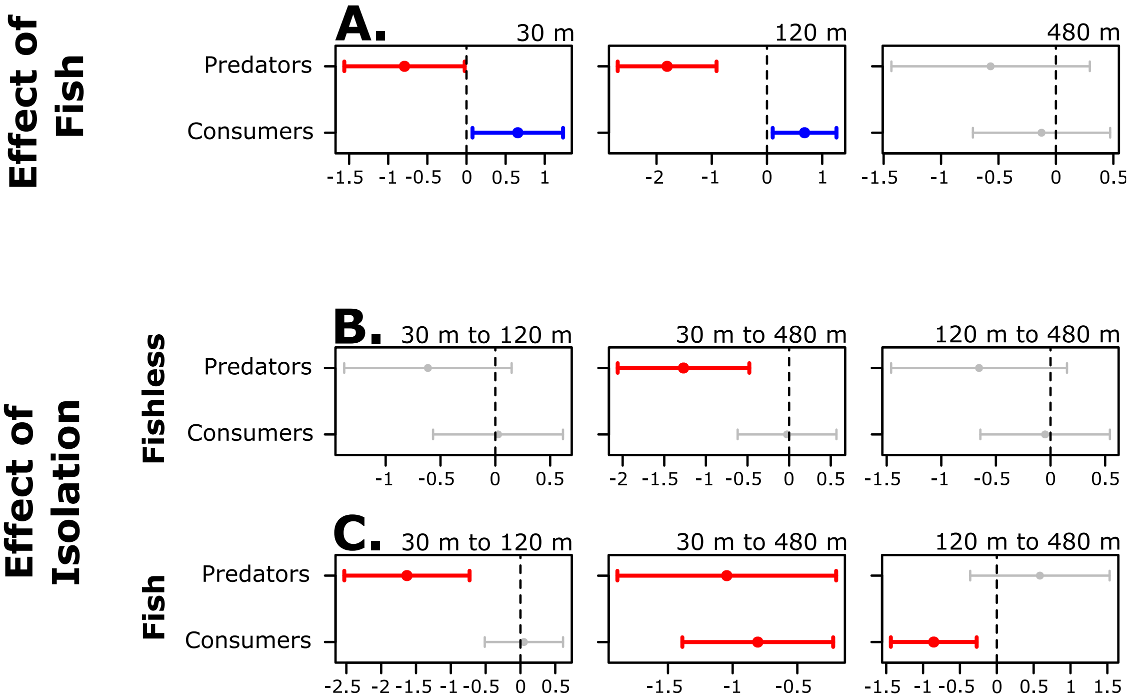

Figure S10.1. 95% Confidence intervals for the effect of fish and isolation on abundance of predators and consumers when comparing pairs of treatments for the second survey of the experiment. Confidence intervals not crossing the zero hatched line were considered significant effects and colored; blue 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 each of the 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.

Chapter 2

Assembly and reassembly of freshwater communities under the influence of agrochemicals in different spatial contexts

ABSTRACT

Many lentic aquatic environments are found embedded in agricultural fields, forming complex metacommunity structures. These habitats are vulnerable to contamination by agrochemicals, which can differently affect local communities, depending on the intensity and variability of species dispersal rates. We conducted a field experiment to assess how agrochemical intensification simulating the conversion of savannas into managed pastures and sugarcane fields affects freshwater community structure at different levels of spatial isolation. We constructed forty-five 1,200-L artificial ponds in a savanna landscape at three different distances from a source wetland (30 m, 120 m, and 480 m). Ponds were spontaneously colonized by semiaquatic insects and amphibians and treated with no agrochemicals (control), fertilizers ('pasture' treatment), or fertilizers and a single pulse of the insecticide fipronil and the herbicide 2,4-D ('sugar cane' treatment) following realistic dosages and application schedules. 'Pasture' communities were not generally different from controls; however, two predatory insects were more abundant in 'pasture' ponds. 'Sugarcane' communities largely diverged from other treatments after insecticide application, which decreased insect abundance, indirectly benefiting amphibian abundance. However, this effect nearly disappeared by the end of the rainy season. The herbicide pulse had no effect on community structure. Spatial isolation changed community structure by increasing the abundance of consumers in the peak of the rainy season due to trophic cascades. However, it did not affect all predatory insects and, surprisingly, amphibians. Thus, spatial isolation did not change the effects of agrochemicals, likely it mostly affected species that were not dispersal limited. Because agrochemical application frequently overlaps with the rainy season in many monocultures, it can strongly affect temporary pond communities. Ponds embedded in pastures might suffer mild consequences of fertilization by favoring the abundance of few predators through *bottom-up* effects. Ponds in sugarcane fields, however, might experience a decline in insect population, followed by an increase in abundance of

amphibians tolerant to environmental degradation. Furthermore, we found no evidence that isolation by distance can change the general effects of chemical intensification. However, this might not be true in real crop fields since they can represent dispersal barriers.

INTRODUCTION

Since the so-called Green Revolution, one of the cornerstones of industrial agriculture is the management of agrochemicals such as pesticides, fertilizers, and soil amendment products (Foley *et al.* 2005, Schiesari & Grillitsch 2011, Schiesari *et al.* 2013). These agrochemicals are applied in crop fields, alone or in combinations, usually following specific schedules along the crop cycle (*e.g.*, Schiesari *et al.* 2013), being able to reach aquatic habitats by direct overspray, drift, and runoff (Matson et al. 1997, Carvalho 2017). Despite the evident ecological importance of the seasonal and predictable release of these molecules designed to have biological effects, until recently, ecotoxicological studies were mostly devoid of ecological realism by emphasizing effects on individuals and populations instead of communities and ecosystems (Relyea *et al.* 2005; Rohr *et al.* 2006). Following this diagnosis, a relatively large number of studies mechanistically addressing direct and indirect effects of contaminants in communities were conducted (*e.g.*, Boone & James 2003, Relyea 2005a, 2012, Relyea & Jones 2009), while the importance of spatial context in modulating the effects of contaminants remains largely unexplored (Schiesari *et al.* 2017, Schiesari *et al.* 2019; but see Trekels *et al.* 2011). Yet, spatial structure is the norm in both natural and modified environments. For instance, many lentic aquatic environments can be found embedded in agricultural fields, forming complex metapopulations and metacommunity structures (da Silva *et al.* 2012; Prado & Rossa-Feres 2014; Schiesari & Corrêa 2016). Therefore, the consequences of agrochemicals in metacommunities must be understood if we are to manage biodiversity in agricultural landscapes successfully.

Chemical intensification can affect aquatic communities via two main pathways: First, it can affect primary productivity due to the use of organic and inorganic fertilizers and herbicides (Peterson *et al.* 1994; Smith *et al.* 1999; Rohr *et al.* 2006; Isbell *et al.* 2013). Second, insecticides can strongly negatively affect most aquatic insects, while indirectly benefiting non-insect taxa (Relyea *et al.* 2005; Rohr *et al.* 2006). The use of nitrogen-phosphorus-potassium (NPK) fertilizers is globally widespread (Foley *et al.* 2005), causing water nutrient enrichment, which leads to the increase of algal biomass and, consequently, increased primary productivity (Smith *et al*. 1999). Increased primary productivity can have several different consequences on higher trophic levels. For instance, it can increase competition, causing the abundance of consumers with a higher ability to deplete resources to increase (Abrams 1988). In the presence of higher trophic levels, however, such effects can propagate through the trophic web via *bottom-up* effects, benefiting predators (Abrams 1993, Slavik *et al.* 2004, Cross *et al*. 2006), which can then change consumer species composition by preferentially preying upon the most vulnerable prey (Leibold 1996, 1999). Herbicides, on the other hand, can have opposite effects of fertilizers. They are usually employed to control weeds in monocultures, but they can also affect non-target aquatic plants, such as green algae and macrophytes. It can lead to negative effects on aquatic primary productivity (Titeux *et al.* 2016), thus possibly reversing the positive effects of nutrient enrichment on higher trophic levels (Rohr *et al.* 2006). However, in a spatially structured landscape, both effects of nutrient enrichment and herbicides may not always be obvious since dispersal can either mitigate or strengthen them. For instance, in the presence of environmental heterogeneity, an increased flux of individuals (*i.e.*, mass effects) may prevent more vulnerable consumers from being excluded from nutrient-enriched habitats (Leibold *et al.* 2004; Leibold & Chase 2018). Similarly, if predators have lower dispersal rates than consumers, species composition may be less affected by predation in isolated habitats, thus changing the consequences of nutrient enrichment on consumers.

In turn, insecticides are predicted to have strong negative effects on higher trophic levels, such as zooplankton and aquatic insects, mostly affecting community structure via *top-down* effects (Rohr *et al.* 2006). The indirect effects will depend on the trophic level and competitive interactions among the species that are differentially vulnerable to the pesticide. If most vulnerable species are (top) predators then, consumers will be indirectly and positively affected by reduced predation, also negatively affecting producers (Rohr *et al.* 2006). Alternatively, if the most vulnerable species are consumers, insecticides can indirectly and negatively affect predators, but positively affect producers.

Because pesticides, including herbicides and insecticides, are usually applied in seasonal pulses, their direct acute effects can be, albeit strong, temporary. Therefore, their effects on communities can be understood as a disturbance, followed by full or partial recovery of the original community structure (*i.e.,* Trekels *et al.* 2011). This means that the indirect effects of pesticides could be highly dependent on spatial processes in a medium-to-long term. For instance, more isolated communities might take longer to recover from an insecticide pulse (Trekels *et al.* 2011). Also, if predators have lower dispersal rates and higher vulnerability to the insecticide than consumers, the indirect positive effects of the insecticide on consumers can last longer in more isolated habitats.

In this study we aimed at understanding the consequences of chemical intensification on pond macroinvertebrate and amphibian community structure in different spatial contexts. We constructed replicated artificial ponds at different distances from a source wetland and experimentally simulated a gradient of chemical intensification by treating artificial ponds as if they were embedded in savannas (*i.e.,* no agrochemical use), pastures (*i.e.,* use of fertilizer) and sugarcane fields (*i.e.,* use of fertilizer and pesticides). We chose to simulate pastures and sugar cane fields because they are two of the most abundant land uses in Brazil, the largest country in the neotropical region. Pastures occupy around 20% of the Brazilian territory (~173 million hectares; MapBiomas 2019), while sugarcane represents the third largest planted area in Brazil (~9 million hectares), only behind soybeans (~30 million hectares) and corn (~16 million hectares; IBGE 2017). More importantly, sugarcane is predicted to expand in the next years, mostly over pasture and savanna areas (MapBiomas 2019), because of the increasing demand for the replacement of fossil fuels for alternative biofuels (Titeux *et al.* 2016).

Freshwater insects and amphibians are known to have variable dispersal rates and sensitivity to agrochemicals across different trophic levels. Semiaquatic predatory insects have smaller populations and longer generation times than consumer insects; thus, they have fewer events of dispersal, requiring more connected patches to maintain viable populations (Shulman & Chase 2007, Chase & Shulman 2009, Hein & Gillooly 2011, Kalinkat *et al.* 2015). Amphibians, different from consumer insects, have limited dispersal ability due to high risk of dissection during terrestrial migration (Marsh *et al.* 1999; Marsh & Trenham 2001; Sinsch *et al.* 2012), and do not suffer acute effects of insecticide pulses. In fact, they can even be indirectly benefited by it (Relyea 2005a). Therefore, we believe that spatial processes may likely play an important role in regulating the consequences of agrochemical use on community structure. We hypothesized that: (H1.1) Fertilization should increase the abundance of predatory insects via bottom-up effects in ‘pasture’ communities, (H1.2) which can, in turn, shift consumer abundances towards a higher abundance of less vulnerable taxa. This effect (H1.3) should be weaker in more isolated habitats where most predatory insects may not be able to establish large populations, thus the importance of fertilizers in causing bottom-up effects may not be fully realized. (H2) The herbicide, on the other hand, should temporally reverse the effects of nutrient increase via a negative bottom-up effect in ‘sugarcane’ communities. (H3.1) We also expect that the insecticide will have the strongest consequences on freshwater communities from ‘sugarcane’ ponds, shifting community structure towards a higher abundance of amphibians due to a lower abundance of insects in general, and amphibian predators in particular. We further hypothesize that, (H3.2) because predatory insects are predicted to be more dispersal limited than consumers, the possible positive indirect effects of the insecticide pulse on consumers (*e.g.*, amphibians) should last longer in more isolated habitats. Finally, (H4) because pesticide pulses could be compared to short term disturbances, they should have strong, but temporary effects on community structure.

METHODS

Experimental design

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) (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 4m long, 1m wide and 40 cm deep. Walls were vertical along the length of the pond; 1m-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 (Figure 1B). Two roof tiles were placed at the waterline in each of the short sides to provide shelter and oviposition habitat for insects and amphibians.

The experiment followed a fully factorial design crossing agrochemical manipulation simulating increasingly intensified land uses (‘savanna´, i.e., the control, ‘pasture’ and ‘sugar cane’) with spatial isolation (three levels of isolation). The isolation treatment was achieved by establishing 15 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 (Figure 1A). 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 agrochemical-by-distance treatment was replicated five times for a total of 45 artificial ponds. The experiment ran from 19-Sep-2017 to 04-Mar-2018 and therefore encompassed the entire rainy season, effectively mimicking the dynamics of temporary ponds that are common in both preserved and converted landscapes. Between 19 and 25-Sep-2017, mesocosms were filled with well water. On 30-Sep-2017, we added to each mesocosm 800g (wet mass) of leaf litter composed of equal amounts of grass and tree leaf litter to provide a source of nutrients and structural complexity for benthic organisms.

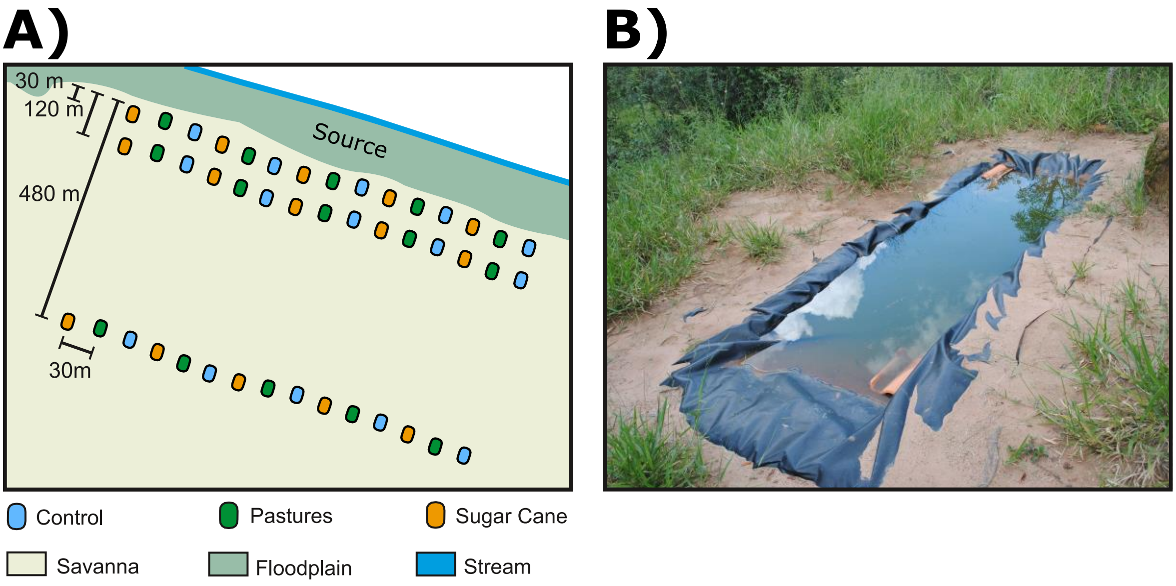

Figure. 1. A. Experimental setup comprising three rows of 15 artificial ponds at 30, 120 and 480m from a source water body (stream and its floodplain). B. One of the naturally colonized pond mesocosms during the experiment.

Agrochemical treatments

For representativeness, we simulated conventional land management practices in Central-South Brazil. Sugarcane is planted in the rainy season from October to March. Between 12 and 18 months later, usually in September or October, the first harvest takes place (´primary cane´, ´plant cane´), followed by four to five annual harvests by ratoon cropping (´ratoon cane´) after which the field is reformed. Sugarcane planting involves laying stalk stumps along furrows together with NPK fertilizers and frequently an insecticide to control termites and beetles. Other insecticides are applied as needed but are not as prevalent as biological control with parasitoid wasps and the fungus Metarhizium is common and effective in sugarcane plantations. Herbicides are sprayed shortly after planting and before sugarcane sprouts (pre-emergence herbicides) and/or a few weeks later (post-emergence herbicides). In subsequent years NPK fertilizers are applied directly on top of the soil, herbicides are either applied over the row or in localized weeds, and insecticides are applied in a shallow line cut over the sugarcane row (Parra *et al.* 2010, Cantarella & Rossetto 2010, R. Rossetto *pers.com.*). Because the sugarcane cycle typically lasts 5 to 6 years, at any given moment 4/5 to 5/6 of all sugarcane fields follow the latter land management practices, Our experimental design followed a ´crop cycle´ design (van Wijngaarden *et al.* 2004, Arts *et al.* 2006) in that molecules, doses and schedule of application were realistically simulated. The ´pasture´ agrochemical treatment was achieved by weekly nutrient additions, whereas the ´sugar cane´ treatment was achieved by identical weekly nutrient additions plus one insecticide and one herbicide application pulses. The manipulated NPK formulation was composed of ammonium nitrate (NH4NO3), monoammonium phosphate (NH4H2PO4) and potassium chloride (KCl) following a 20-05-20 NPK ratio (20% of N, 5% of P2O5 and 20% of K2O) at a dose of 80 kg/ha of N, 20 kg/ha of P2O5 and 80 kg/ha of K2O. This dosage is compatible with the management of both moderately intense pastures (Santos 2010) and sugarcane plantations (Rossetto *et al.* 2008). Dosage per hectare was scaled to the area of each pond mesocosms (4 m^2, *i.e.*, 4 x 10^{-4} ha) and divided into equal weekly doses to simulate a linear dissolution over 52 weeks (*i.e.,* one year), which is the usual interval for new fertilizer applications. An amount of 3.08 g of the NPK 20-05-20 formulation was added weekly from 30-Oct-2017 (the simulated planting/harvesting date) until the end of the experiment, for a total of 15 applications. Therefore, by the end of the experiment, each pond from pasture and sugar cane treatments had received a total of 46.2 g of NPK 20-05-20.

Selected pesticide active ingredients were among the top-selling insecticides and herbicides in the State of São Paulo, and that are registered for use in sugarcane plantations in Brazil (AGROFIT 2020). These were fipronil, a broad-spectrum phenylpyrazole insecticide commonly used for controlling moths, ants, beetles and termites, and 2,4-D, an alkylchlorophenoxy selective systemic herbicide used for controlling broadleaf weeds. Fipronil is the top-selling insecticide registered for use in sugarcane in the State of São Paulo and is also registered for use in Belgium, the Netherlands, USA, and Australia (PPDB 2020). 2,4-D is the second top-selling herbicide registered for use in sugarcane in the state of São Paulo and is also used in Australia and 27 countries of the European Community (PPDB 2020). We opted for this active ingredient instead of the top-selling glyphosate because we did not have a validated methodology for quantifying glyphosate at the time. The toxicity of fipronil is moderate to algae and moderate to high to zooplankton and sediment-dwelling aquatic insects. The toxicity of 2,4-D is low to algae, moderate to macrophytes, and low to zooplankton (PPDB 2020).

The commercial products selected for manipulation were REGENT ® 800 WG (BASF; active ingredient fipronil at an 800 g/kg concentration), and DMA ® 806 BR (Dow AgroSciences. active ingredient 2,4-D at an 806 g/L concentration). Pesticides were applied at the maximum doses recommended by the manufacturer for pest and post-emergence weed control in sugarcane plantations (250 g/ha of REGENT ® 800 WG; 1,5 L/ha of DMA ® 806 BR), in all cases scaled to the area of each pond mesocosms (4 m², *i.e.*, 4 x 10^{-4} ha). We made one single application pulse of the insecticide on 30-Oct-2017 (the simulated planting/harvesting date) and one single application pulse of the herbicide five weeks later, on 04-Dec-2017, which is a common time frame for the application of post-emergence herbicides (R. Rossetto *pers. com*). Pesticide applications were made by diluting 0,1 g of REGENT ® 800 WG (*i.e.,* 80 mg of fipronil) and 0,6 ml of DMA ® 806 BR (*i.e.*, 483,6 mg of 2,4-D) in 400 ml of well water and applying this solution directly into each 'sugar cane' treatment pond. After each application, we gently steered the water with a wood stick and collected a 20 ml water sample into a 300 ml composite sample of the 15 ´sugarcane´ treatment ponds to measure pesticide concentration. We did not attempt to thoroughly mix mesocosms to avoid mechanical damage to the established community, which was to be sampled a few days later. One last composite water sample was also collected at the end of the experiment. Water samples were stored in ice, protected from light, and transported to the lab for pesticide dosage within the same day or the next day. Both fipronil and 2,4-D were measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Predicted environmental concentrations (PECs) at the time of application were 66.7 µg/L fipronil and 403 µg/L 2,4-D. Actual concentrations for fipronil were 15.6 µg/L at the application date (30-Oct-2017), 2.9 µg/L five weeks later (04-Dec-2017), and below detection limits (<5.9 ng/L) by the end of the experiment (04-Mar-2018). Actual concentrations for 2,4-D were 337.8 µg/L µg/L (04-Dec-2017, application date) and below quantification limits (40.6 ng/L) 04-Mar-2018.

To monitor effects of pesticides and nutrient enrichment we took *in vivo* measures of the biomass of chlorophyll-a using an Aquafluor portable fluorometer (Turner Designs, San Jose, CA, USA), before and after each pesticide pulse (28-Oct-2017 and 03-Nov-2017 for the insecticide; 02 and 08-Dec-2017 for the herbicide). We also took water samples of each pond at the end of the experiment to measure total phosphorus (TP) concentrations in water. In this case, water samples were stored frozen at -20 C until TP concentration was measured using a spectrophotometer Shimadzu (Kyoto, Japan) ASC-

5 Automatic Sample Changer, with absorbance at 880 nm. We also conducted monthly measures of basic water quality parameters (temperature, pH, conductivity, dissolved oxygen) with a YSI ProPlus Multiparameter Water Quality Meter (Yellow Springs, OH, USA).

Macroinvertebrate and amphibian surveys

Because community composition changes drastically in the earliest phases of colonization, we proposed to conduct biodiversity surveys 20, 40, 80, and 160 days from the start of the experiment. Actual sampling dates were 13 to 19-Oct-2017, 03 to 10-Nov-2017, 12 to 19-Dec-2017and 24-Feb-2018 to 03-Mar-2018 (24, 45, 84, and 158 days from the start of the experiment). Samples were taken by pipe sampling, which provided quantitative per-unit-area information on species composition and abundances. The sample was taken by quickly thrusting the pipe through the water column and into the sediments to seal the sample area. We then swept all the pipe bottom (area 0,102 m^2) four times and the pelagic area three times with a hand net (mesh size 1.5 mm). We took four samples per pond at each sampling survey. After samples were cleaned of sediment and debris, tadpoles were immediately euthanized and preserved in 10% buffered formalin and invertebrates in 70% ethanol. We counted and identified all aquatic macroinvertebrates to the genus level, with an exception for the Chironomidae and Ceratopogonidae families, which were identified to subfamily and family levels, respectively. Amphibians were assigned to species level, except for Scinax tadpoles, which were identified to the genus level.

Data analysis

To test the hypothesis that community structure is influenced by the agrochemical treatments, 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). Before this analysis, we removed all species with three or fewer occurrences in each survey, 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 (Supplement 1). Because our treatments are expected to change in its effects with time, we analyzed each sampling survey separately. To test whether different treatments and their interactions had a significant effect on community structure, we performed likelihood ratio tests to test if the progressive addition of terms to a simpler model provided a statistically better fit. We did not consider the agrochemical treatments treatments in the first survey because it was previous to the beginning of the agrochemical treatments. Those tests were always assessed by comparing the best model so far against the next more complex model. 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. P-values were computed using the PIT-trap bootstrap resample procedure from 10,000 bootstrap resamples, which operates on probability integral transform residuals (Warton *et al.* 2017). Those analyses were implemented using functions *manyglm()* and *anova.manyglm()* from package 'mvabund' version 4.0.1 (Wang *et al.* 2012, 2019). To test if responses to agrochemicals and isolation were mediated by traits related to taxonomic class (*i.e.,* insect VS amphibian) or trophic level (*i.e.,* strict predator VS consumer), we further tested if the inclusion of the categorical traits amphibian/insect and 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 supplement 2). This trait-based approach is called the model-based fourth corner solution (Brown *et al.* 2014) and was implemented using functions *traitglm()* and *anova.traitglm()* from package 'mvabund' version 4.0.1 (Wang *et al.* 2012). To assess whether individual taxa, or taxa grouped by traits, respond to the different treatments, we looked at 95%

confidence intervals of estimated parameters for each taxon, or group of taxa, in each treatment.

To visualize data, 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). These analyses were implemented using the function *gllvm()* from package 'gllvm' version 1.1.3 (Niku et al. 2019). All analyses were implemented in software R version 3.6.1 (R Core Team 2019).

RESULTS

Mesocosms were colonized by amphibians and aquatic and semiaquatic insects comprising six orders and 22 families (Anura: Hylidae, Leptodactylidae, and Microhylidae; Odonata: Aeshnidae, Coenagrionidae, Gomphidae, and Libellulidae; Coleoptera: Dytiscidae, Elmidae, Hydrophilidae, and Noteridae; Diptera: Ceratopogonidae, Chaoboridae, Chironomidae, and Culicidae; Ephemeroptera: Baetidae and Caenidae; Hemiptera: Corixidae, Naucoridae, Nepidae, Notonectidae, and Veliidae). The most abundant families, each with more than 2000 individuals sampled in the entire experiment, were Chironomidae (total abundance N=23445 individuals), Hylidae (N=3079), Libellulidae (N=3057) and Culicidae (N=2762). The rarest families, each with less than 10 individuals, were Aeshnidae, Microhylidae, Noteridae, Coenagrionidae, Gomphidae, Nepidae, and Corixidae. More detailed information is available in Supplement 2.

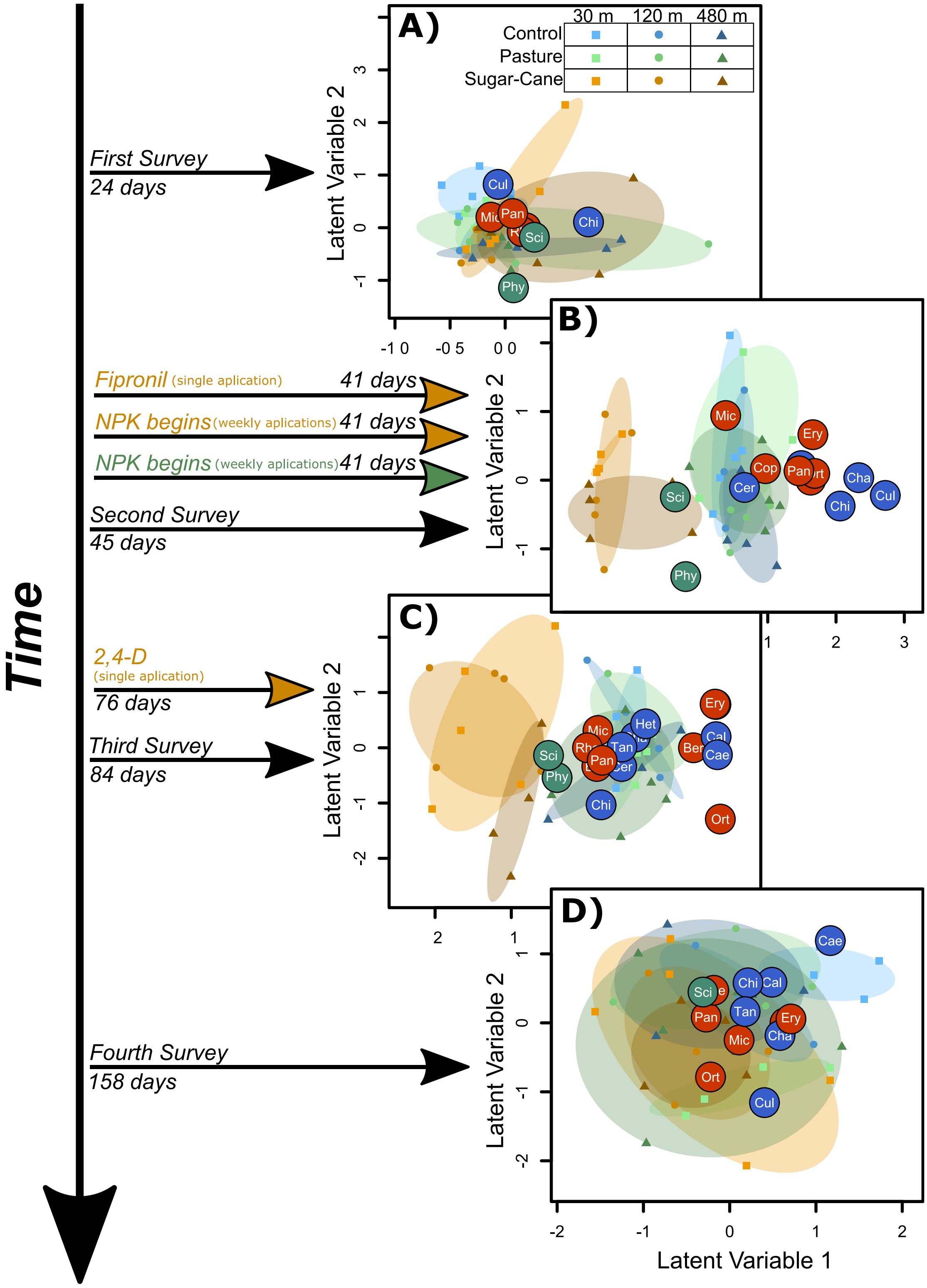

Figure 2. Model-based unconstrained ordinations showing pond communities (symbols) and species (bubbles) in each of the four sampling surveys (A to D, respectively). Red bubbles are predatory insects, blue bubbles are non-predatory insects, and green bubbles are amphibians. Green arrowheads indicate chemical treatments applied in pasture ponds and yellow in sugar cane ponds. Note that chemical treatments are represented in different colors in the first survey for reference only. Abbreviations of names of taxa are provided in Supplement 2.

We generally found that the ‘pasture’ and control treatments were not significantly different from each other, whereas the ‘sugarcane’ treatment differed from both control and ‘pasture’ treatments right after application, and gradually became similar to the ‘pasture’ treatment, but not to the control (Figure 2). We found no interactive effects among agrochemical treatments and spatial isolation (Table 1). Specifically, the \`sugarcane\` treatment strongly differed from the control in all surveys (second survey: $p < 0.001$; third survey: $p < 0.001$; fourth survey: $p = 0.004$), but it only differed from \`pasture\` in the second and third surveys (second survey: $p < 0.001$; third survey: $p < 0.001$; fourth survey: $p = 0.373$). The ´pasture´ treatment did not differ from the control in any survey (second survey: $p = 0.443$; third survey: $p = 0.069$; fourth survey: $p = 0.373$).

As expected, the differences among the ‘sugarcane’ treatment and the others were mediated by the different responses of insects and amphibians in two surveys after the insecticide application (Table 1). Specifically, insects were negatively affected by the ‘sugarcane’ treatment, whereas amphibians were either not affected (Figure 3B-C) or positively affected by it (Figure 3E-F). Even though communities as a whole were not affected by nutrient addition alone (*i.e.*, ‘pasture’ treatment), we still observed that *Orthemis* dragonflies were positively affected by the ‘pasture’ treatment in the second and third surveys (Figure 5d and 5e), and *Pantala* dragonflies only in the second survey (Figure 5a). Additionally, *Pantala* was also positively affected by the ‘sugarcane’ treatments in the last survey (Figure 5c).

Table 1. Summary of likelihood ratio tests of models explaining community structure. All values of deviance considering each sampling survey separately, or all sampling surveys together are relative to the best simpler previous model. Bold values represent a significant improvement in model fit ($p < 0.05$). ´Taxonomic class´ represents a test for different responses of amphibians and insects, whereas ´trophic level´ represents a test for different responses of predators and consumers to a specific factor (*i.e.*, Agrochemical treatment or Isolation).

	Diff. Df.	Deviance	p
First Survey			
1 – Isolation	**2**	**48.94**	**0.004**
2 - Isolation + (Isolation: Taxonomic class) (Compared to 1)	**2**	**15.096**	**0.044**
3 - Isolation + (Isolation: Taxonomic class) + (Isolation:Trophic Level) (Compared to 2)	2	2.691	0.289
Second Survey			
4 - Agrochemical	**2**	**231.73**	**<0.001**
5 - Agrochemical + Isolation (Compared to 4)	**2**	**73.84**	**0.003**
6 - Agrochemical * Isolation (Compared to 5)	4	76.81	0.056
7 - Agrochemical + Isolation + (Agrochemical: Taxonomic class) (Compared to 5)	**2**	**66.14**	**<0.001**
8 - Agrochemical + Isolation + (Agrochemical: Taxonomic class) + (Agrochemical:Trophic Level)(Compared to 7)	2	3.641	0.198
9 - Agrochemical + Isolation + (Agrochemical: Taxonomic class) + (Isolation: Taxonomic class) (Compared to 7)	2	8.508	0.092
10 - Agrochemical + Isolation + (Agrochemical: Taxonomic class) + (Isolation:Trophic) (Compared to 7)	**2**	**10.94**	**0.016**
Third Survey			
11 - Agrochemical	**2**	**194.48**	**<0.001**
12 - Agrochemical + Isolation (Compared to 11)	**2**	**85.20**	**0.001**
13 - Agrochemical * Isolation (Compared to 12)	4	86.81	0.131
14 - Agrochemical + Isolation + (Agrochemical: Taxonomic class) (Compared to 12)	**2**	**48.45**	**<0.001**
15 - Agrochemical + Isolation + (Agrochemical: Taxonomic class) + (Agrochemical:Trophic Level)(Compared to 14)	2	1.148	0.659
16 - Agrochemical + Isolation + (Agrochemical: Taxonomic class) + (Isolation: Taxonomic class) (Compared to 14)	2	1.363	0.625
17 - Agrochemical + Isolation + (Agrochemical: Taxonomic class) + (Isolation:Trophic) (Compared to 14)	2	3.345	0.305
Fourth Survey			
18 - Agrochemical	**2**	**58.46**	**0.018**
19 - Agrochemical + Isolation (Compared to 18)	**2**	**65.02**	**0.002**
20 - Agrochemical * Isolation (Compared to 18)	4	84.38	0.085
21 - Agrochemical + Isolation + (Agrochemical: Taxonomic class) (Compared to 18)	2	0.573	0.777
22 - Agrochemical + Isolation + (Agrochemical:Trophic) (Compared to 18)	2	4.182	0.228
23 - Agrochemical + Isolation + (Isolation: Taxonomic class) (Compared to 18)	2	4.114	0.165
24 - Agrochemical + Isolation + (Isolation:Trophic) (Compared to 18)	2	2.35	0.434

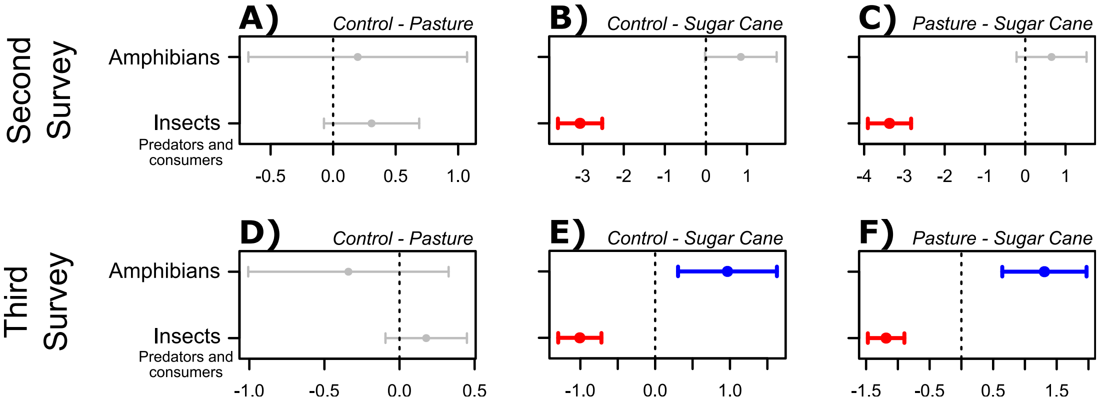

Figure 3. 95% Confidence intervals for the maximum likelihood estimates (MLE) of the effect of the agrochemical treatments on the abundance of amphibians and insects when comparing pairs of treatments for the second (A to C) and third surveys (D to F). Confidence intervals not crossing the zero hatched line were considered significant effects and colored; blue bars represent an increase and red bars a decrease in abundance from the reference treatment. A and D are effects of the 'pasture' treatment relative to control. B and E are the effects of 'sugarcane' treatment relative to control. C and F are the effects of the 'sugarcane' treatment compared to the 'pasture' treatment.

The main differences among isolation treatments were between highly isolated communities (*i.e.*, 480m) and communities at low or moderate isolation from the source wetland (*i.e.*, 30 and 120m). Communities from 30 m were always different from those in 480 m (first survey: p = 0.007; second survey: p = 0.028; third survey: p = 0.023; fourth survey: p = 0.039), but never from those in 120 m (first survey: p = 0.065; second survey: p = 0.484; third survey: p = 0.185; fourth survey: p = 0.187). Also, communities in 120 m were different from 480 m, but only in the two last surveys (first survey: p = 0.330; second survey: p = 0.484; third survey: p = 0.010; fourth survey: p = 0.039).

The effects of isolation were, in the first survey, mediated by different responses of amphibians and insects to isolation (Table 1), and in the second survey by different responses of consumers and predators (Table 1). Specifically, amphibians were positively affected by isolation in the first survey (Figure 4A-C), whereas consumers (including amphibians and insects) were positively affected only in the second survey (Figure 4D-F). Chironomids were the only ones that continued to be positively affected by isolation in the third survey (Figure 5N). However, this effect disappeared by the end of the experiment (Figure 5O). Predatory taxa had idiosyncratic responses to isolation. *Berosus* beetles and *Erithrodiplax* dragonflies exhibited either tendencies or significant decreases in abundance with isolation (Figure 5G-L), whereas *Pantala* and *Orthemis* dragonflies exhibited either no change or significant increases in abundance along the isolation gradient (Figure 5A-B and D-E).

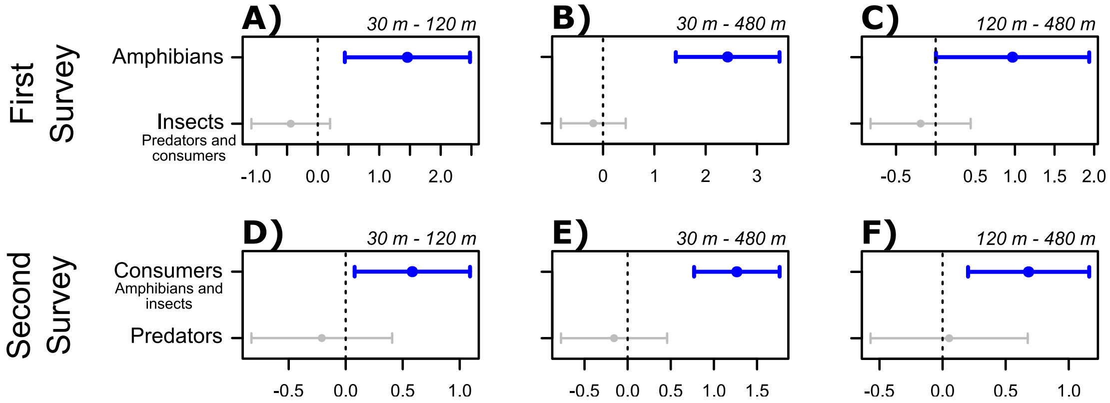

Figure 4. 95% Confidence intervals for the maximum likelihood estimates (MLE) of the effect of isolation on the abundance of amphibians and insects in the first survey (A to C), and consumers and predators in the second survey (D to F) when comparing pairs of treatments. Confidence intervals not crossing the zero hatched line were considered significant effects and colored; blue bars represent an increase in abundance from the reference treatment. A and D are the effects of 120 m compared to 30 m. B and E are effects of 480 m compared to 30 m. C and F are the effects of 480 m compared to 120 m.

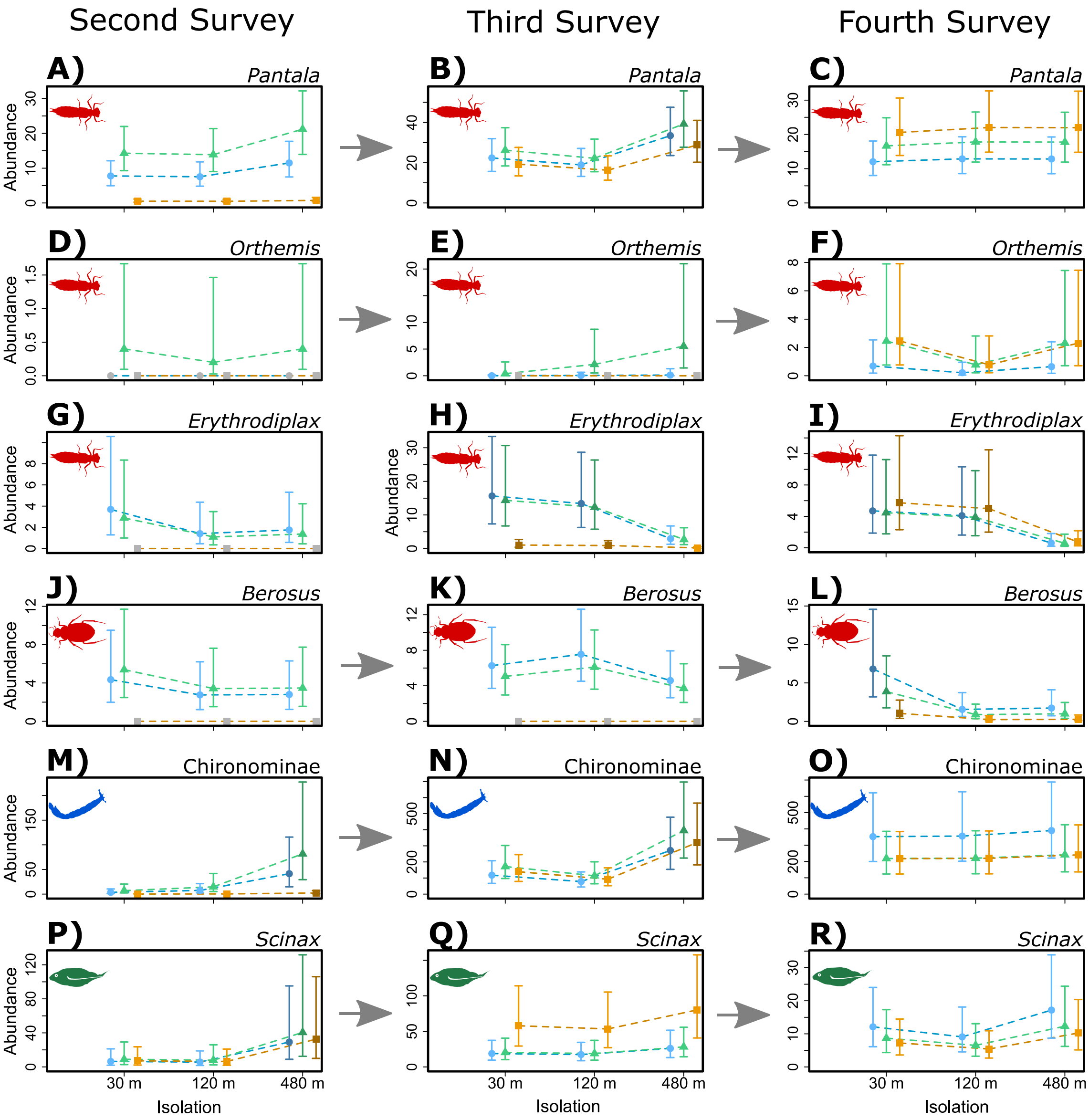

Figure 5. Maximum likelihood estimates (MLE) of abundances and corresponding 95% confidence intervals for three of the most representative predators (top four rows), insect-consumer (fifth row) and amphibian taxa (sixth row) from the second (left column), third (middle column) and fourth surveys (right column). Gray symbols indicate absolute absence (zero abundance) of a taxon in a given treatment. Blue circles are MLEs for control treatments, green triangles are MLEs for 'pasture' treatments, and orange squares are MLEs for sugar cane treatments. MLEs that are not contained inside the 95% confidence interval of other estimates are considered to be significantly different. The actual estimated differences are provided in supplement 7. MLEs of abundance for all species are provided in supplement 6. Symbols are jittered around manipulated distances in the x-axis for improved visualization.

DISCUSSION

We were able to assess how freshwater communities change as a consequence of chemical intensification in different spatial contexts by following community assembly and reassembly after the cumulative application of fertilizers and the pesticide pulses. We saw that ‘pasture’ communities were not generally different from controls, even though some predatory insects seemed to be more abundant in ‘pasture’ ponds. ‘Sugarcane’ communities, however, largely diverged from ‘control’ and ‘pasture’ communities, but this effect lost strength with time. Yet, at the end of the rainy season, ‘sugarcane’ and ‘control’ communities were still significantly different. Spatial isolation showed a generally positive effect on consumers, as expected, and idiosyncratic responses of predatory insects. Amphibians were surprisingly not affected by isolation. Furthermore, spatial isolation did not change the general effects of the chemical treatments. Thus we will address the main effects of both chemical treatments and isolation separately.

Agrochemical treatments

Because the ‘pasture’ treatment only received the fertilization regime, we expected a general increase in predatory insect’s abundance(H1.1; Abrams 1993, Slavik *et al.* 2004), likely followed by an increase in the abundance of consumers that are less vulnerable to predation (H1.2; Leibold 1999, Davis *et al.* 2009), in comparison to control ponds. We indeed observed that two of the most important predatory insects in our system, *Pantala* and *Orthemis* dragonflies, appeared to generally positively respond to the fertilization regime in the ‘pasture’ treatment, especially right after the fertilization regime began (*i.e.*, second survey). However, we found no evidence of any increase in the abundance of consumers when comparing ‘pasture’ to ‘control’ treatments. Such patterns have been observed before. For instance, Jahnke *et al.* (2001) found that the majority of the taxa positively correlated with a gradient of nutrient concentration in wetlands were predacious dytiscid beetles. It could also be that the increase in consumer production was buffered by predator consumption via *top-down* regulation (Abrams 1993), thus consumer abundance was unaffected. Still, even though noticeable, the increase in abundance of both these dragonflies were mild and not enough to yield significant differences among ‘pasture’ and ‘control’ communities. We believe this is because temporary ponds are often in the hypertrophic portion of the primary productivity gradient. For instance, Schiesari & Corrêa (2016) observed that TP concentration in ponds embedded in either savannah, pasture or sugar cane landscapes had values of TP above 1000 ug L^{-1}, which are way higher than expected for eutrophic lakes (*i.e.*, between 30 and 100 ug L^{-1}, Smith et al. 1999). Indeed, even though the average measured TP values were higher in 'pasture' relative to 'control' ponds, they were not significantly different and almost always above 100 ug L^{-1} (see Supplement 3). Still, even in this extreme of the gradient, we were able to observe that intensification of pastures by using fertilizers can potentially affect predatory insects, likely through bottom-up effects.

'Sugarcane' communities diverged from both and control and 'pasture' communities in all sampling surveys. We hypothesized that it would be mainly caused by the insecticide application, which would have an indirect positive effect on amphibian abundance by negatively affecting predatory insects (H3.1). Indeed, the insecticide fipronil clearly caused a massive death of aquatic and semiaquatic insects. The known fipronil EC50 for aquatic insects is of about 1ug L^{-1}, a lower concentration than the one found in 'sugarcane' ponds even one month after the pesticide pulse. Amphibians, however, have an estimated LC50 higher than 800mg L^{-1} (ELG Espíndola, *pers. com*), a concentration more than 10 thousand times higher than our manipulated concentration. And indeed, their abundance was not affected right after the fipronil application. In fact, as we expected, we observed an increase in amphibian abundance one month after the pesticide pulse (*i.e.*, third survey), likely because of the reduced predation pressure of predatory insects. Similar effects have been observed in other experimental work conducted in more controlled mesocosm experiments with different insecticides (*i.e.*, carbaryl: Relyea 2005a, malathion: Relyea *et al.* 2005, endosulfan: Rohr & Crumrine 2005). For instance, Relyea (2005a) found that the use of the insecticide Sevin (active ingredient carbaryl) can reduce the biomass of predators, causing an increase in the biomass of herbivores (including tadpoles). We believe these results can be generalized by any other monocultures. More importantly, they might even be stronger in crops that, different from sugarcane, heavily relies on the use of insecticides. Soybean fields, for example, are typically subject to three to four applications of insecticides, for a combination of 13 active ingredients, within a crop cycle typically lasting less than four months (Schiesari & Grillitsch 2011, Schiesari *et al.* 2013). Indeed, similar patterns of high mortality of predatory insects and a higher abundance of amphibians have been observed in soybean fields, when compared to forest habitats (Negri 2015). This is not to say that insecticide applications in agricultural fields may not be detrimental to anuran larvae in any way. For instance, (Relyea & Diecks 2008) found that insecticide malathion can indirectly reduce time to metamorphose in amphibians, making them more vulnerable to pond drying. Rather, we argue that the indirect positive effect of insecticides through reduced predation might have a greater consequence on amphibian abundances than possible negative direct or indirect effects.

Because our experiment aimed at observing the net effects of chemical intensification simulating a full crop cycle, we could only infer the separated effects of insecticides and herbicides on community structure by chronology in the application. That being said, we did not observe any clear acute effects of the herbicide 2,4-D on species abundance patterns after the application, which is not surprising. (Relyea 2005a) also found no differences in general abundance patterns of predatory insects, amphibians or snail herbivores that were treated with similar concentrations of 2,4-D. These results are consistent with past toxicity studies with amphibians ($LC50_{96h}$ varying from 28.8 to 574.2 mg L^{-1}, depending on the species, Freitas *et al.* 2019), zooplankton ($EC50_{48h}$ of 134.2 mg L^{-1} for *Daphnia magna*, PPDB 2020) and fish ($LC50_{96h}$ of 100 mg L^{-1} for *Pimephales promelas*, PPDB 2020), which show acute effects with concentrations close to a thousand times higher than what we manipulated. We also did not observe differences in the response of chlorophyll-a biomass to herbicide application (see supplement 4). 2,4-D is known to have moderate toxicity to macrophytes but low toxicity to algae (Peterson *et al.* 1994; PPDB 2020), which significantly decreases its potential to cause negative *bottom-up* effects (H2) in our simulated ecosystems (and temporary ponds in general, perhaps). However, the non-lethal effects of 2,4-D could still have played an important role in our experiment. 2,4-D has been found to decrease the swimming speed of tadpoles at the same concentrations that we manipulated (Freitas *et al.* 2019), possibly making amphibians more susceptible to predation. Thus, it could have contributed to the increase in the abundance of dragonflies in sugar cane treatments by the end of the experiment (which will be discussed below). However, we do not know what systemic effects 2,4-D could have on predatory insects. For instance, other pesticides have been found to increase prey survival in the presence of predators because it decreases their predatory potential (Hanlon & Relyea 2013). It is also important to mention that, even though we found no evidence that 2,4-D can cause significant changes in macroinvertebrate and amphibian communities, many works have shown that glyphosate-based herbicides (top-selling herbicide in the state of São Paulo) that contain the surfactant polyethoxylated tallowamine in their formulation, such as Roundup ®, are highly toxic to amphibians (see Mann & Bidwell 1999, Relyea 2005a, 2005b, Relyea & Jones 2009, and Moutinho 2013 for sugarcane herbicides). Therefore, we cannot discard other possible harmful effects of herbicides on freshwater communities.

We also expected the effect of the pesticide pulses to be temporary (H4), allowing the recovery of communities after pesticide degradation. We indeed observed that the insecticide made communities a lot different, as discussed above, but this effect lost strength with time (Figure 2). More importantly, most of the patterns we observed in our experiment were dependent on the timing of the pesticide pulses and fertilization. For instance, the positive effect of insecticides on amphibian abundance happened only one month after insecticide application (*i.e.,* third survey). By the end of the experiment (*i.e.,* about four months after insecticide pulse), insect populations had already recovered, and amphibian populations were reduced back to numbers similar to those found in control ponds. Yet, sugar cane communities were still different from controls, but not from 'pasture' ponds. At the end of the experiment, the two predatory dragonfly taxa that showed patterns of increased abundance in 'pasture' ponds, *Pantala* and *Orthemis,* showed similar patterns in 'sugarcane' ponds. These two taxa did not just recover their population size, they actually had higher abundances in 'sugarcane' ponds than in control ponds. We believe there could be to non-mutually excluding explanations to these differences. First, nutrient enrichment might have caused the abundance of these dragonflies to increase, the same way that it did in 'pasture' ponds (*i.e.,* bottom-up effects). Indeed, TP values measured in 'sugarcane' ponds at the end of the experiment were significantly higher than control ponds, but not different from 'pasture' ponds. Second, the increased dragonfly abundance could also be a classic case of predator-prey dynamics (Lotka 1926, Gilpin 1973), where we observe a time lag in the responses of tadpoles to the decrease in dragonfly abundance, followed by an increase in dragonfly abundance in the last survey (Figure 5). Those two effects may have acted together to accentuate the differences between control and 'sugarcane' communities, even after insecticide degradation.

Even though the insecticide effects were temporary, they are absolutely relevant in temporary ponds given that the very existence of these habitats is synchronized with pesticide application in sugar cane fields and other rainfed crops, such as soybean (*e.g.,* Petter *et al.* 2007) and corn (*e.g.,* Preez *et al.* 2005). For instance, some of the most abundant and important species observed in our mesocosms have aquatic life cycles of less than three months, such as mosquitoes, midges, dragonflies and amphibians (Oliver 1971, Nebeker 1973, Ciota et al. 2014, Hamada *et al.* 2014). Thus, the processes happening at the peak (*i.e.,* December and January) of the rainy season, which was the period where the effects of the treatments were the clearest, can strongly affect patterns of adult insect and amphibian emergence, and therefore, regional abundance patterns.

Spatial isolation

We observed that the effect of isolation was mediated by trophic level, where consumers were benefited by the reduction in the abundance of predatory insects. However, this effect was only clear in the second survey. In the third survey, among consumers, only chironomid midges, the most abundant taxa in our experiment, kept being positively affected by isolation. However, by the end of the experiment, all positive effects of isolation on consumers had disappeared. Different from consumers, predatory insects had idiosyncratic responses to isolation with a few taxa, such as *Berosus* beetles and *Erythrodiplax* dragonflies, almost always being negatively affected, and *Pantala* and *Orthemis* dragonflies being not affected, or even positively affected. These patterns confirm the findings of our previous experiment (Pelinson *et al.* 2019), which had a duration of about 80 days, corresponding to the third survey in this experiment. We observe that the lower dispersal rates of some predatory insects can benefit consumers in more isolated habitats, likely due to trophic cascades (Shulman & Chase 2007; Chase & Shulman 2009; Hein & Gillooly 2011; Pelinson *et al.* 2019). Additionally, the absence of the most dispersal limited predators in more isolated habitats can even benefit those that are not dispersal limited, likely through reduced competition (Pelinson *et al.* 2019). However, the reason why most of the positive effects of isolation disappeared by the end of the experiment are unclear to us. It could possibly be a limitation of our experimental design. Because our experimental ponds were not isolated from each other, it could be that as local populations of consumers increased in size; dispersal among experimental ponds grew in importance for local abundance patterns. Such an effect may not be true in natural habitats. For instance, work made on natural ponds and wetlands found that consumers are more abundant in ponds that are highly isolated from any other aquatic habitat (Chase & Shulman 2009).

One unexpected pattern we observed was that amphibians were generally not affected by spatial isolation. In fact, they were even positively affected in the earliest stage of community assembly. This is surprising given the overwhelming evidence that amphibians have limited dispersal ability due to their high risk of dissection during terrestrial dispersal (*e.g.,* Marsh *et al.* 1999, Marsh & Trenham 2001, Rittenhouse & Semlitsch 2007, Semlitsch 2008, Sinsch *et al.* 2012). We believe that there could be two joint explanations to such pattern. First, habitat selection by amphibians is subject to context dependence (Resetarits 2005), where adult amphibians should perceive habitat quality differently depending on the surrounding context (see Resetarits & Silberbush 2015). Specifically, amphibians are known to strongly avoid habitats containing fish (Resetarits & Wilbur 1991; Resetarits 2005; Vonesh *et al.* 2009). In this case, ponds that are closer to the creek and its marshy floodplains could be perceived as having a comparatively lower quality (*i.e.,* higher risk of predation by fish), whereas isolated ponds would be perceived as high-quality habitats since they are far away from the creek. Second, because we conducted our experiment in a relatively dense savannah landscape with sparse trees, the terrestrial matrix could have been a suitable habitat for amphibian adults to use for shelter and foraging, especially for generalist amphibians with adaptations to live and reproduce in dryer habitats (Haddad & Prado 2005, Vasconcelos *et al.* 2014). It would mean that ponds that are closer or further away from the source (*i.e.,* the bigger aquatic habitats) were actually embedded in equally suitable terrestrial habitats. This might be an important deviation between our manipulated environmental scenario and those observed in true agricultural landscapes. For instance, da Silva *et al.* (2012) found a strong reduction in adult anuran abundance in pastures after 50 m of distance from forest fragments. True open canopy agricultural landscapes might vary in permeability to dispersal (*e.g.,* Hansen *et al.* 2018), with open canopy ones, such as pastures, being likely drier and more hostile habitats to dispersal if compared to dense savannahs or forest fragments (*e.g.,* Rothermel & Semlitsch 2002, Watling & Braga 2015).

Finally, we found no evidence that spatial isolation can play an important role in mediating the effects of chemical intensification on community structure (H1.3 and H3.2). The expectations we had on spatial isolation mediating these effects were based on the assumption that predatory insects have lower dispersal rates than consumers. We expected that the bottom-up effects of nutrient enrichment would be stronger in less isolated habitats because predatory insects would be more abundant in lower isolation (H1.3). We also expected that because predatory insects are dispersal limited, there would be a delay in predator recolonization in more isolated ponds. Thus the indirect effect on consumers would last longer (H3.2). However, as discussed above, not all predatory insects were limited by our isolation treatment. In fact, the only two predators that were indirectly affected by nutrient enrichment, *Pantala* and *Orthemis* dragonflies, were not affected by spatial isolation. Additionally, more than any other predatory taxa, the reduction in the abundance of these dragonflies after the insecticide pulse was likely the main cause of the subsequent increase in amphibian abundance. Dragonflies are known to be both one of the most important amphibian predators in temporary ponds (Heyer *et al.* 1975, Wellborn *et al.* 1996) and, in general, good dispersers (McCauley 2006, McCauley *et al.* 2008). Therefore, we believe that the lack of dispersal limitation of the taxa who experienced most of the indirect effects of the chemical treatments (*i.e.*, dragonflies and amphibians) was the cause of the similar effects of chemical intensification on different spatial contexts.

CONCLUSION

Here we show the net consequences of chemical intensification involving only fertilization, as used in intensive pastures, and a combined fertilization regime and seasonal pesticide pulses, as used in sugarcane fields, on freshwater macroinvertebrate and amphibian communities. We show that, because insecticide and fertilizer application frequently overlap with the rainy season, it can strongly affect freshwater communities in temporary ponds formed close to or embedded in pastures and crop fields. Specifically, nutrient enrichment in pasture ponds can potentially favor predatory insects through *bottom-up* effects, whereas in landscapes dominated by sugarcane fields, or any other monoculture that requires the use of insecticides, we might observe a decrease of aquatic insect populations, followed by an increase in amphibian populations. We also confirmed the effects of spatial isolation on macroinvertebrate community structure found in previous experiments (see Pelinson *et al.* 2019). Furthermore, we found no evidence that the effects of chemical intensification are strongly dependent on spatial isolation of ponds, likely because the most important predatory insects and consumers in our system were not dispersal limited and, thus, equally indirectly affected by chemical treatments in all isolation distances. However, experiments and observational data addressing spatial isolation on truly altered landscapes are still lacking, since different crop types might impose different dispersal limitations to different freshwater taxa.

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Appendix - Chapter 2

SUPPLEMENT 1

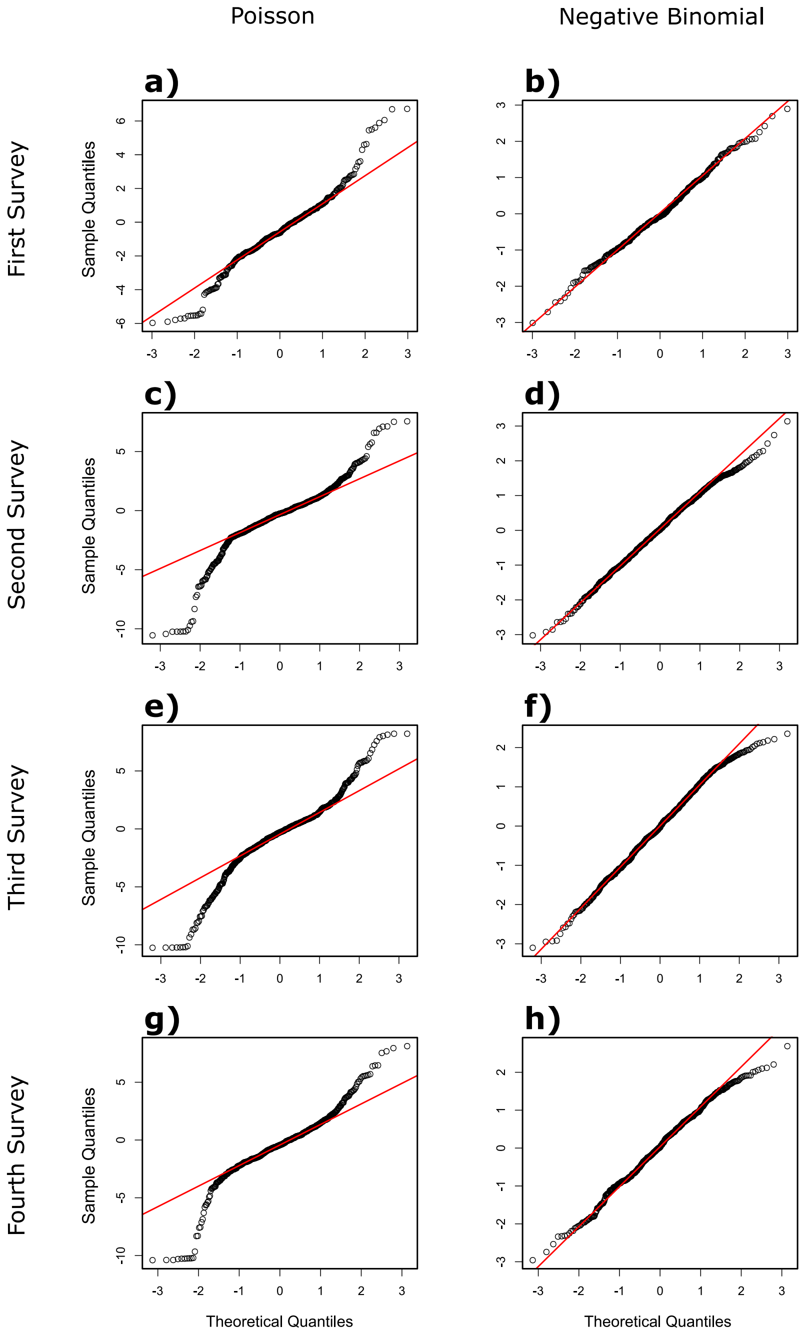

Figure S1.1. Normal Q-Q-Plots of residuals of each sampling survey using Poisson and Negative Binomial distributions.

SUPPLEMENT 2

Table S2.1. Family, Order, Class, Trophic level and Total Abundance of all taxa collected during all sampling surveys.

Taxa	**Family**	**Order**	**Class**	**Abbreviation**	**Trophic Level**	**Total abundance**	**Reference for Trophic Level**
Scinax sp.	Hylidae	Anura	Amphibia	Sci	consumer	3079	Annibale et al. 2019
Physalaemus nattereri	Leptodactylidae	Anura	Amphibia	Phy	consumer	1831	Annibale et al. 2019
Elachistocleis sp.	Microhylidae	Anura	Amphibia	Ela	consumer	1	Annibale et al. 2019
Rhantus	Dytiscidae	Coleoptera	Hexapoda	Rha	predator	46	Ramírez and Gutiérrez-Fonseca 2014
Copelatus	Dytiscidae	Coleoptera	Hexapoda	Cop	predator	9	Ramírez & Gutiérrez-Fonseca 2014
Derovatellus	Dytiscidae	Coleoptera	Hexapoda	Der	predator	5	Ramírez & Gutiérrez-Fonseca 2014
Bidessonotus	Dytiscidae	Coleoptera	Hexapoda	Bid	predator	2	Ramírez & Gutiérrez-Fonseca 2014
Laccophilus	Dytiscidae	Coleoptera	Hexapoda	Lac	predator	2	Ramírez & Gutiérrez-Fonseca 2014
Hypodessus	Dytiscidae	Coleoptera	Hexapoda	Hyp	predator	1	Ramírez & Gutiérrez-Fonseca 2014
Liodessus	Dytiscidae	Coleoptera	Hexapoda	Lio	predator	1	Ramírez & Gutiérrez-Fonseca 2014
Heterelmis	Elmidae	Coleoptera	Hexapoda	Het	consumer	15	Ramírez & Gutiérrez-Fonseca 2014
Berosus	Hydrophilidae	Coleoptera	Hexapoda	Ber	predator	390	Ramírez & Gutiérrez-Fonseca 2014

Tropisternus	Hydrophilidae	Coleoptera	Hexapoda	Tro	predator	4	Ramírez & Gutiérrez-Fonseca 2014
Thermonectus	Hydrophilidae	Coleoptera	Hexapoda	The	predator	1	Ramírez & Gutiérrez-Fonseca 2014
Hydrocanthus	Noteridae	Coleoptera	Hexapoda	Hyd	predator	1	Ramírez & Gutiérrez-Fonseca 2014
Ceratopogonidae	Ceratopogonidae	Diptera	Hexapoda	Cer	consumer	40	Aussel and Linley 1994, Ramírez and Gutiérrez-Fonseca 2014
Chaoborus	Chaoboridae	Diptera	Hexapoda	Cha	consumer	235	ARCIFA 2000, Ramírez and Gutiérrez-Fonseca 2014
Chironominae	Chironomidae	Diptera	Hexapoda	Chi	consumer	21560	Ramírez & Gutiérrez-Fonseca 2014
Tanypodinae	Chironomidae	Diptera	Hexapoda	Tan	consumer	1885	Henriques-Oliveira et al. 2003, Ramírez and Gutiérrez-Fonseca 2014
Culex	Culicidae	Diptera	Hexapoda	Cul	consumer	2762	Ramírez & Gutiérrez-Fonseca 2014
Callibaetis	Baetidae	Ephemeroptera	Hexapoda	Cal	consumer	118	Ramírez & Gutiérrez-Fonseca 2014
Caenis	Caenidae	Ephemeroptera	Hexapoda	Cae	consumer	374	Ramírez & Gutiérrez-Fonseca 2014
Sigara	Corixidae	Hemiptera	Hexapoda	Sig	predator	6	Ramírez & Gutiérrez-Fonseca 2014
Limnocoris	Naucoridae	Hemiptera	Hexapoda	Lim	predator	10	Ramírez & Gutiérrez-Fonseca 2014
Pelocoris	Naucoridae	Hemiptera	Hexapoda	Pel	predator	1	Ramírez & Gutiérrez-Fonseca 2014
Curicta	Nepidae	Hemiptera	Hexapoda	Cur	predator	3	Ramírez & Gutiérrez-Fonseca 2014

Buenoa	Notonectidae	Hemiptera	Hexapoda	Bue	predator	249	Ramírez & Gutiérrez-Fonseca 2014
Notonecta	Notonectidae	Hemiptera	Hexapoda	Not	predator	13	Ramírez & Gutiérrez-Fonseca 2014
Microvelia	Veliidae	Hemiptera	Hexapoda	Mic	predator	1495	Ramírez & Gutiérrez-Fonseca 2014
Anax	Aeshnidae	Odonata	Hexapoda	Ana	predator	1	Ramírez & Gutiérrez-Fonseca 2014
Oxyagrion	Coenagrionidae	Odonata	Hexapoda	Oxy	predator	3	Ramírez & Gutiérrez-Fonseca 2014
Progomphus	Gomphidae	Odonata	Hexapoda	Pro	predator	3	Ramírez & Gutiérrez-Fonseca 2014
Pantala	Libellulidae	Odonata	Hexapoda	Pan	predator	2419	Ramírez & Gutiérrez-Fonseca 2014
Erythrodiplax	Libellulidae	Odonata	Hexapoda	Ery	predator	521	Ramírez & Gutiérrez-Fonseca 2014
Orthemis	Libellulidae	Odonata	Hexapoda	Ort	predator	112	Ramírez & Gutiérrez-Fonseca 2014
Tholymis	Libellulidae	Odonata	Hexapoda	Tho	predator	5	Ramírez & Gutiérrez-Fonseca 2014

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SUPPLEMENT 3

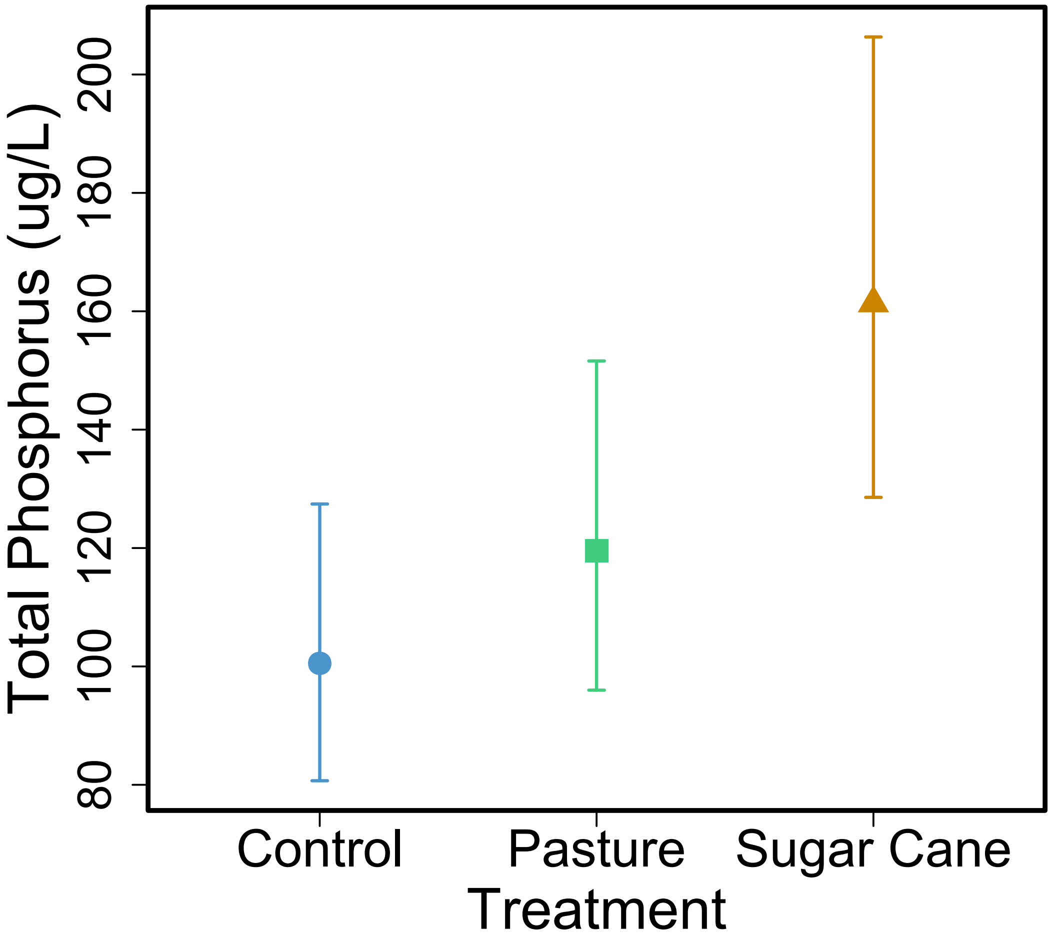

Figure S3.1. Mean total phosphorus concentration in water by the end of the experiment. Error bars are 95% confidence intervals from a linear model using Gamma error distribution. We analyzed the effect of land use treatment on TP concentration through likelihood ratio tests (Dif. Df: 2; Residual Deviance: 7.7084; p value: 0.0156). Post-hoc pairwise comparisons showed that sugar cane is significantly different from control ponds (p: 0.02), but not from pasture ponds (p: 0.186). Controls were also not different from pasture ponds (p: 0. 544).

SUPPLEMENT 4

Chlorophyll-a increased in all land use treatments after the application of both fipronil and 2,4-D (Table S4.1; Figures S4.1 and S4.2). Thus, this increase was not a consequence of our land use treatments. The increase in Chlorophyll-a biomass after the fipronil application only happened in the 30 m (p <0.001) and 120 m (p < 0.001) isolation treatments, not in the 480 m treatment (p = 0.356; Figures S4.1). After the 2,4-D pulse, the increase was similar in all isolation treatments (Figures S4.2). At this stage of the experiment Chlorophyll-a biomass was higher in sugarcane ponds (Table S4.1; Figures S4.2), if compared to control (p = 0.002) and pasture ponds (0.003). Control and pasture ponds had similar values of chlorophyll-a (p = 0.142).

Table S4.1. Summary of likelihood ratio tests of mixed effect models (using pond ID as random intercept) describing changes in chlorophyll-a biomass before and after fipronil and 2,4-D application. All values of deviance are relative to the best simpler previous model. Bold values represent a significant improvement in model fit (p < 0.05).

	Difference of . Df	**deviance**	**p**
Fipronil			
1 - Date	**1**	**167.46**	**<0.001**
2 - Date + treatment (Compared to 1)	2	163.63	0.147
3 - Date + isolation (Compared to 1)	2	161.96	0.064
4 - Date * treatment (Compared to 1)	4	162.49	0.291
5 - Date * isolation (Compared to 1)	**4**	**150.36**	**0.002**
5 - Date * treatment * isolation (Compared to 5)	12	132.57	0.122
2,4-D			
1 - Date	**1**	**196.44**	**0.009**
2 - Date + treatment (Compared to 1)	**2**	**185.77**	**0.005**
3 - Date + treatment + isolation (Compared to 2)	2	184.68	0.578
4 - Date * treatment (Compared to 2)	2	183.72	0.359
5 - Date * treatment (Compared to 2)	14	172.55	0.509

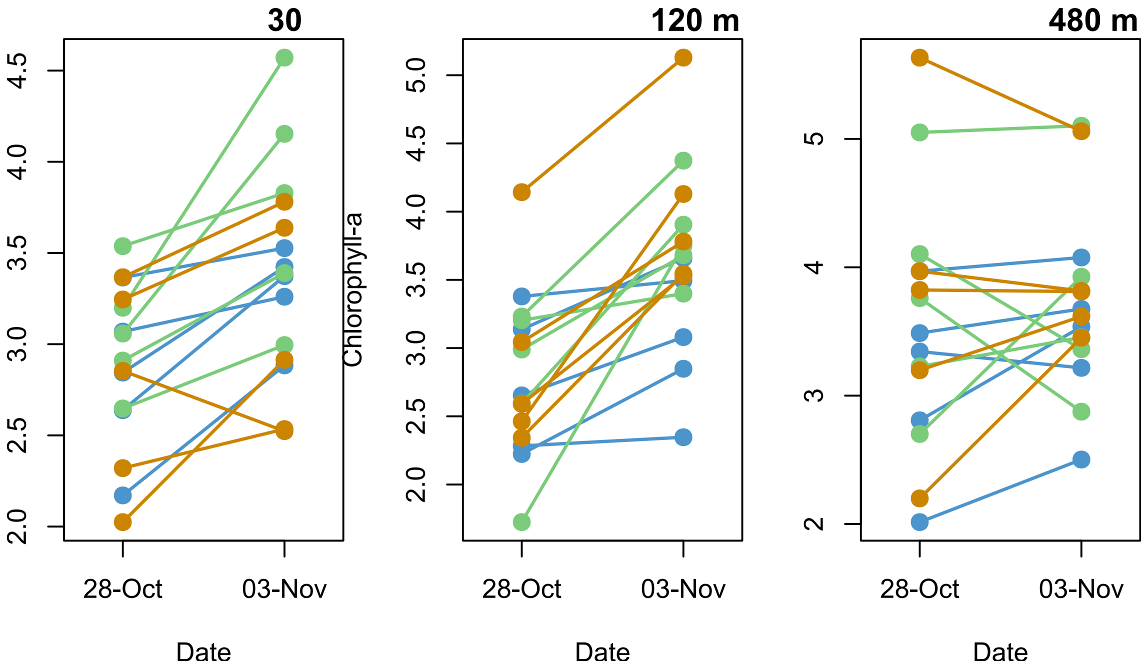

Figure S4.1. Chlorophyll-a change before (28-Oct) and after (03-Nov) fipronil application in different isolation levels and land use treatments. Yellow: sugarcane; green: pasture; blue: control.

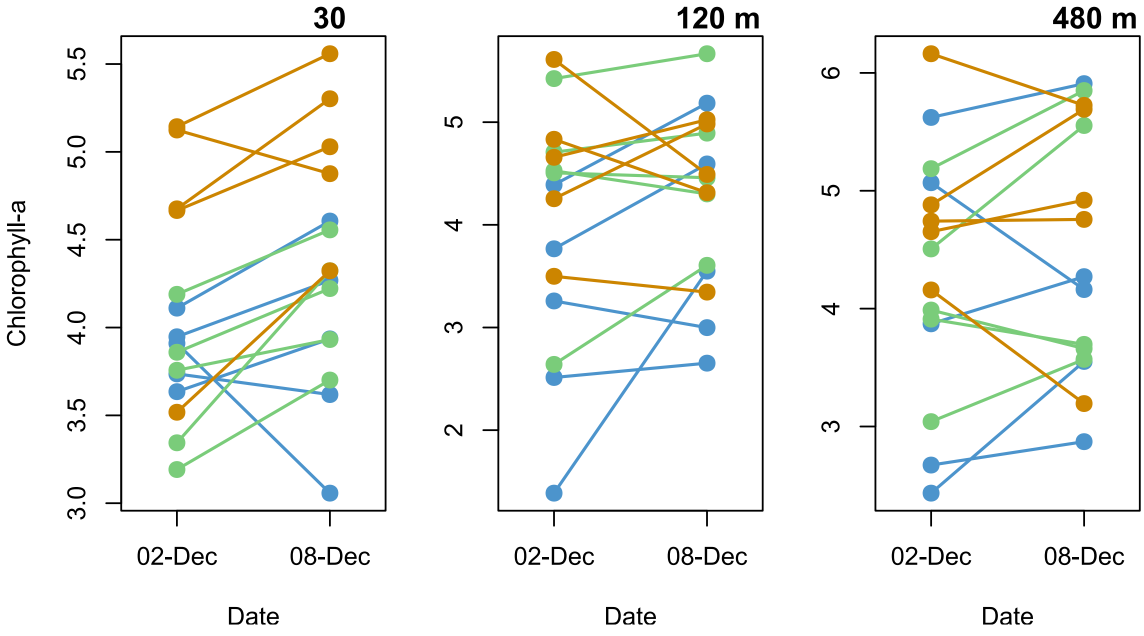

Figure S4.2. Chlorophyll-a change before (02-Dec) and after (08-Dec) 2,4-D application in different isolation levels and land use treatments. Yellow: sugarcane; green: pasture; blue: control.

SUPPLEMENT 5

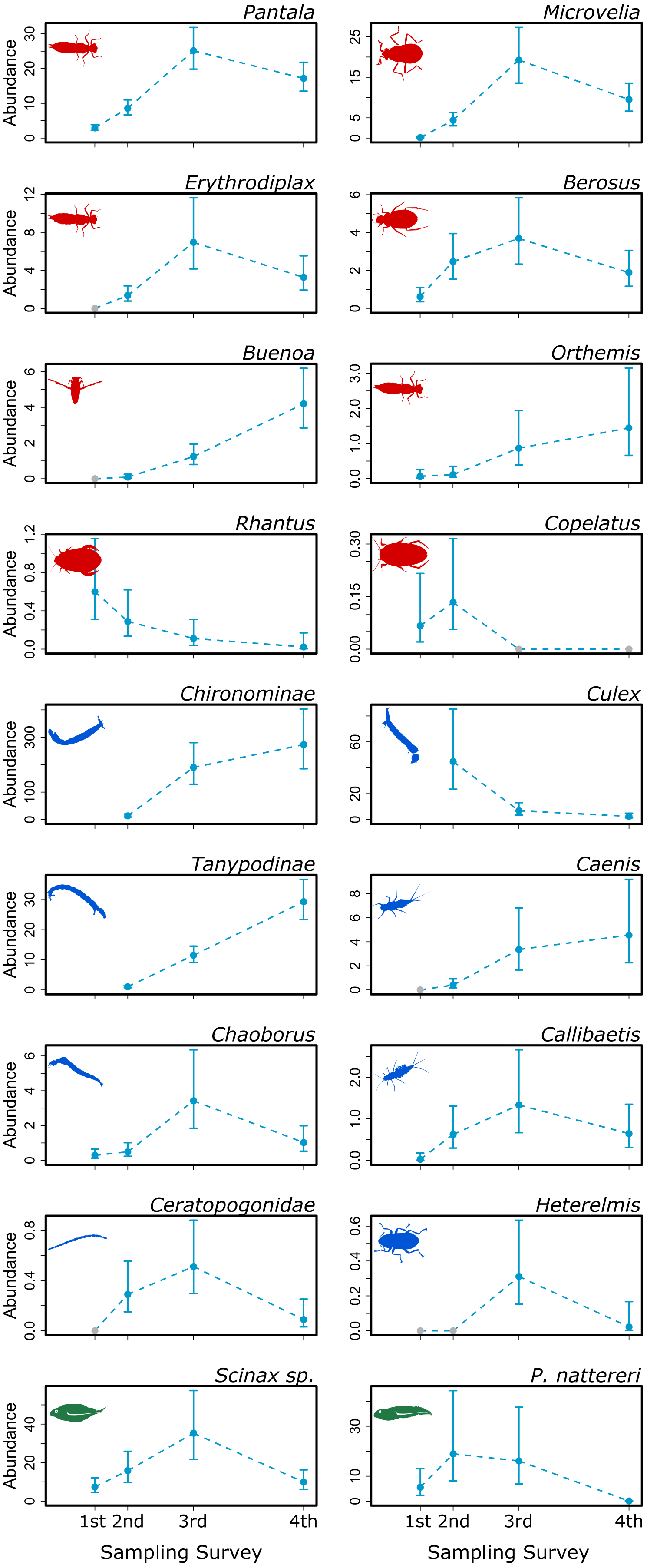

Figure S5.1. Effect of time on species abundances according to maximum likelihood estimates of abundance and their 95% confidence intervals. Grey symbols indicate absolute absence (zero abundance) of a taxon in a given survey. Maximum likelihood estimates (dots) that are not contained inside the 95% confidence interval of other estimates were considered to be different. Abundances for Culicidae, Chironominae and Tanypodinae were only comparable among the last three surveys because of different sampling methods, therefore they were omitted for the first survey here.

SUPPLEMENT 6

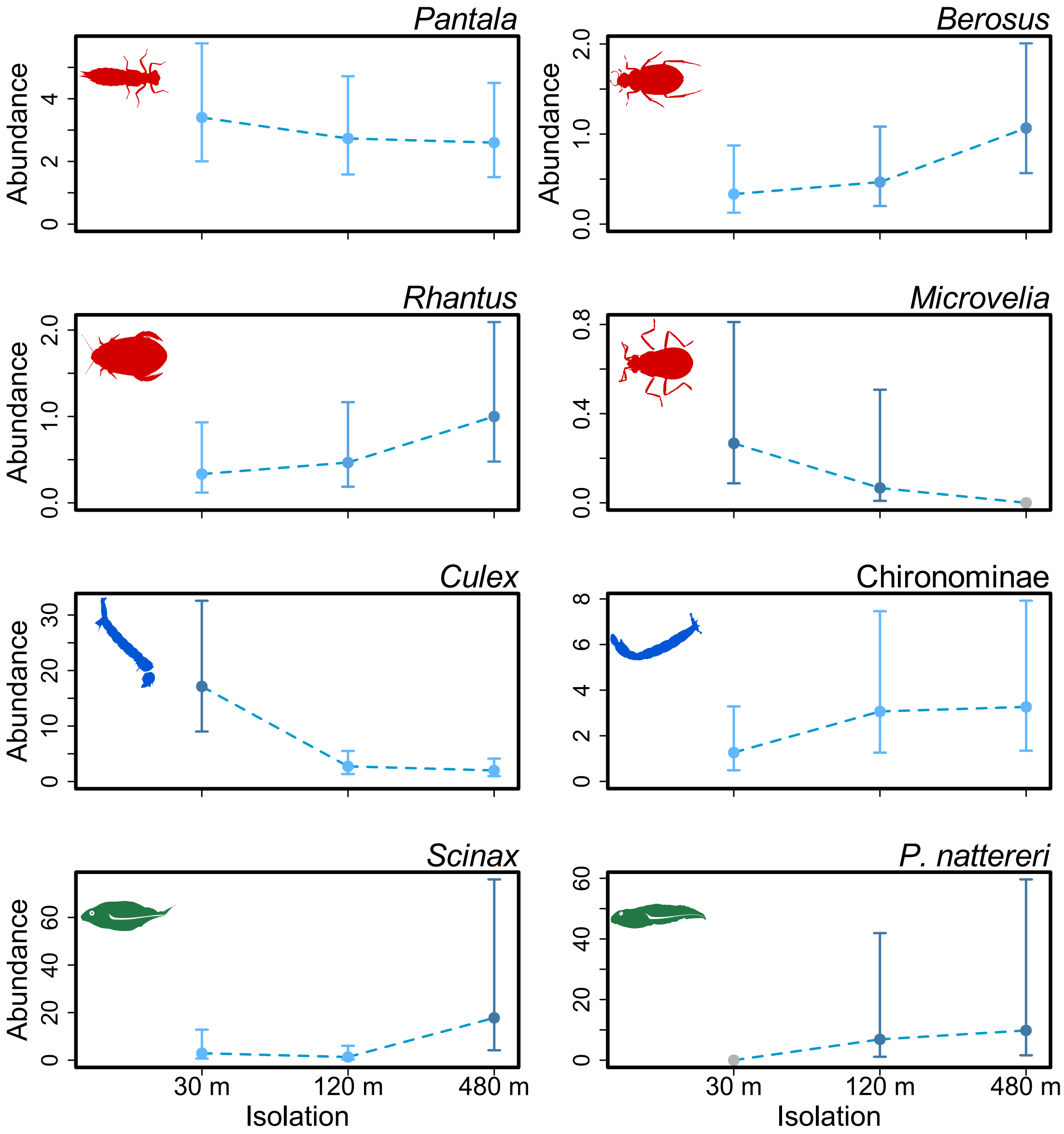

Figure S6.1. Effect of isolation on species abundances in the first sampling survey according to maximum likelihood estimates (MLE) of abundance and their 95% confidence intervals for Model 1 in Table 1 of the manuscript. Grey symbols represent absolute absence (zero abundance) of a taxon in a given treatment. 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 Supplement 6.

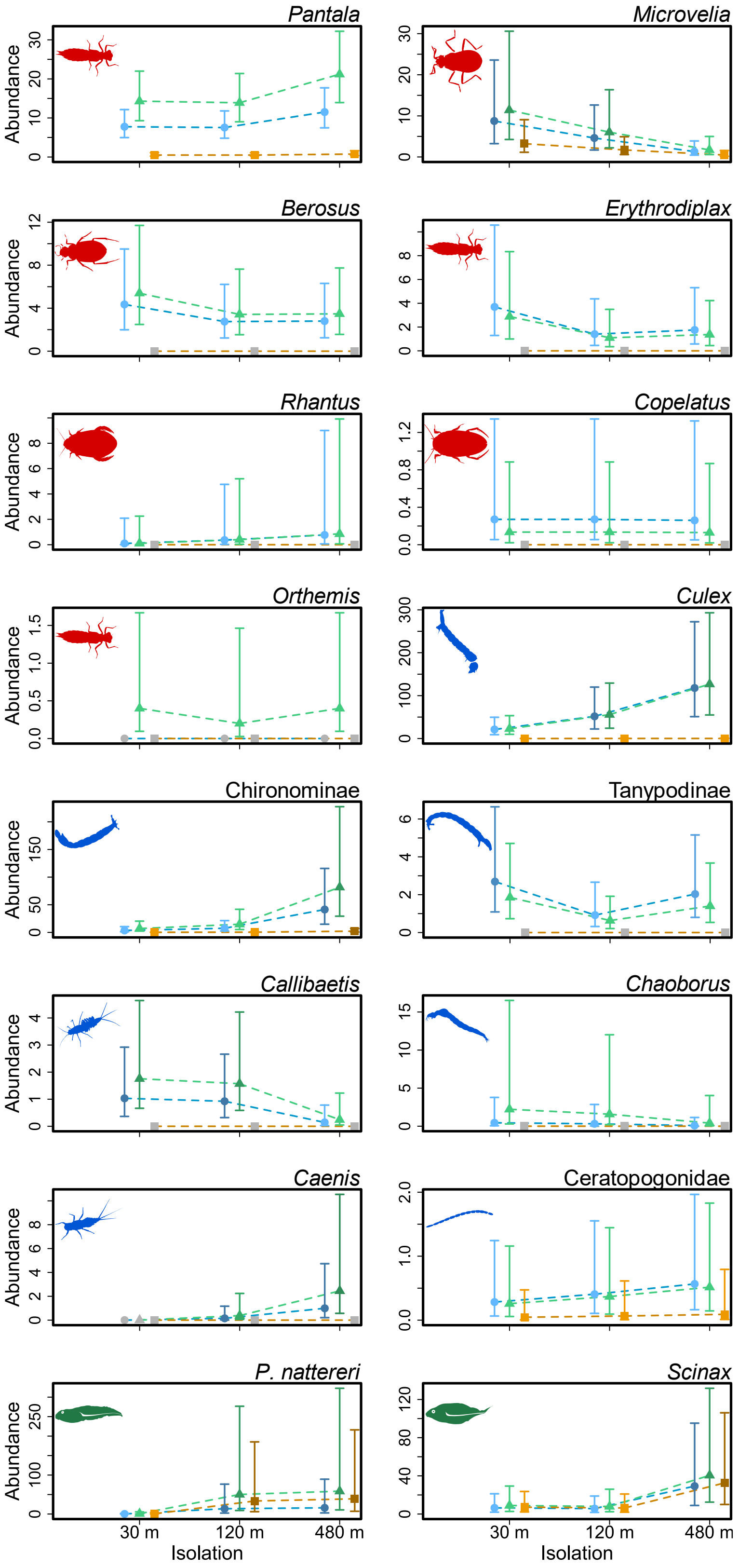

Figure S6.2. Effect of land use treatments and isolation on species abundance in the second sampling survey according to maximum likelihood estimates (MLE) of abundance and their 95% confidence interval for Model 5 in Table 1 of the manuscript. Blue circles, green triangles and yellow squares represent control, pasture and sugarcane estimates. Grey symbols represent absolute absence (zero abundance) of a taxon in a treatment. The actual estimated differences are provided in Supplement 6.

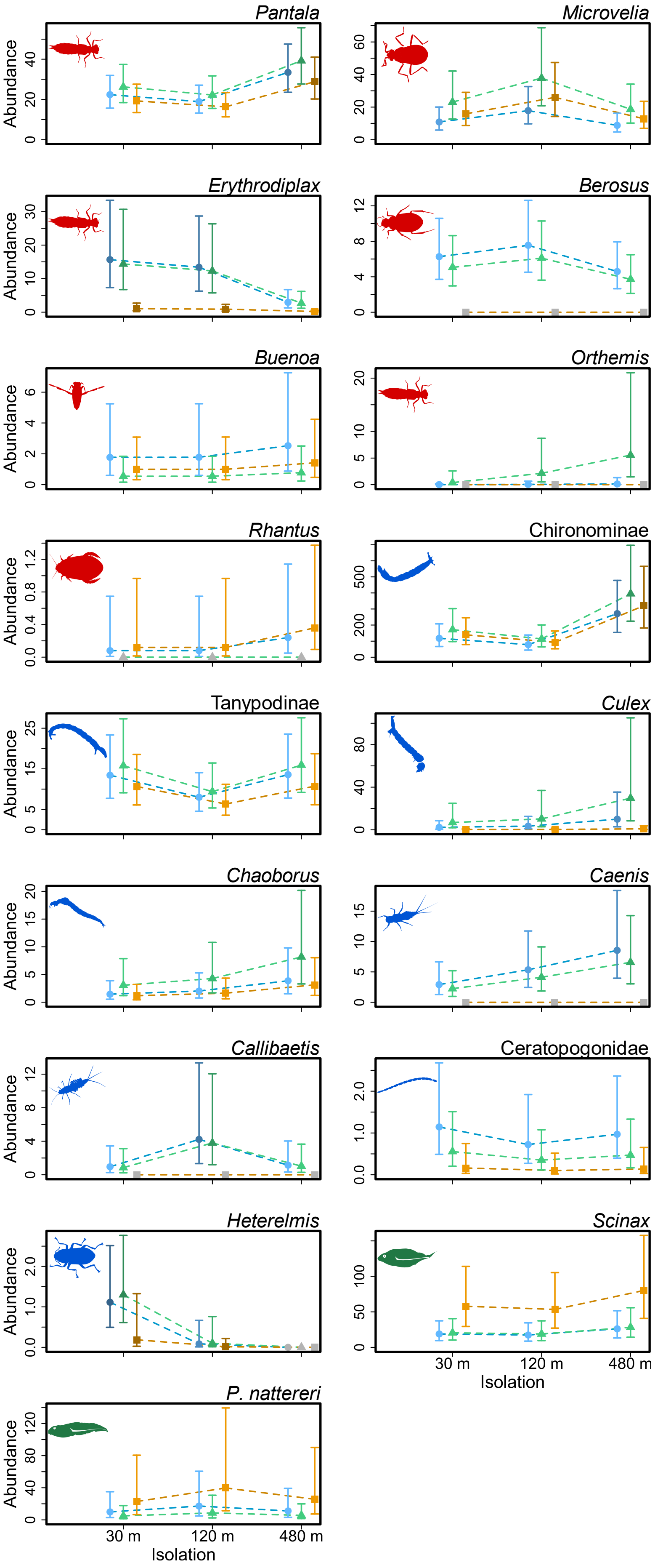

Figure S6.3. Effect of land use treatments and isolation on species abundance in the third sampling survey according to maximum likelihood estimates (MLE) of abundance and their 95% confidence interval for Model 12 in Table 1 of the manuscript. Blue circles, green triangles and yellow squares represent control, pasture and sugarcane estimates. Grey symbols represent absolute absence (zero abundance) of a taxon in a treatment. The actual estimated differences are provided in Supplement 6.

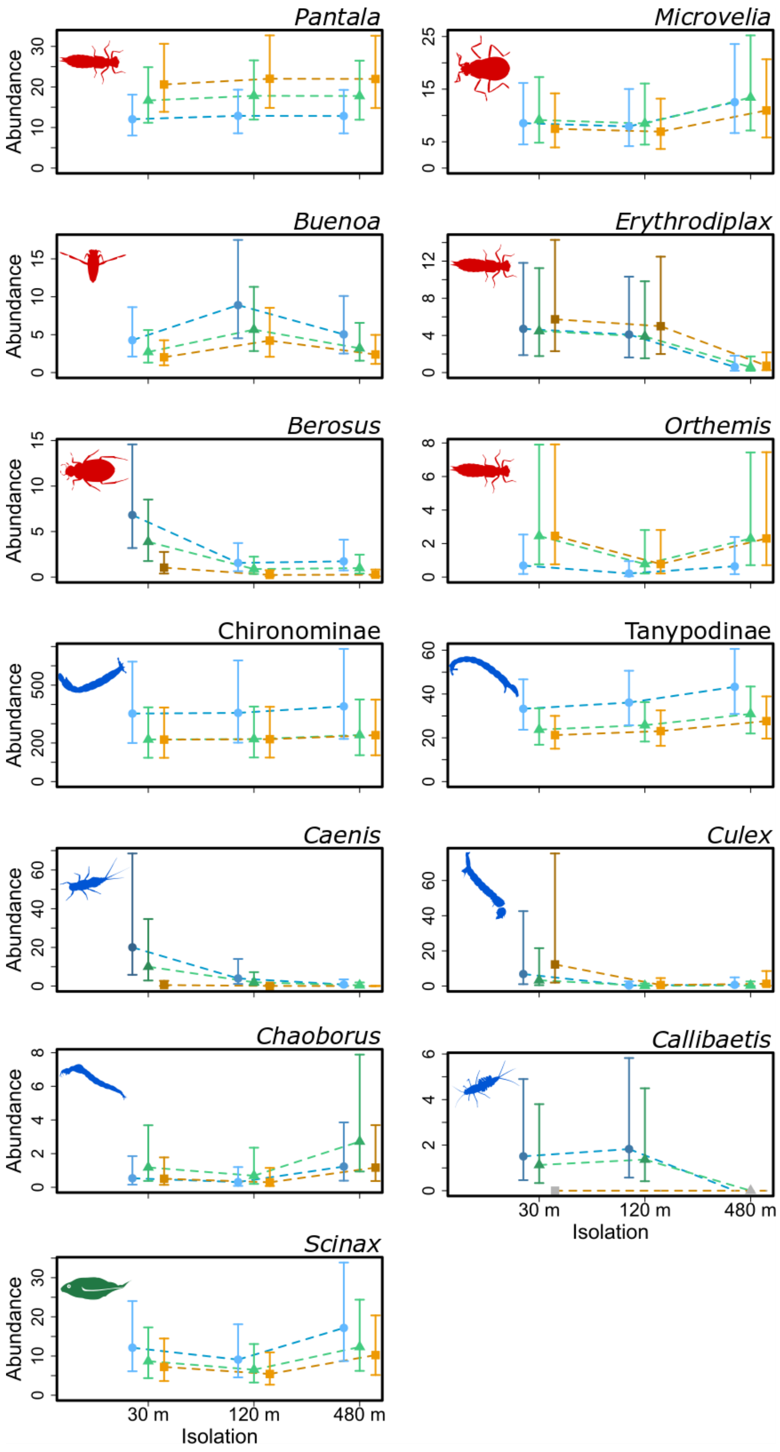

Figure S6.4. Effect of land use treatments and isolation on species abundance in the fourth sampling survey according to maximum likelihood estimates (MLE) of abundance and their 95% confidence interval for Model 19 in Table 1 of the manuscript. Blue circles, green triangles and yellow squares represent control, pasture and sugarcane estimates. Grey symbols represent absolute absence (zero abundance) of a taxon in a treatment. The actual estimated differences are provided in Supplement 6.

SUPPLEMENT 7

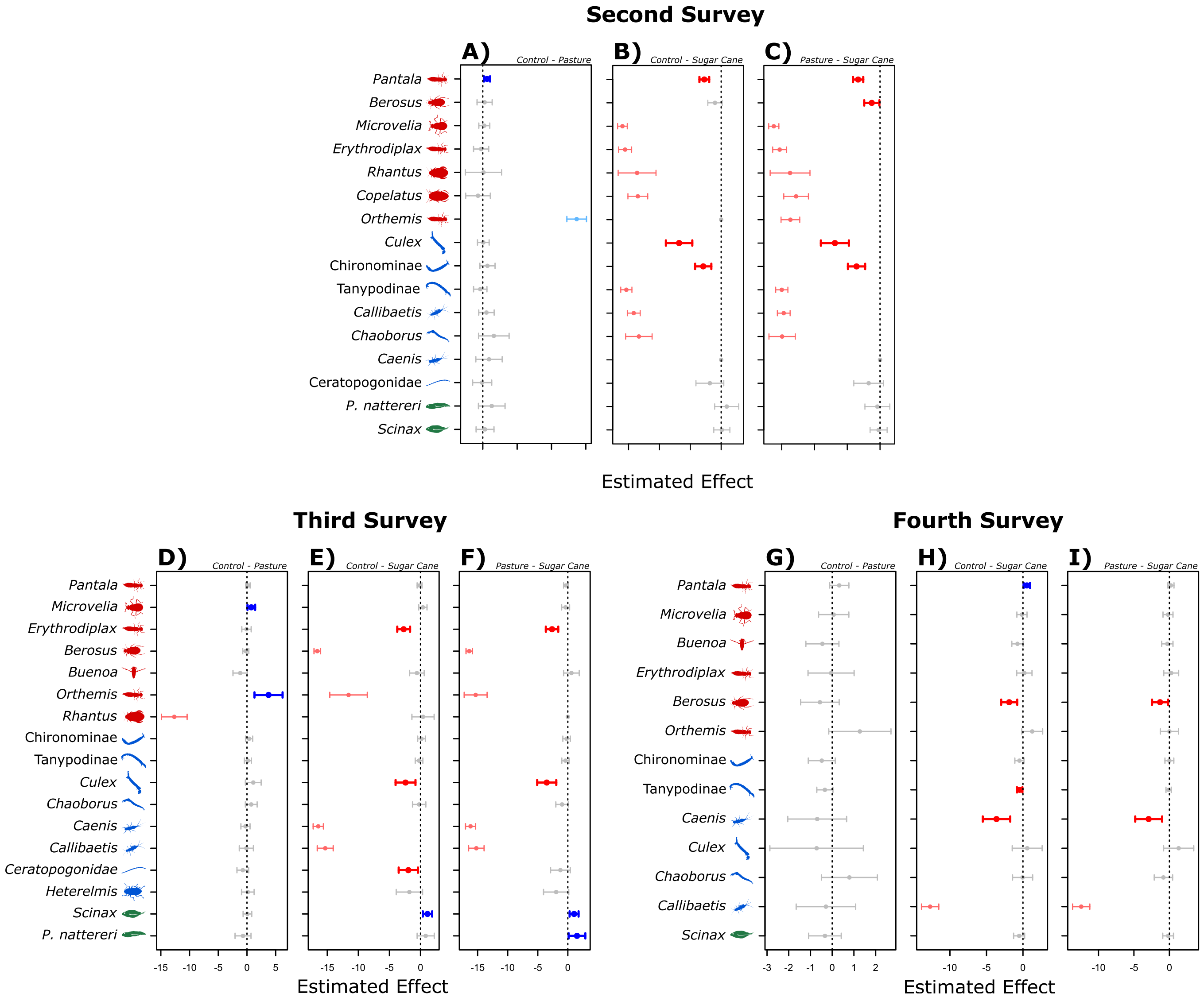

Figure S7.1. Confidence intervals for the effect of land use treatments on abundance for each taxon estimated from Models 5, 12 and 19 in Table 1. Taxa are ordered from top to bottom: predators, insect consumers and amphibians, from the most to less abundant taxon. Bars which the 95% confidence interval does not cross the zero-line are colored. Blue bars mean an increase in abundance from the reference treatment to the other. Red bars mean a decrease in abundance from the reference treatment to the other. Lighter blue bars mean that a taxon was absent from the reference treatment. Lighter red bars mean that a taxon was absent from the treatment which the reference is being compared to. A, B and C are effects for the second survey, D, E and F are for the third, and G, H and I are for the fourth. A, D and G are effects of pasture compared to control. B, E and H are effects of sugar cane compared to control. C, F and I are effects of sugar cane compared to pasture.

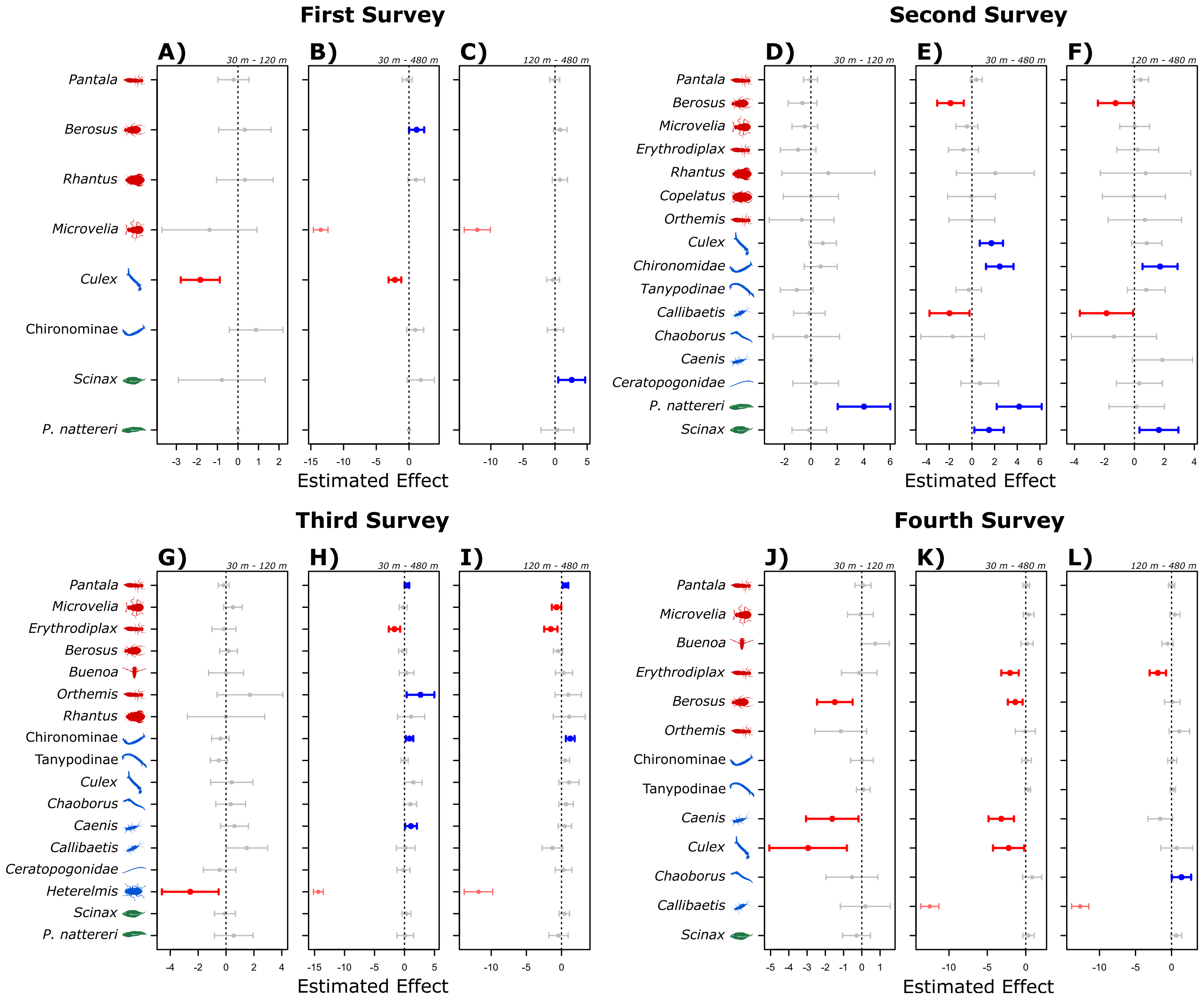

Figure S7.2. Confidence intervals for the effect of isolation on abundance for each taxon estimated from Models 1, 5, 12 and 19 in Table 1. Taxa are ordered from top to bottom: predators, insect consumers and amphibians, from most to less abundant. Bars which the 95% confidence interval does not cross the zero-line are colored. Blue bars mean an increase in abundance from the reference treatment to the other. Red bars mean a decrease in abundance from the reference treatment to the other. Lighter blue bars mean that a taxon was absent from the reference treatment. Lighter red bars mean that a taxon was absent from the treatment which the reference is being compared to. A, B and C are effects for the first survey, D, E and F are for the second, G, H and I are for the third and J, K and L are for the fourth. A, D, G and J are effects of 120 m compared to 30 m. B, E, H and I are effects of 480 m compared to 30 m. C, F, I and L are effects of 480 m compared to 120.

Chapter 3

Presence of top predator reverses the effects of dispersal limitation on beta-diversity

ABSTRACT

In the absence of environmental heterogeneity, variation among communities (*i.e.*, beta-diversity) is attributed to demographic stochasticity (*i.e.*, drift) and historical contingency (*e.g.*, priority effects). Consequences of demographic stochasticity are highly dependent on community size, gamma, and alpha diversity, which, along with historical contingency, can be strongly affected by dispersal limitation and presence of predators. We used freshwater insect communities to experimentally test whether and how the presence of generalist predatory fish and dispersal limitation (*i.e.*, isolation by distance from a source habitat) can change the relative importance of ecological drift and historical contingency in beta-diversity patterns. We were able to tease apart the effects of ecological drift from historical contingency by comparing observed to expected patterns of beta-diversity generated by null models. We expected that, because fish tend to prey on a defined subset of species and predatory insects have lower dispersal rates than consumers, both fish and spatial isolation would have negative effects on beta-diversity by reducing gamma diversity. Instead, we found that, because consumers are the majority of individuals in these communities, gamma diversity is unaffected. We also expected that, if historical contingency was important in this system, unequal dispersal rates would negatively affect beta-diversity because species dispersal rates would determine community composition in isolated habitats. However, historical contingency caused beta-diversity to increase with isolation, likely because most consumers have similar dispersal rates. Interestingly, and in stark opposition to fishless ponds and evidence from other systems, when predatory fish was present, beta-diversity was actually negatively affected by isolation, and it was not due to historical contingency. Instead, because most predatory insects cannot reach highly isolated ponds, consumers increase in abundance, thus increasing community size, which decreases the importance of demographic stochasticity, negatively affecting beta-diversity.

INTRODUCTION

The reasons why communities differ from each other in species composition and abundances is a central question in community ecology (Vellend 2016). Ecologists have traditionally considered that environmental variation in local conditions is the main driver of beta-diversity. Yet, considerable variation in community composition and structure arises even among communities under similar environmental conditions (Vellend *et al.* 2014; Vellend 2016; Leibold & Chase 2018). Beta-diversity among habitats with similar environmental conditions is now recognized as mostly a consequence of processes such as demographic stochasticity (*i.e.,* stochastic events of birth and death; ecological drift; (Vellend 2010, 2016) and historical contingency in colonization leading communities to multiple structures (Vellend *et al.* 2014; Fukami 2015).

The consequences of ecological drift on beta-diversity can be enhanced or dampened by any processes affecting gamma diversity (*i.e.,* regional richness), alpha diversity (*i.e.,* local richness), and community size (*i.e.,* total number of individuals in a community; Mouquet & Loreau 2003; Chase 2007, 2010; Chase *et al.* 2009). For instance, processes that increase regional but not local richness can result in higher beta-diversity simply because they increase the set of possible different community compositions (*i.e.,* a statistical inevitability; Chase *et al.* 2009; Chase & Myers 2011). Species abundance distributions (*i.e.,* community size) are also important in this context. Small populations are more likely affected by stochastic events of birth and death than larger populations. Thus, communities where species are less abundant tend to have higher beta-diversity as well (Myers *et al.* 2015).

If historical contingency is important in a given biological system, the order of arrival of species have a strong deterministic effect on community structure (Chase 2003; Shurin *et al.* 2004; Fukami 2015). For instance, if the identity of the first colonizers of a community is sufficiently random, the colonization sequence in different communities will follow different deterministic orders, and community structures will differ more than would be expected by simple demographic stochasticity (Vellend *et al.* 2014; Fukami 2015). In this case, because the order of arrival of species is random, species that arrive first have a competitive advantage over the others, thus leading communities to different structures. However, if the order of the first colonizers is deterministically determined to be the same in multiple communities, their structures will be more similar to each other than would be expected by demographic stochasticity (Vellend *et al.* 2014).

Dispersal limitation can mitigate or strengthen the effects of both ecological drift and historical contingency on beta-diversity (Chase & Myers 2011; Vellend *et al.* 2014); Figure 1A and 1B). If species are similarly dispersal limited, recolonization after stochastic local extinctions might not happen, and ecological drift will have greater importance in community structure, increasing beta-diversity (Mouquet & Loreau 2003; Leibold *et al.* 2004; Figure 1A). This effect can be further reinforced if historical contingency is important in determining community structure since the first colonizers will have more time to grow in abundance before the arrival of possible competitors (Vellend *et al.* 2014; Fukami 2015; but see Vannette & Fukami 2017; Figure 1A). Alternatively, if species have different dispersal abilities, poor dispersers can be excluded from a landscape where habitats are isolated, decreasing gamma (Hendrickx *et al.* 2009) and beta-diversity (Chase & Myers 2011; Figure 1B). In this second scenario, if historical contingency is important, unequal dispersal rates can make beta-diversity even lower because the order of arrival of species in all communities would be determined by dispersal ability (Vellend *et al.* 2014; Figure 1B).

The presence of predators, like many strong environmental filters, is also an important driver of beta-diversity (Chase *et al.* 2009). If the presence of predators can decrease community size through consumption, it can both increase beta-diversity by increasing the importance of drift on the community structure and decrease the importance of historical contingency by reducing competition (Orrock and Fletcher 2005; Figure 1C). Predators can also decrease the importance of colonization sequence by suppressing competitive dominants, not necessarily affecting community size. If there is a trade-off among vulnerability to predation and competitive ability, predators might preferentially prey upon more vulnerable taxa allowing only weak competitors to persist irrespective of the order of arrival (Leibold 1996, 1999; Louette & de Meester 2007). Additionally, if predators preferentially prey upon a subset of species in a community, they can lead these prey to be regionally extinct, thus decreasing gamma and beta diversity (Chase *et al.* 2009; Figure 1C).

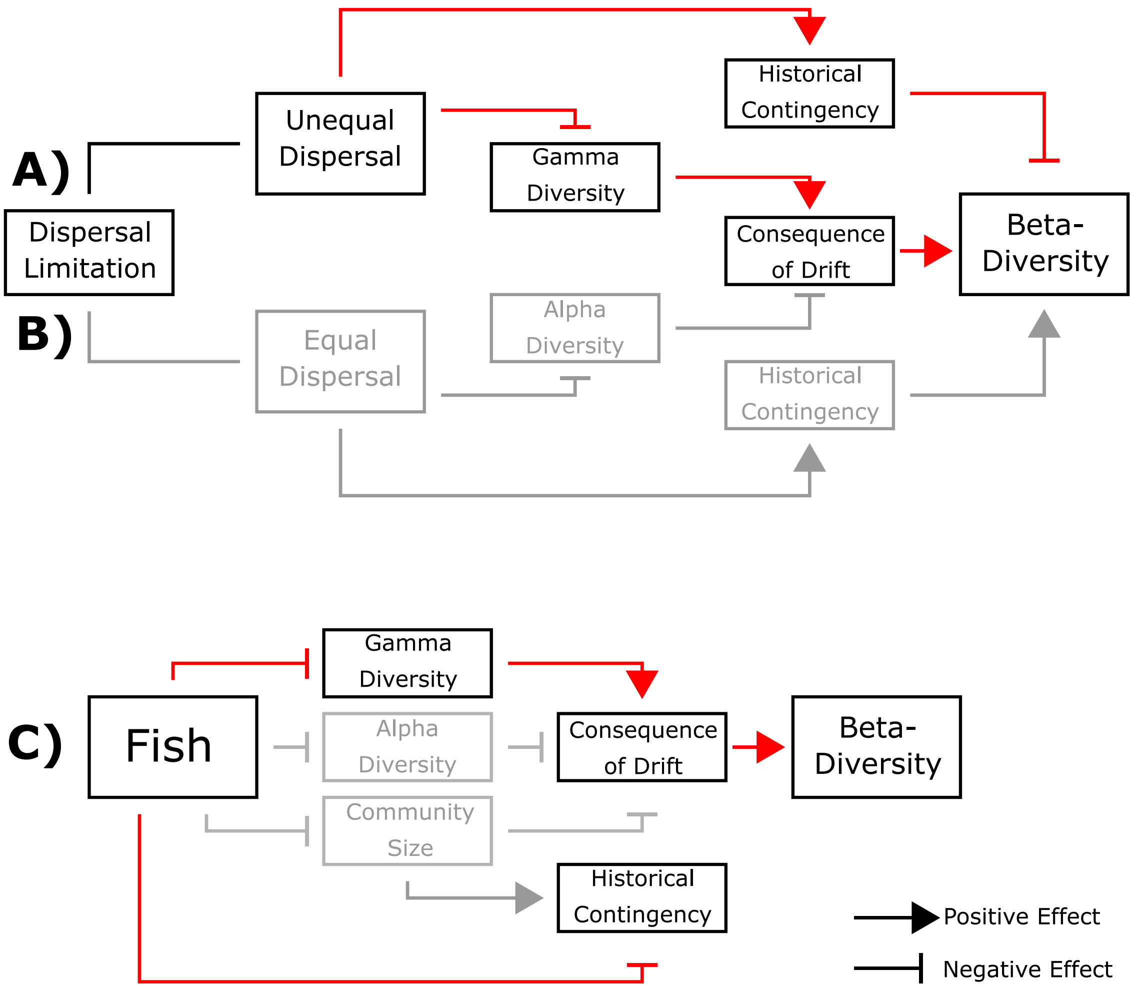

Figure 1. Different ways that dispersal limitation (A and B) and the presence of predatory fish (C) can affect beta-diversity. Colored paths (*i.e.*, non-grey) are those we believe are more likely to affect beta-diversity in freshwater macroinvertebrate communities. Red paths rep0resent a final negative effect of the path on beta-diversity.

One way to tease apart the deterministic effect of deterministic processes affecting community variability, including historical contingency, from ecological drift is by using null models to compute how much community variability would be expected in randomly reassembled communities under the same conditions of community size, gamma and alpha diversity (Chase *et al.* 2011). In this case, the deviations of the observed beta diversity from the expected, termed beta-deviation, would be caused by any deterministic effect not included in the null model, such as historical contingency (Chase *et al.* 2011; Kraft *et al.* 2011; Vannette & Fukami 2017).

Here we used freshwater aquatic and semi-aquatic insects to test how the presence of generalist predatory fish and dispersal limitation can change the relative importance of ecological drift and deterministic effects other than environmental heterogeneity (e.g., historical contingency) on Beta-diversity patterns. Fish are usually visually oriented predators; thus they tend to preferentially prey upon more conspicuous taxa (Diehl 1992; Goyke & Hershey 1992; Wellborn *et al.* 1996; Pelinson *et al.* 2019) including large-bodied predatory insects and, eventually, the most abundant consumers (Pelinson *et al.* 2019). Therefore, (H1), we believe that fish can more likely decrease beta-diversity by preferentially preying on a defined subset of prey, thus decreasing gamma diversity and promoting the homogenization of communities (Figure 2A). Freshwater insects are also a good example of a system where species have different dispersal rates. For instance, predatory insects are known to have smaller population sizes and greater generation times if compared to consumers; thus they have fewer events of dispersal (Shulman & Chase 2007; Chase & Shulman 2009; Hein & Gillooly 2011; Pelinson *et al.* 2019), what can cause many of them to be excluded from highly isolated ponds. Thus, (H2) we expect highly isolated habitats to have lower beta-diversity (Figure 2A) because poor dispersers would not be able to reach such patches frequently. Also, (H3) If colonization sequence is important in determining community structure, spatial isolation should make beta-diversity lower than would be expected by simple demographic stochasticity because the order of arrival of species will be determined by dispersal rates (Figure 2B). Finally, (H4) if historical contingency is indeed important in this system, the presence of fish should decrease it by always benefiting species that are less vulnerable to predation, irrespective of the order of arrival (Figure 2B).

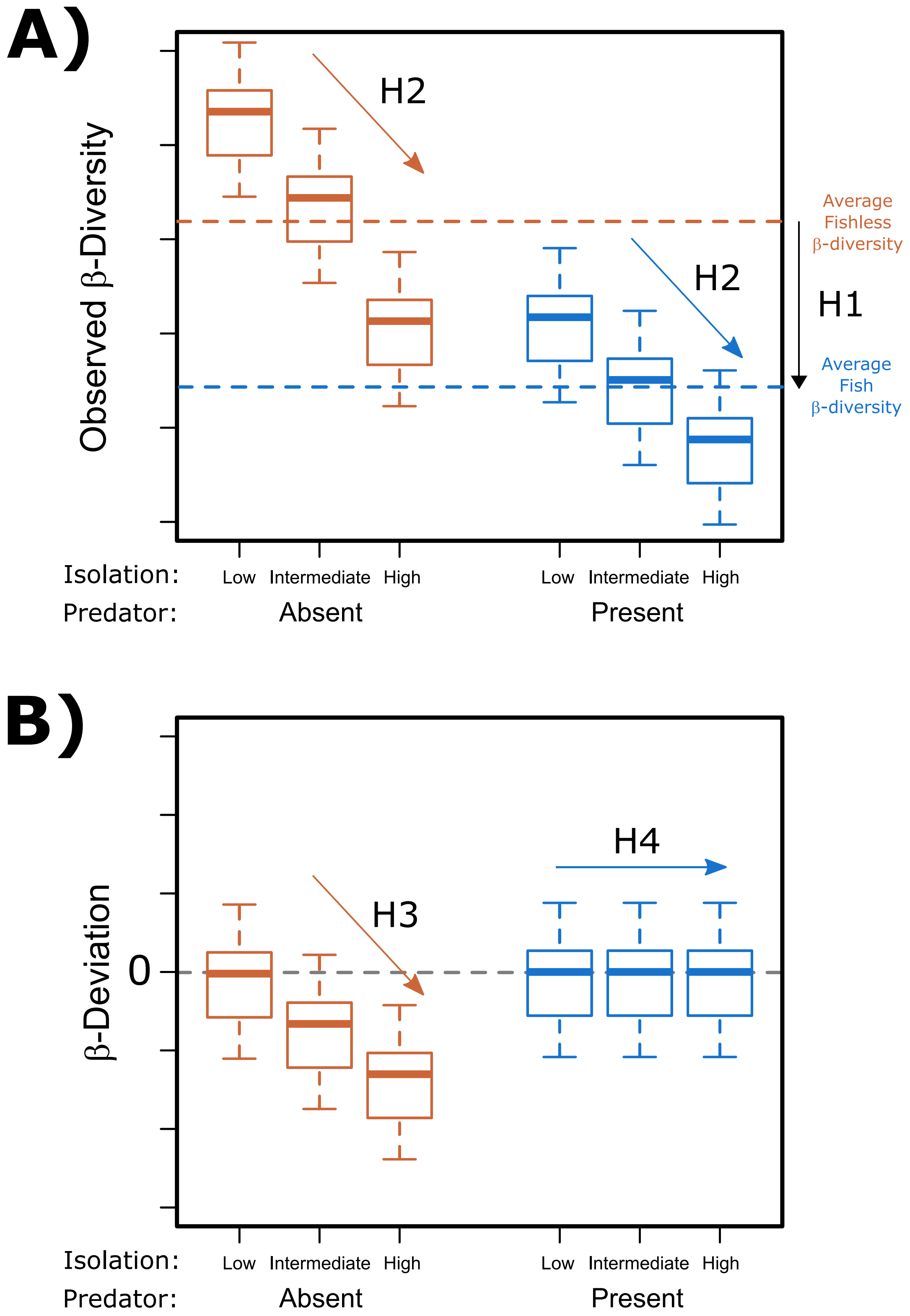

Figure 2. Conceptual boxplot of our *a priori* expectations of results of observed beta-diversity (A) and beta-deviation (B) for each isolation distances in ponds with (blue boxes) and without fish (orange boxes).

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 4m long, 1m wide and 40 cm deep. Walls were vertical along the length of the pond; 1m-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 oviposition habitat for insects and amphibians. Three 30 cm-long, ten cm-wide PVC pipes were placed in the water to provide shelter for fishes. The experiment followed a fully factorial design crossing fish presence (presence/absence) with spatial isolation (three levels of isolation). The manipulated fish was the Redbreast Tilapia (*Coptodon rendalli*, standard length 99.2 mm ± 5.9 mm, wet mass 40.2 g ± 8.8 g, mean ± SD) at a density of one individual per pond. The isolation treatment was achieved by establishing eight 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. 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. This experiment ran from 18-Jan-2017 to 24-Apr-2017. Between 18 and 25-Jan-2017 mesocosms were filled with well water. Fish were added on 29-Jan-2017. We conducted three sampling surveys of freshwater macroinvertebrates after ~3 weeks (18 to 23-Feb-2017), ~8 weeks (23 to 27-Mar-2017) and ~12 weeks (20 to 24-Abr-2017) of the experiment. Previously, we used this experiment to address how predators and preys change their abundance patterns according to spatial isolation and presence of predators, thus more detailed information of the experimental design is available in the Methods and Supplementary Material of Pelinson et al. (2019).

Data analysis

We ignored the first survey in our interpretation of results because stochasticity is predicted to be high in the early stages of community assembly, frequently overriding effects of treatments, which indeed occurred (see supplement 2). We compared the log-transformed total abundance of individuals (*i.e.,* community size) and observed richness across treatments for the two last surveys together through ANOVAs using the identity of ponds as a random effect term. We also performed *posthoc* pairwise permutational t-test comparisons to assess differences among specific treatments correcting p values for false discovery rate. Unfortunately, we lost samples from four ponds in the third survey; therefore, in the third survey, treatments with fish in 30 m, 120 m, and 480 m, and without fish in 480 m, had only three replicates (see Pelinson et al. 2019). We, therefore, compared gamma diversity in each treatment by computing the effective number of species (*i.e.,* Hill numbers, Chao et al. 2014; Hsieh et al. 2016), that is, we computed the number of species in each treatment for a similar number of sampled ponds through sample-based rarefaction and extrapolation (Colwell et al. 2012).

To test the hypothesis concerning how beta-diversity is affected by isolation and presence of fish, we used distances from each replicate pond (*i.e.*, communities) to their group spatial centroid in multivariate space as response variables (Anderson *et al.* 2006). This is achieved by placing a distance matrix of any measure of dissimilarity between pairs of observations into a multivariate Euclidean space through principal coordinate analysis (PCoA; Anderson 2006). In this case, greater distances to the group spatial centroid reflect larger beta-diversity. We used the Bray-Curtis distance on abundance data to compute the dissimilarity matrices. To not confound effects of location (*i.e.*, differences among treatments) and dispersion (*i.e.*, differences within treatments) in multivariate space we computed distances to centroid for each crossed treatment separately for all tests (*e.g.*, ponds with and without fish, in different isolations, in different surveys, were considered different treatments even when we tested only for differences among levels of one specific factor, such as fish and fishless ponds). To tease apart the community dissimilarity caused by variations in community size, gamma, and alpha diversity from deterministic processes leading to homogenization or divergence of structures, we used a null model approach to calculate the expected community similarity in the absence of such deterministic processes in 1,000 simulated communities. We used a null model that shuffles individuals across communities but preserves the number of absences in the matrix (*i.e.,* matrix fill), total species abundances in each community (*i.e.*, row sums) and total regional abundance of each species (*i.e.*, column sums). We did that for each treatment separately to keep gamma and average alpha diversity constant in each treatment. By not allowing individuals occurring in a given treatment to occur in any other treatment, we were able to assess the expected stochasticity in a landscape that supposedly only contains ponds of the specific condition described by its assigned treatment. Then we calculated how much distances to centroids deviates from expected distances (*i.e.,* beta-deviation; Chase *et al*. 2011, Kraft *et al.* 2011) by subtracting the average expected distance to the centroid of each community from its observed distance and dividing by the standard deviation of the expected distance. This method enables statistical tests of treatment effects on beta deviation (see Vannette & Fukami 2017).

We tested for differences of observed distances to centroid and beta-deviations among treatments also through ANOVAs with pond identity as a random effect term and *posthoc* pairwise comparisons with corrected p-values. The analyses were performed in R version 3.6.1 (R Core Team 2019). ANOVAs were performed using function *Anova()* from package ‘car’ version 3.0-4 (Fox & Weisberg 2019). Pairwise comparisons were done by using function *pairwise.perm.t.test()* from package ‘RVAideMemoire’ version 0.9-73 (Hervé 2019). Sample-based rarefaction and extrapolations were done using function *iNEXT()* from package ‘iNEXT’ version 2.0.19 (Hsieh *et al.* 2016). Distances to centroid were computed using function *betadisper()* from package ‘vegan’ version 2.5-5 (Oksanen *et al.* 2019). The null communities were generated using function *permatswap()* also from ‘vegan’. We also used the same null model used by Siqueira et al. (2019), but results followed the same general patterns (see supplement 5).

RESULTS

Community size grew significantly from the second to the third survey, and this effect was particularly strong in the most isolated (*i.e.*, 480 m) ponds (Table 1; Figure 3). Alpha diversity (*i.e.*, local richness) was negatively affected by the presence of fish (Table 1; Supplement 4), and gamma diversity (*i.e.*, treatment richness) was generally not different among treatments, except for fishless ponds in the intermediate isolation treatment in the last survey, which had the highest number of species in our experiment (see supplement 3).

Table 1. ANOVA table of linear mixed models for pond total abundance (log-transformed) and local richness.

		Abundance		**Local Richness**	
	Df	F	p	F	P
Fish	1	0.042	0.84	**8.545**	**0.01**
Isolation	2	0.196	0.824	0.008	0.992
Sampling Survey	**1**	**20.547**	**<0.001**	0.104	0.751
Fish : Isolation	2	0.339	0.717	1.5	0.252
Fish : Sampling Survey	1	3.73	0.072	0.075	0.788
Isolation : Sampling Survey	**2**	**5.471**	**0.016**	2.154	0.148
Fish : Isolation : Sampling Survey	2	0.165	0.849	1.776	0.2

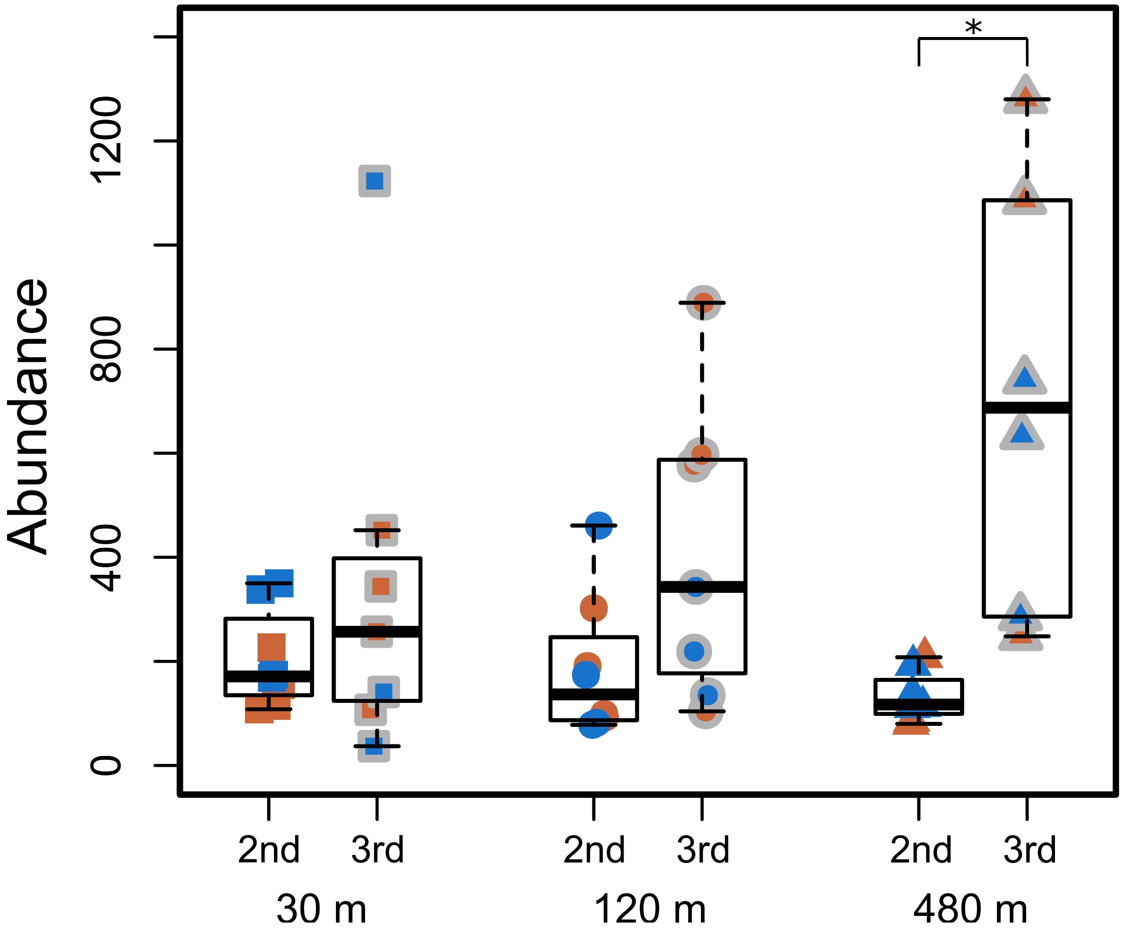

Figure 3. Box plot of abundance (*i.e.*, community size) by sampling survey in each isolation treatment. Squares, circles, and triangles are respectively 30 m, 120 m, and 480 m isolation treatments. Orange represents fishless ponds, whereas blue represents ponds with fish. Symbols without a grey border are from the second survey, whereas symbols with a gray border are from the third survey. Asterisks show significant differences among pairs of treatments. Because we were interested in assessing the effects of sampling surveys in different isolation treatments, pairwise comparisons were only performed between the second and third surveys for each isolation treatment.

Both observed beta-diversity and beta-deviation were significantly explained by an interaction between spatial isolation and the presence of fish; no other term or interaction influenced beta-diversity and beta-deviation (Table 2). Observed beta diversity significantly increased from low to intermediate isolation in fishless ponds, but none of them were different from the most isolated treatment (Figure 4A). The values of beta-deviation for fishless ponds followed a similar pattern, except that in this case, beta-deviations both from the intermediate and highest isolation treatments were significantly higher than those of the lowest isolation treatment. (Figure 4B). Additionally, values of beta deviation for fishless ponds went from values close to 0 in 30 m isolation (*i.e.*, expected by the null models) to positive (higher beta-diversity than expected by null models) in 120 m and 480 m of isolation. When we considered only ponds with fish, we observed a significant decrease in observed beta-diversity from 30 m to 480 m of isolation (Figure 4A). More importantly, values of beta-deviation for ponds with fish did not differ significantly among isolation treatments with values always close to the 0 line (*i.e.* observed beta-diversity is similar to expected by the null models; Figure 4B).

Table 2. Anova table of linear mixed models for values of observed beta-diversity and beta-deviation.

		Beta Diversity		**Beta-Deviation**	
	Df	F	p	F	p
Fish	1	1.305	0.269	4.131	0.058
Isolation	2	1.073	0.364	2.899	0.083
Sampling Survey	1	1.931	0.184	1.236	0.282
Fish : Isolation	2	**8.669**	**0.003**	**3.877**	**0.041**
Fish : Sampling Survey	1	0.051	0.824	0.339	0.568
Isolation : Sampling Survey	2	3.452	0.057	0.714	0.504
Fish : Isolation : Sampling Survey	2	2.634	0.102	1.039	0.376

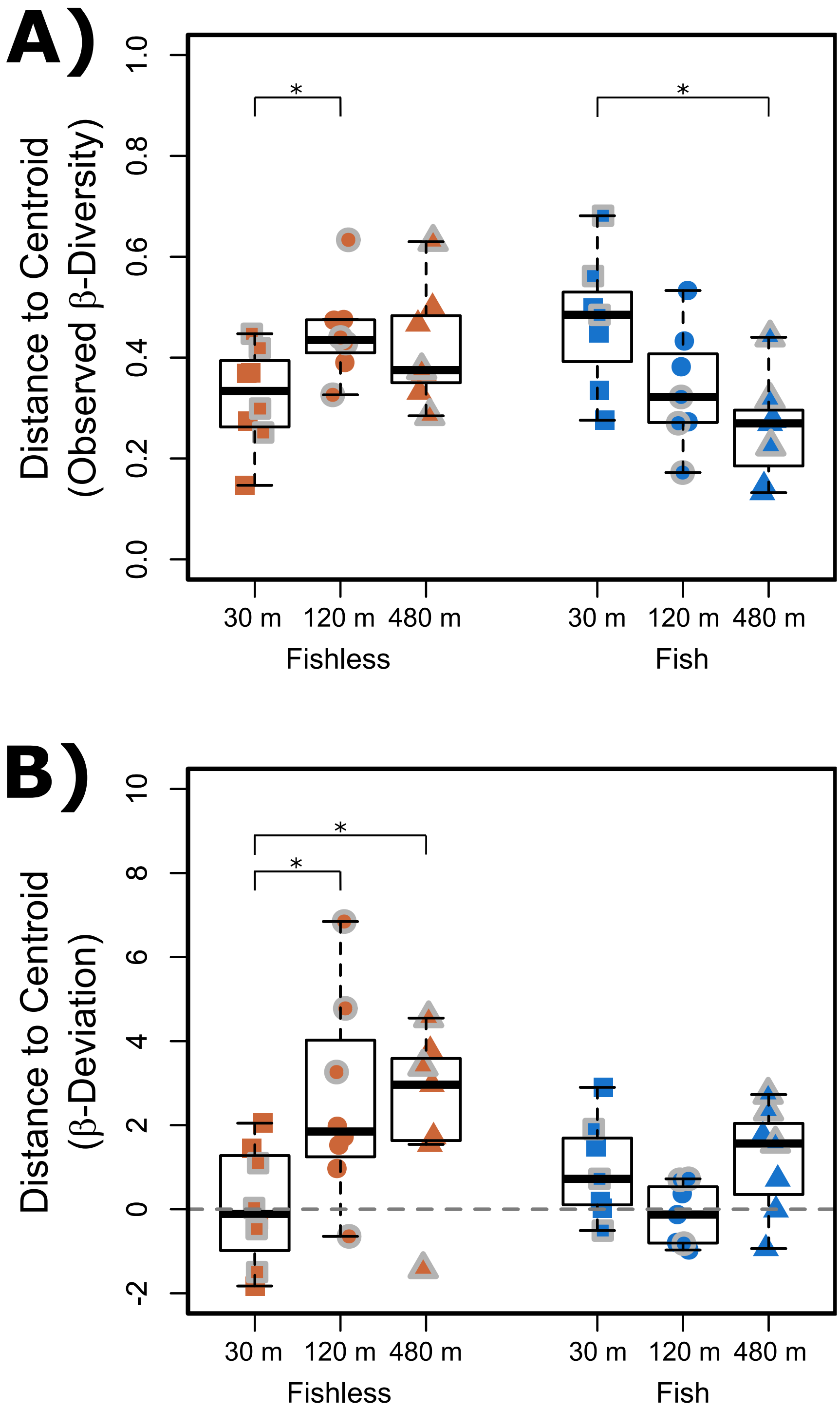

Figure 4. Box plots of values of distance to the centroid of observed dissimilarity values based on Bray-Curtis distance (A) and distance to centroid based on beta-deviation measures (B). Squares, circles, and triangles are 30 m, 120 m, and 480 m isolation treatments, respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey, whereas the ones with a gray border are from the third survey. Asterisks show significant differences among pairs of treatments. Because we were interested in assessing different effects of isolation in ponds with and without fish, pairwise comparisons were only made between isolation treatments within fish and fishless ponds.

DISCUSSION

Few works have paid attention to how much beta-diversity is due to pure ecological drift and deterministic processes not related to environmental heterogeneity, such as historical contingency (*e.g.*, Vellend *et al.* 2014, Vannette & Fukami 2017). Because our treatments did not have any environmental heterogeneity, the only source of variability among local communities (*i.e.*, beta-diversity) was either ecological drift or any other processes not related to environmental heterogeneity. Additionally, because we accounted for stochastic community assembly using null models, we could assess how much variability was generated by processes not related to ecological drift as beta-deviation. Thus, here we provide empirical evidence of how beta-diversity changes in a scenario of increasing dispersal limitation and the presence or absence of a generalist top predator.

Predatory fish are predicted to decrease beta-diversity because they could exclude a defined subset of vulnerable taxa, leading to a decline in gamma diversity (H1; Chase *et al.* 2009). Contrary to expected, however, fish had no direct effect on gamma or beta-diversity (Figure 5B). Fish was expected to mainly decrease the number of taxa of predatory insects, which indeed happens if we restrict our communities to predatory insects (Supplement 7). However, because of the numerical dominance of consumer insects in our experiment (85% of the individuals and 66% of the taxa in the experiment), when we consider all taxa, the decrease in the regional richness of predatory insects is not enough to yield significant differences among ponds with and without fish. Also considering all taxa, fish directly reduced alpha diversity (Figure 5B), which is predicted to increase beta-diversity, if gamma is unchanged (Chase *et al.* 2009). However, the observed differences might have been too small to have any consequences on beta diversity (2 species on average, see supplement 7). Also, differences in richness are usually caused by rare species, which have a low abundance and, therefore, low contributions to abundance-based beta-diversity metrics, such as the Bray-Curtis index (Barwell *et al.* 2015). Thus, community size appears to be the main driver of beta-diversity in our experiment. We previously observed that even though tilapias consume both predatory insects and consumers, the indirect positive effects of fish on consumers due to trophic cascades can offset its negative effects (Pelinson *et al*. 2019), thereby keeping community size and average beta-diversity relatively unchanged (Figure 5B).

We also predicted that beta diversity would decrease with spatial isolation because unequal dispersal rates would decrease regional richness (H2). In stark opposition to these predictions, we found a positive (albeit weak) effect of isolation on beta-diversity in fishless ponds. Indeed, most of the evidence and theoretical models of single trophic level metacommunities points out to a positive effect of dispersal limitation on beta-diversity (Mouquet & Loreau 2003, Astorga *et al.* 2012, Grainger & Gilbert 2016). This effect is, in general, due to either the decrease in the local richness that low dispersal confers (Mouquet & Loreau 2003, Chase & Myers 2011, Leibold & Chase 2018), which we did not observe, or to an increase in the importance of historical contingency. However, an important assumption for these predictions is that all species within a metacommunity have similar dispersal rates, which is not our case. If species have a lot of variation in dispersal rates, historical contingency is actually expected to decrease beta-diversity because the order of arrival of species is be determined by dispersal ability (H3; Vellend *et al.* 2014). Yet, we observed that more isolated fishless ponds had higher beta-diversity than what is expected only by demographic stochasticity (*i.e.,* higher beta-deviation), which is compatible with the predictions for communities where species have similar dispersal rates. We believe that, because consumers drove most of the patterns that we observed, it is plausible to assume that dispersal rates of most species in our community were similar. Thus, the order of species arrival in highly isolated habitats could have been enough random for historical contingency to lead communities to different structures (Vellend *et al.* 2014, Fukami 2015; Figure 5A).

Historical contingency is likely to play an important role in community variability when organisms have rapid life cycles, thus being able to establish large populations and not allowing invasion by species arriving later (*e.g.,* Louette & de Meester 2007, Leopold *et al.* 2017, Vannette & Fukami 2017). Because most freshwater insects inhabiting temporary ponds spend only part of their life cycle in water, a demographic preemption, as mentioned above, is unlikely because it is dependent on many repeated colonization events. Alternatively, priority effects in this system could arise due to post or pre-colonization species interactions. Competition can affect freshwater insect biomass and time to metamorphose (Blaustein & Margalit 1996) but is unlikely to change abundance patterns. Predation (including intraguild predation), by contrast, is common among freshwater insects (*e.g.,* Wissinger & McGrady 1993, Wissinger *et al.* 1996, Fincke 1999), as individuals arriving first become large enough to prey upon those arriving later.

Pre-colonization habitat selection could also yield similar consequences in community structure (Fukami 2015). Numerous works have shown that the decision of colonization is highly dependent on the identity and density of species that have already colonized a habitat (*e.g.,* Resetarits 2005, Sadeh *et al.* 2009, Kraus & Vonesh 2010, Pintar & Resetarits 2017, Trekels & Vanschoenwinkel 2019). For instance, several different species of mosquitoes have been shown to detect and avoid oviposition in ponds with both competitors (Blaustein & Kotler 1993) and predators (backswimmers: Eitam *et al.* 2002, Kiflawi *et al.* 2002, Blaustein *et al.* 2004, Binckley & Resetarits 2005, Negrão 2019; dragonflies: Stav *et al.* 1999, Negrão 2019; a combination of predators: Negrão, 2019). Therefore, the infrequent arrival, especially of predatory insects, in more isolated habitats could have strong and fast consequences on the choice of oviposition of consumers, leading more isolated habitats to be more variable than would be expected in the absence of such constraints.

The same effect of historical contingency was not observed in ponds with fish, as expected (H4). We expected that fish would erase the effect of historical contingency on community structure by preying upon the most vulnerable species irrespective of the order of arrival. We do know that predatory fish had a strong negative effect on predatory insects (Pelinson *et al.* 2019), which are more vulnerable to predation because their larger body size makes them easier to see. We also know that fish had a negative effect on consumers, dampening the indirect positive effect that the absence of predatory insects would have on consumer abundance (Pelinson *et al.* 2019). Thus, fish probably prevented both predatory insects and some consumers from becoming more abundant than others. However, we, again, do not know to what degree these effects were a result of direct predation, habitat selection, or both. For instance, fish can strongly affect the chance of oviposition of both primary and secondary consumers (*i.e.,* predatory insects; Vonesh *et al.* 2009). Either way, even though we don’t know exactly by what mechanism, we show that the presence of fish can override the deterministic effects of the order of arrival of aquatic insect’s on community structure (Figure 5B).

In the absence of the effects of historical contingency, beta-diversity indeed decreased with isolation in ponds with fish. More importantly, this decrease disappeared after accounting for demographic stochasticity with null models. Thus, it could be solely attributed to the effects of demographic stochasticity. However, this effect was still not a consequence of a decrease in regional richness. We believe that regional richness did not change with isolation because, as discussed above, most of our beta-diversity patterns were driven by insect consumers, which do not appear to be dispersal limited. Rather, the decrease in beta-diversity was likely due to an increase in community size with isolation. Other work has shown that because most predatory insects are dispersal limited and consumers are not, consumer populations tend to increase in more isolated habitats due to trophic cascades (Shulman & Chase 2007, Chase & Shulman 2009, Pelinson *et al.* 2019). This was likely what we observe here. Because community size is larger in more isolated habitats, random events of birth and death have little weight on general abundance patterns and, thus, community structure (Figure 5A).

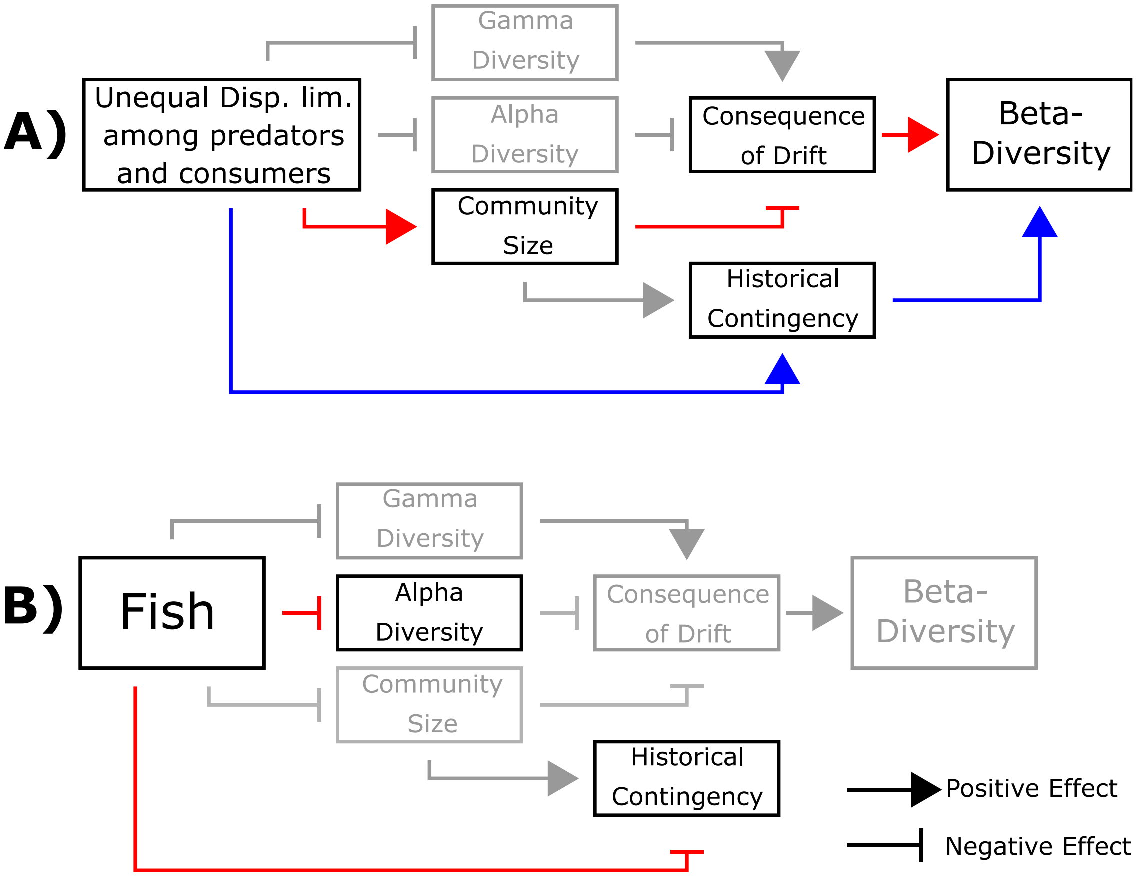

Figure 5. Different ways that dispersal limitation (A) and the presence of predatory fish (B) can affect freshwater macroinvertebrate beta-diversity. Colored paths (*i.e.*, non-grey) are those we found empirical evidence for in this work. Red paths represent a final negative effect of the path on beta-diversity. Blue paths represent a final positive effect on beta-diversity.

Finally, here we show that when predatory fish is absent, dispersal limitation can have positive effects on beta-diversity likely because of an increase in the importance of historical contingency in generating different community structures, even though we could not identify if it is due to pre (*i.e.*, habitat selection) or post-colonization (*i.e.*, direct predation) mechanisms. When fish is present, however, effects of historical contingency disappear, and the unequal dispersal rates among predatory insects and consumers cause community size to increase with isolation due to trophic cascades (Shulman & Chase 2007; Chase & Shulman 2009; Hein & Gillooly 2011; Pelinson *et al.* 2019). Such an increase in community size also decreases beta-diversity by reducing the consequences of demographic stochasticity on community structures. Therefore, we argue that considering multiple trophic levels is of fundamental importance when trying to understand patterns of community variability since it can substantially change how different processes can affect beta-diversity.

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Appendix - Chapter 3

SUPPLEMENT 1

Expected beta diversity used to compute beta-deviations showed in the manuscript.

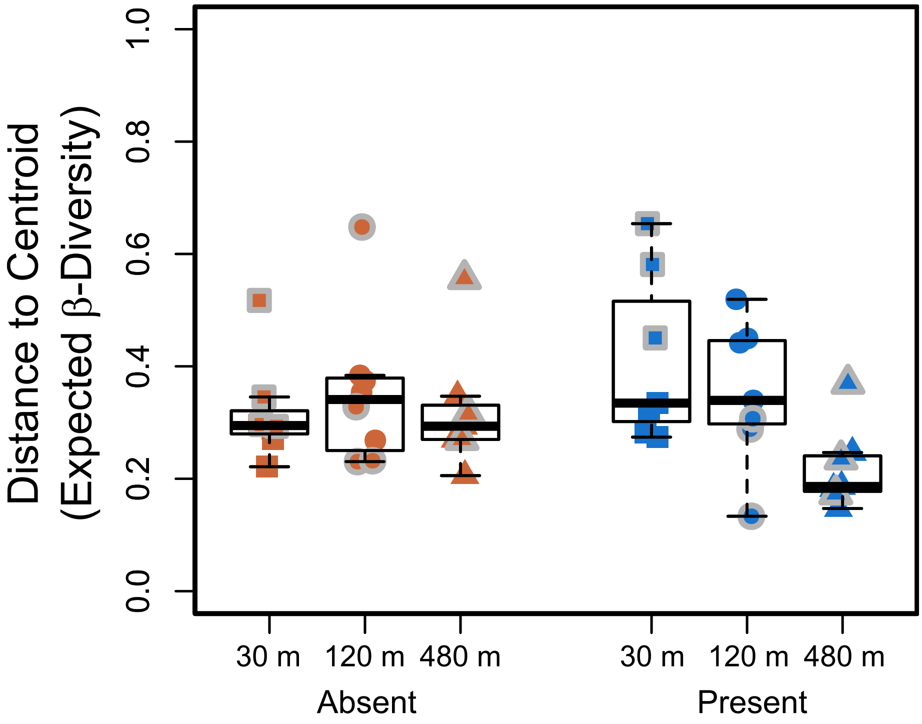


Figure S1.1. Box plots of values of expected distance to the centroid of 1000 randonly generated communities keeping community sizes, mean alpha diversity per treatment and number of species per treatment constant. Squares, circles, and triangles are 30 m, 120 m and 480 m isolation treatments respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey whereas the ones with a gray border are from the third survey. Asterisks show significant differences among pairs of treatments. Because we were interested in accessing different effects of isolation in ponds with and without fish, pairwise comparisons were only done between isolation treatments within fish and fishless ponds. Pairwise comparisons between among treatments for ponds with and without fish sepparetely showed that 30m treatments were significantly different from 480 m for ponds with fish.

Table S1.1. Anova table of linear mixed models for values of expected beta-diversity.

	Df	F	p
Fish	1	0.102	0.753
Isolation	**2**	**4.023**	**0.038**
Sampling Survey	1	2.756	0.116
Fish : Isolation	**2**	**5.022**	**0.02**
Fish : Sampling Survey	1	0.412	0.53
Isolation : Sampling Survey	**2**	**5.234**	**0.017**
Fish : Isolation : Sampling Survey	2	2.811	0.089

SUPPLEMENT 2

Results for the first sampling survey only.

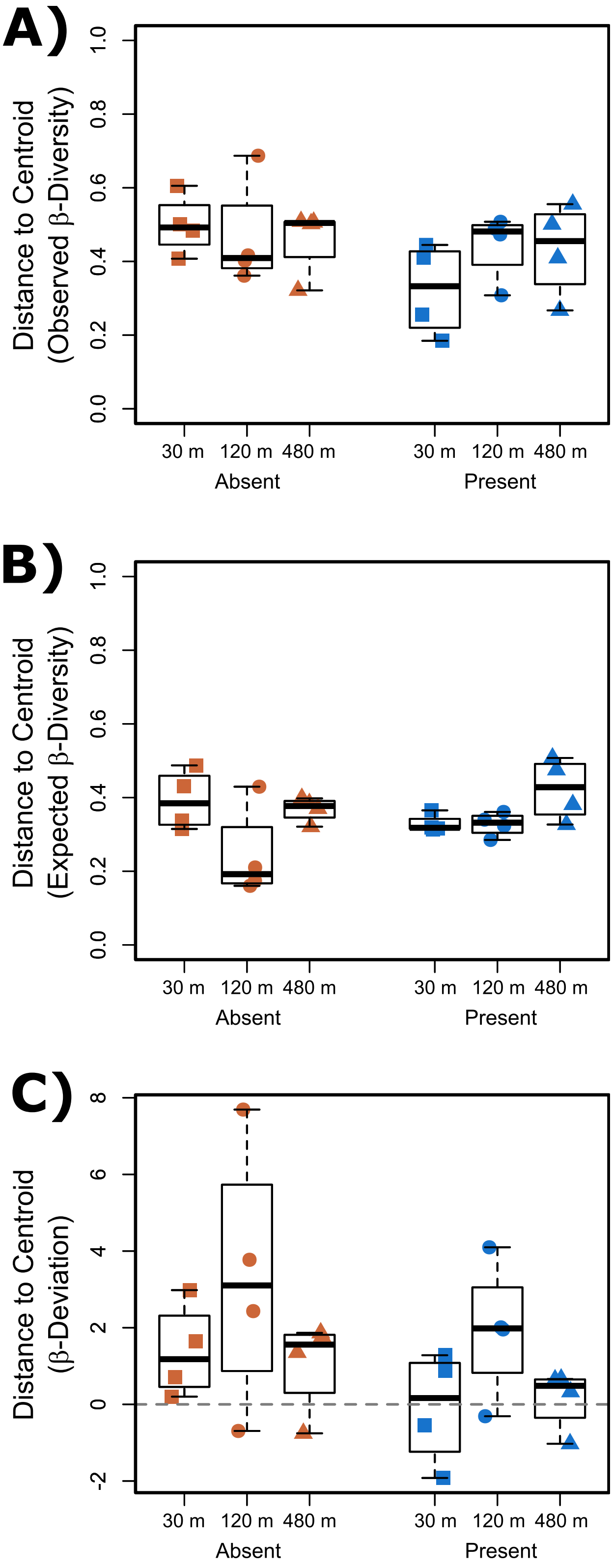


Figure S2.1. Box plots of values of distance to the centroid of observed dissimilarity values based on Bray-Curtis distance (A), expected distance according to 1000 simulations of null communities (B), and distance to centroid based on beta-deviation measures (C) for only the first sampling survey. Squares, circles, and triangles are 30 m, 120 m and 480 m isolation treatments respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey whereas the ones with a gray border are from the third survey.

Table S2.1. Anova table of linear mixed models for values of observed, expected beta-diversity and beta-deviation for the first sampling survey.

		Observed Beta Diversity		**Expected Beta-Diversity**		**Beta-Deviation**	
	Df	F	p	F	p	F	p
Fish	1	2,611	0.124	0.690	0.417	2,643	0.121
Isolation	2	0.338	0.717	4,737	0.022	3,009	0.075
Fish : Isolation	2	1,190	0.327	2,296	0.129	0.050	0.952

SUPPLEMENT 3

Gamma diversity.

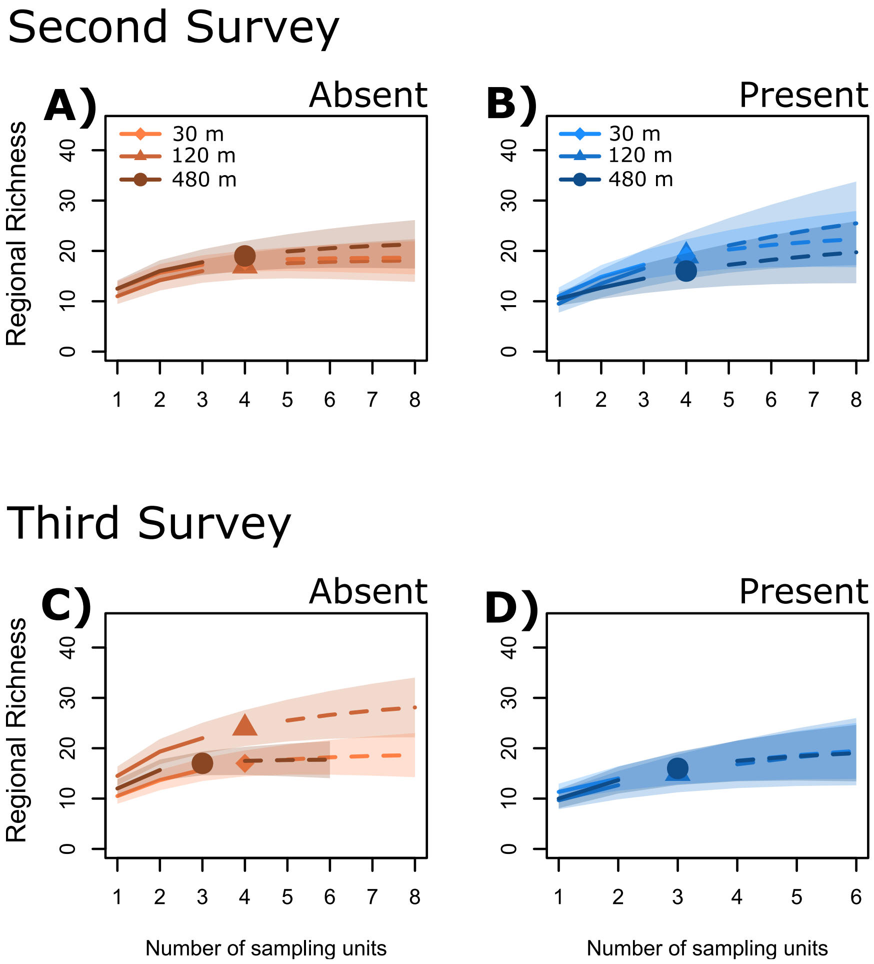
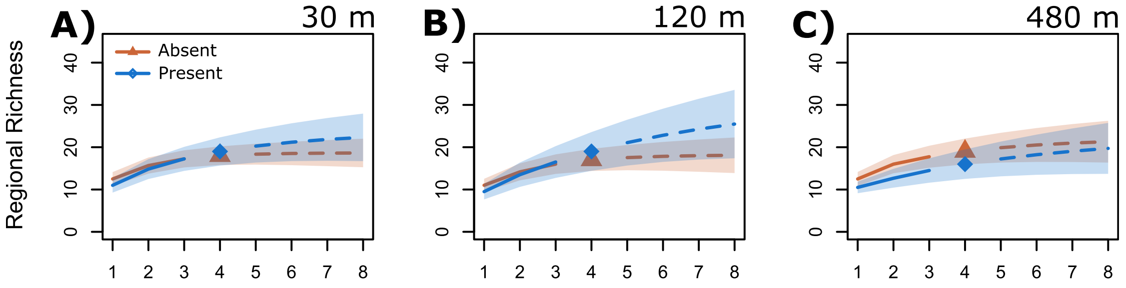


Figure S3.1. Rarefaction curves of gamma diversity in fish and fishless ponds in each isolation treatment, for both second and third surveys separately. Shaded areas are 95% confidence intervals for gamma diversity. Not superposed confidence intervals indicate significant differences. Dotted lines represent extrapolated and solid lines interpolated gamma diversity along increasing number of sample units. Diamonds, triangles and circles are the actual observed gamma diversity in each treatment.

Second Survey



Third Survey

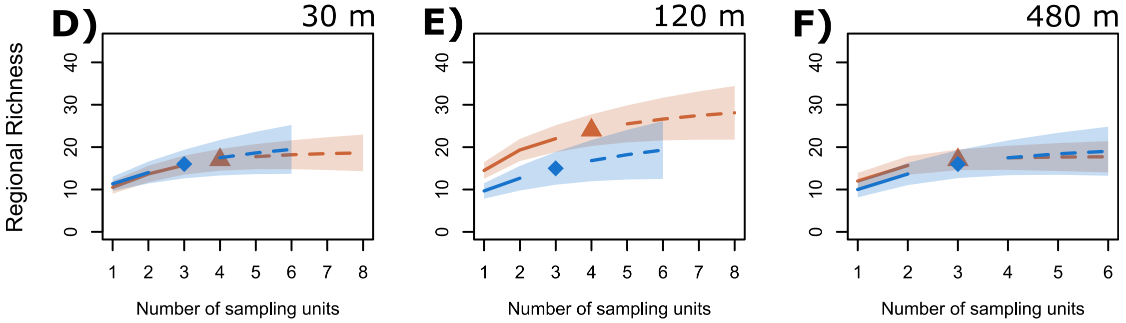


Figure S3.2. Same as figure 1 but now comparing fish and fishless treatments in different isolation treatments.

SUPPLEMENT 4

Local species richness.

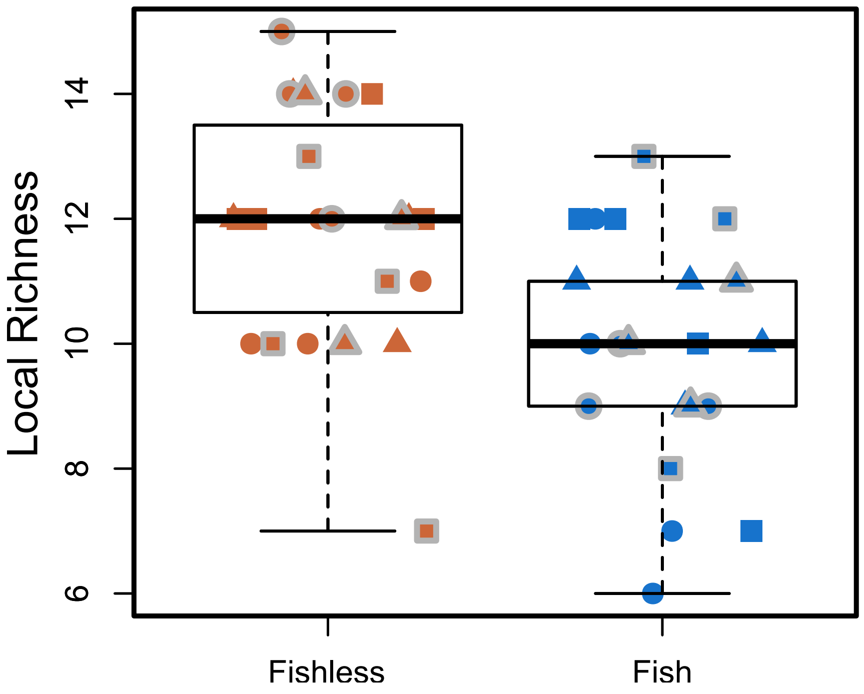


Figure S4.1. Box plot of local species richness in ponds with and without fish. Squares, circles, and triangles are 30 m, 120 m and 480 m isolation treatments respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey whereas the ones with a gray border are from the third survey.

SUPPLEMENT 5

Same analysis as presented in the manuscript, but using the algorithm used by Siqueira et al. (2019).

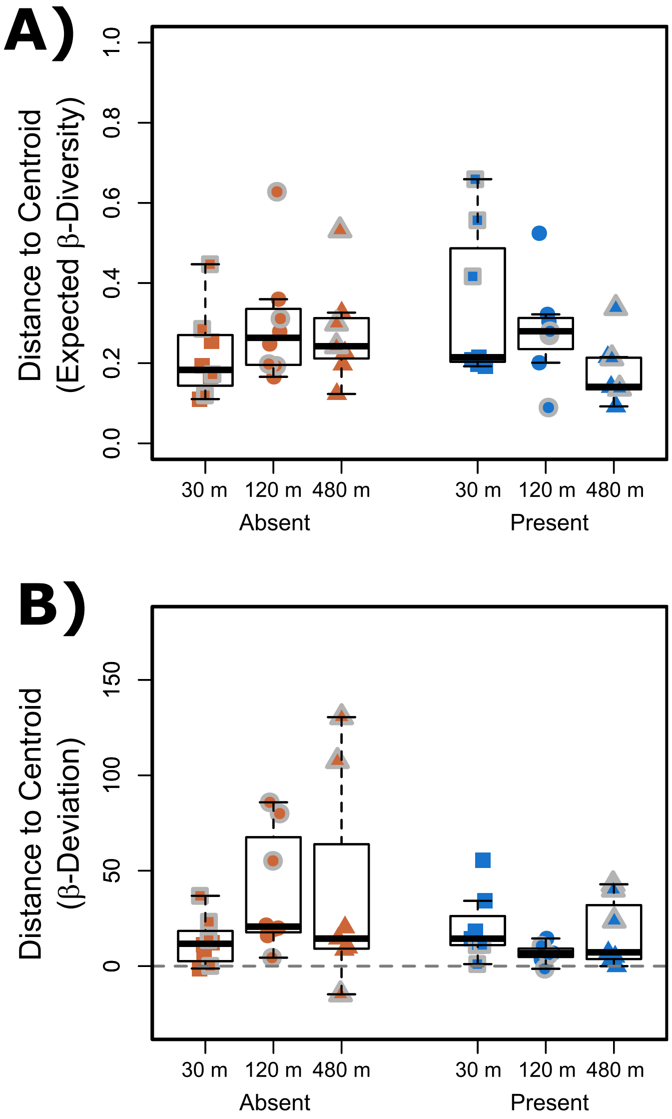


Figure S5.1. Box plots of values of expected beta-diversity based on 1000 randomly generated communities using the same null model used by Siqueira *et al.* (2019), but keeping community sizes, mean alpha diversity per treatment and number of species per treatment constant (A), and beta-deviation measures (B). Squares, circles, and triangles are 30 m, 120 m and 480 m isolation treatments respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey whereas the ones with a gray border are from the third survey. Pairwise comparisons did not show significant differences among treatments.

Table S5.1. Anova table of linear mixed models for values of expected beta-diversity and beta-deviation using the same null model used by Siqueira *et al.* (2019).

		Expected Beta Diversity		**Beta-Deviation**	
	Df	F	p	F	p
Fish	1	0.214	0.65	3.712	0.071
Isolation	2	1.079	0.363	1.159	0.338
Sampling Survey	1	**6.981**	**0.017**	**6.843**	**0.018**
Fish : Isolation	2	**4.276**	**0.032**	2.348	0.126
Fish : Sampling Survey	1	0.001	0.975	**5.277**	**0.035**
Isolation : Sampling Survey	2	3.135	0.07	**4.208**	**0.033**
Fish : Isolation : Sampling Survey	2	**3.654**	**0.048**	0.003	0.997

REFERENCE

Siqueira, T., Saito, V.S., Bini, L.M., Melo, A.S., Petsch, D.K., Landeiro, V.L., *et al.* (2019). Community size affects the signals of ecological drift and niche selection on biodiversity. *Biorxiv*, 515098.

SUPPLEMENT 6

Repeating analyses but with a null model that does not keep gamma diversity of each treatment constant.

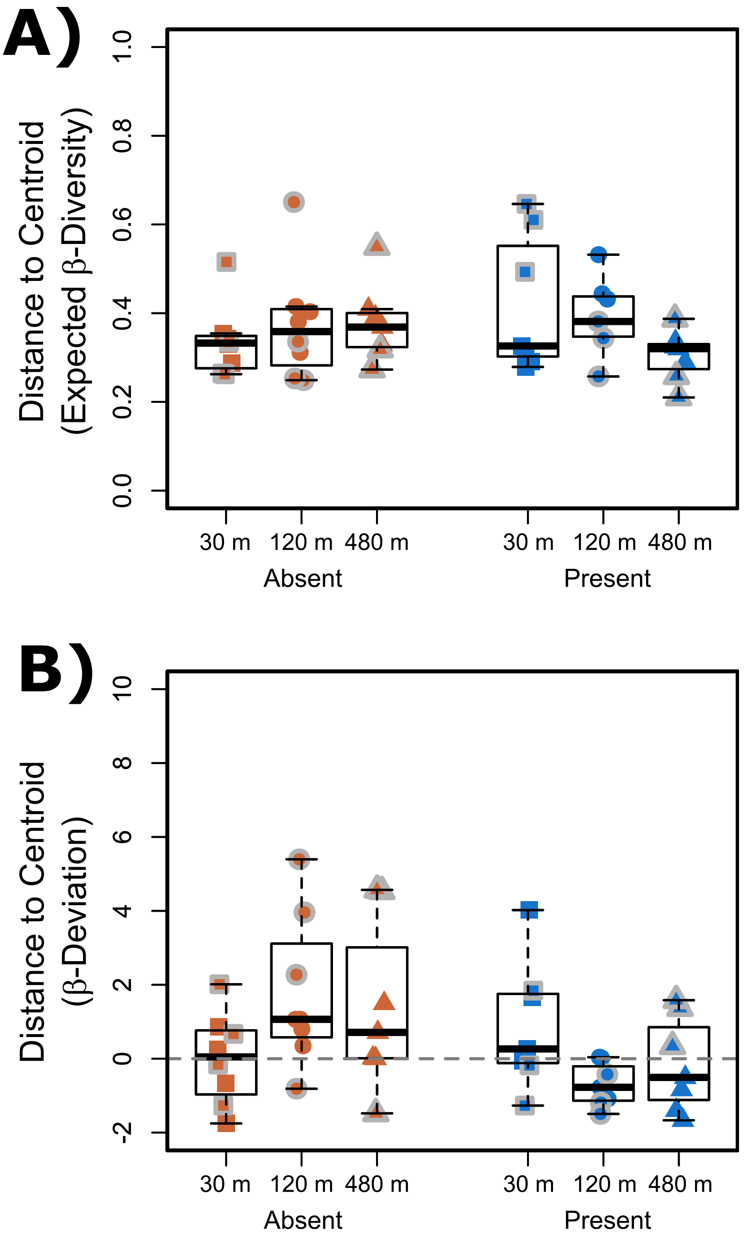


Figure S6.1. Box plots of values of expected beta-diversity based on 1000 randomly generated communities keeping community sizes, but not mean alpha diversity per treatment and number of species per treatment (A), and beta-deviation measures (B). Squares, circles, and triangles are 30 m, 120 m and 480 m isolation treatments respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey whereas the ones with a gray border are from the third survey. Pairwise comparisons did not show significant differences among treatments for beta deviation.

Table S6.1. Anova table of linear mixed models for values of expected beta-diversity and beta-deviation using with a null model that does not keep gamma diversity of each treatment constant.

		Expected Beta Diversity		**Beta-Deviation**	
	Df	F	p	F	p
Fish	1	0.292	0.596	3.661	0.073
Isolation	2	0.871	0.437	0.072	0.931
Sampling Survey	1	0.332	0.572	3.031	0.101
Fish : Isolation	2	2.864	0.085	**4.327**	**0.03**
Fish : Sampling Survey	1	0.656	0.43	2.741	0.117
Isolation : Sampling Survey	2	**4.43**	**0.029**	2.514	0.112
Fish : Isolation : Sampling Survey	2	**4.203**	**0.033**	0.858	0.442

SUPPLEMENT 7

Repeating results for predators and consumers separately.

Table S7.1. ANOVA table of linear mixed models for pond total abundance (log-transformed) and local richness for predatory insects only.

		Abundance		**Richness**	
	Df	F	p	F	p
Fish	1	**47.613**	**<0.001**	**20.153**	**<0.001**
Isolation	2	**14.861**	**<0.001**	**6.692**	**0.007**
Sampling Survey	1	2.203	0.157	0.569	0.462
Fish : Isolation	2	1.263	0.308	1.639	0.223
Fish : Sampling Survey	1	**5.51**	**0.032**	1.427	0.25
Isolation : Sampling Survey	2	1.743	0.207	1.595	0.234
Fish : Isolation : Sampling Survey	2	1.42	0.271	5.497	0.015

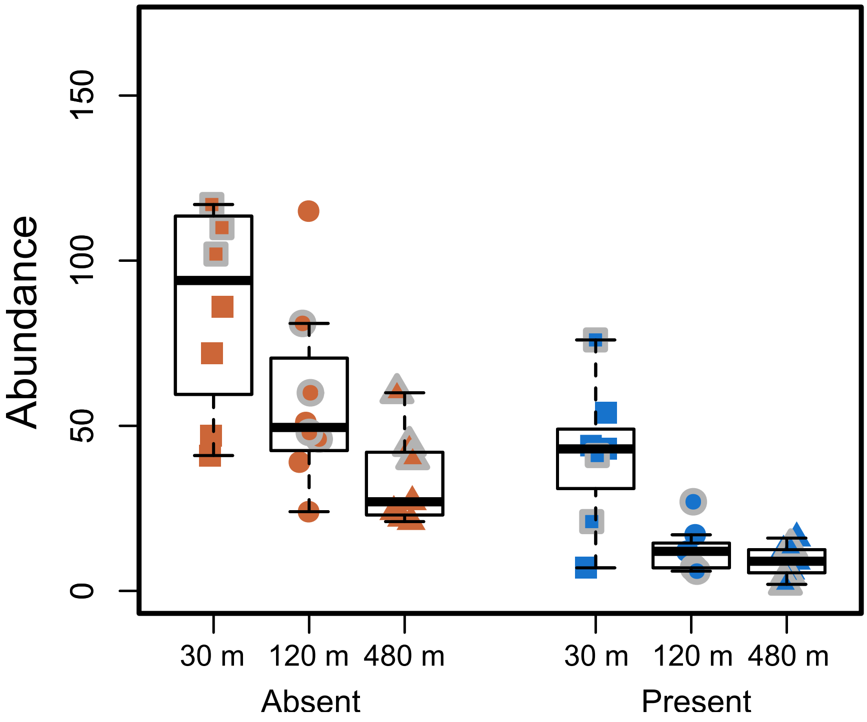


Figure S7.1. Box plot of abundance (i.e. community size) for predatory insects only. Squares, circles, and triangles are 30 m, 120 m and 480 m isolation treatments respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey whereas the ones with a gray border are from the third survey. Pairwise comparisons among isolation treatments showed that both 30 m and 120 m treatments were significantly different from 480 m.

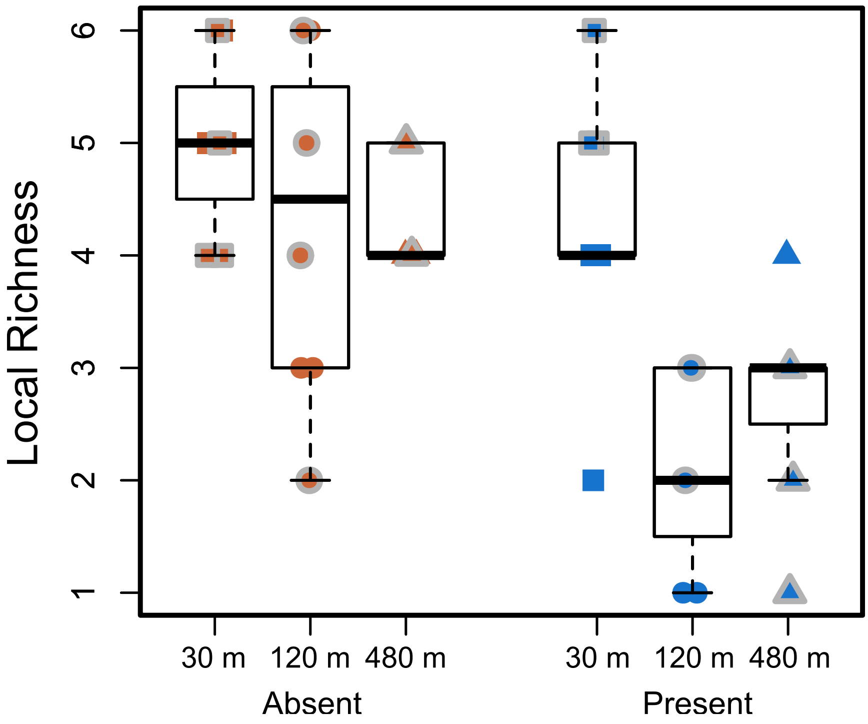
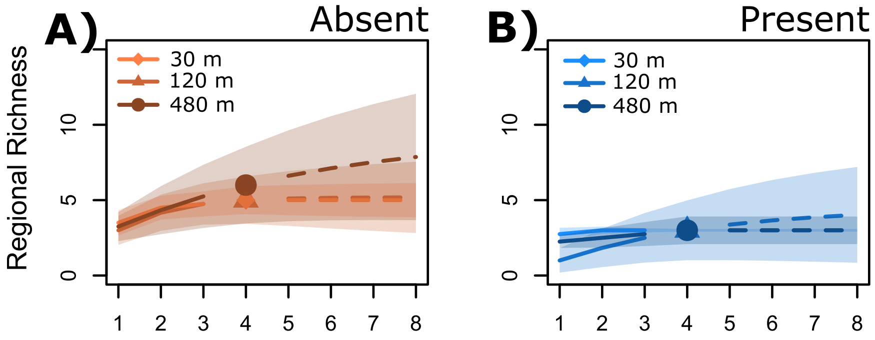


Figure S7.2. Box plot of local richness for predatory insects only. Squares, circles, and triangles are 30 m, 120 m and 480 m isolation treatments respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey whereas the ones with a gray border are from the third survey. Pairwise comparisons among isolation treatments showed that both 30 m and 120 m treatments were significantly different from 480 m.

Second Survey



Third Survey

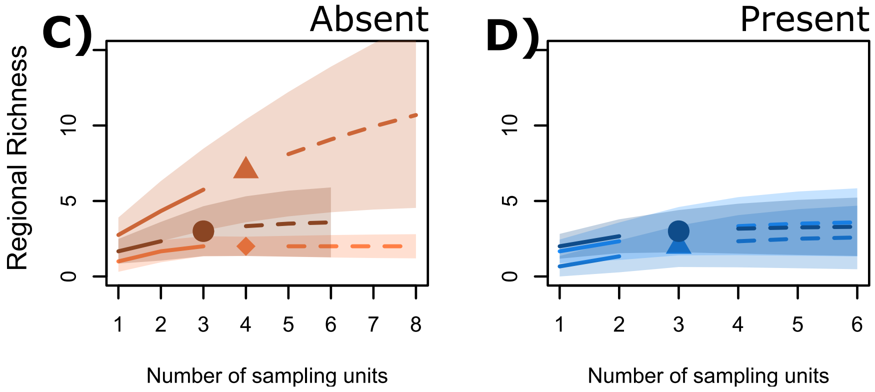


Figure S7.3. Rarefaction curves of regional richness of predatory insects in fish and fishless ponds in each isolation treatment, for both second and third surveys separately. Shaded areas are 95% confidence intervals for gamma diversity. Not superposed confidence intervals indicate significant differences. Dotted lines represent extrapolated and solid lines interpolated gamma diversity along increasing number of sample units. Diamonds, triangles and circles are the actual observed regional richness in each treatment.

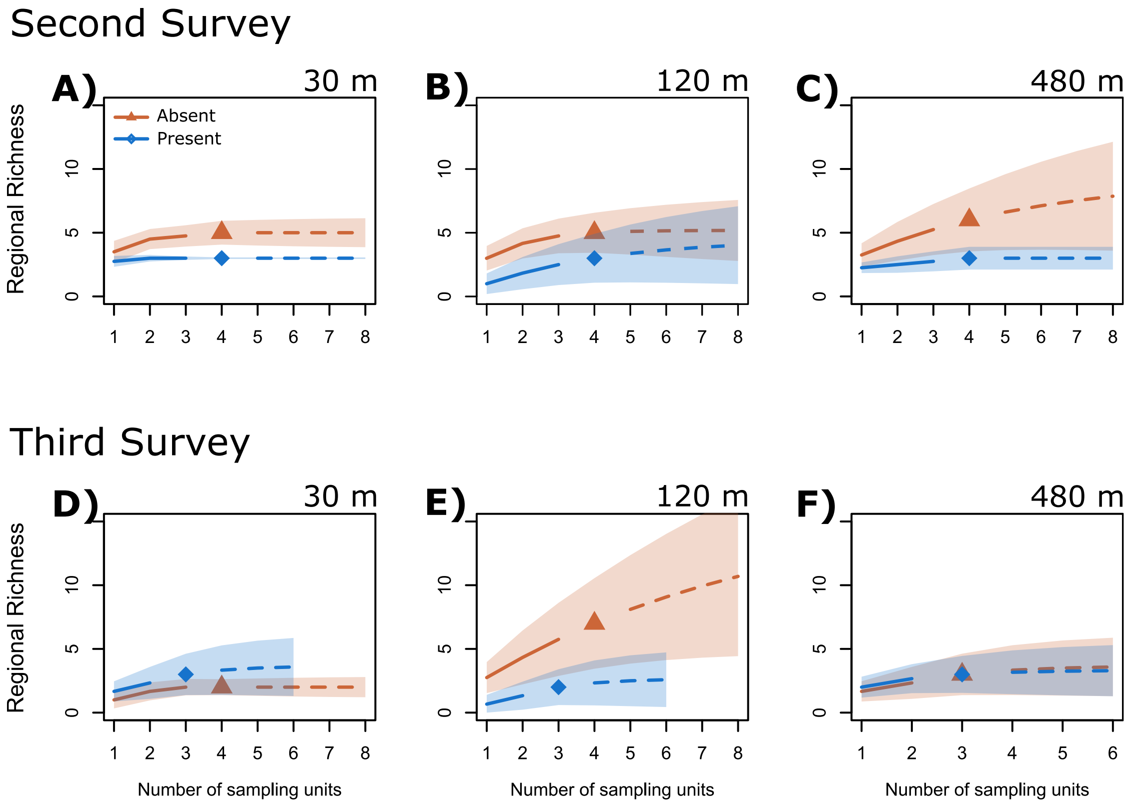


Figure S7.4. Same as figure 3 but now comparing fish and fishless treatments in different isolation treatments.

Table S7.2. ANOVA table of linear mixed models for pond total abundance (log-transformed) and local richness for consumers only.

		Abundance		**Richness**	
	Df	F	p	F	p
Fish	1	0.002	0.964	0.964	0.34
Isolation	2	1.295	0.3	2.74	0.094
Sampling Survey	1	18.42	0.001	0.047	0.831
Fish : Isolation	2	1.173	0.333	0.353	0.708
Fish : Sampling Survey	1	1.638	0.219	0.241	0.63
Isolation : Sampling Survey	2	4.314	0.032	3.3	0.062
Fish : Isolation : Sampling Survey	2	1.175	0.334	2.414	0.12

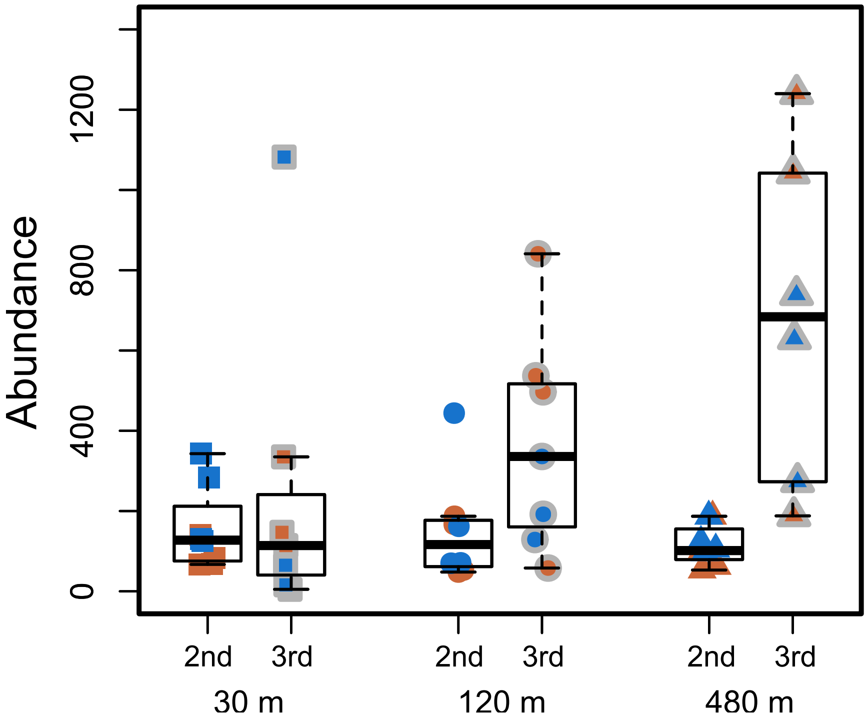


Figure S7.5. Box plot of abundance (i.e. community size) for consumers only. Squares, circles, and triangles are 30 m, 120 m and 480 m isolation treatments respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey whereas the ones with a gray border are from the third survey. Pairwise comparisons between second and third surveys for each isolation distance showed that abundance were only significantly different in the 480 m ponds.

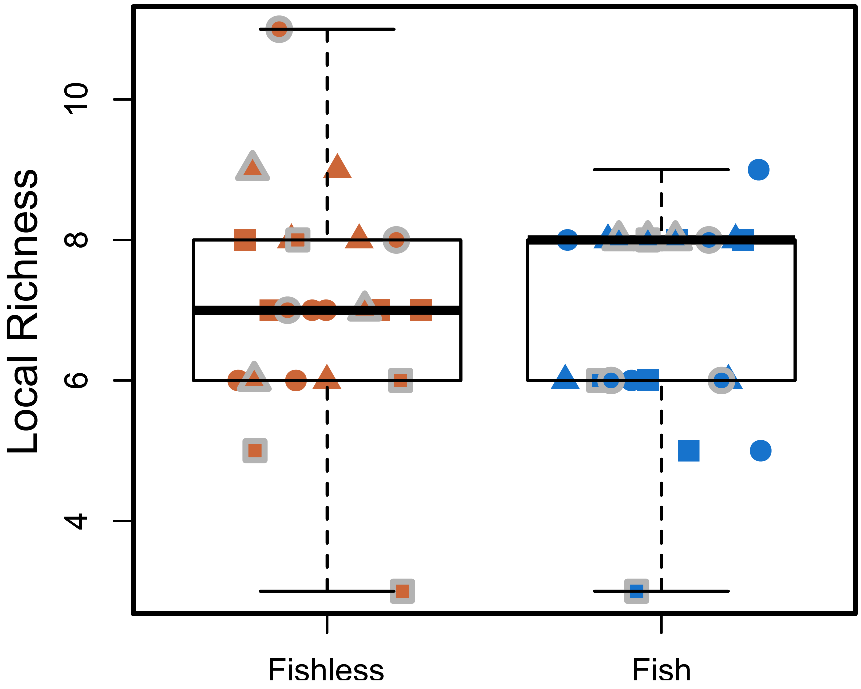
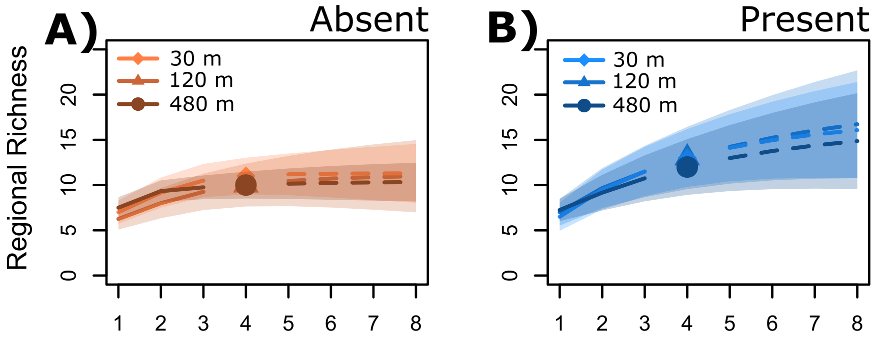


Figure S7.6. Box plot of local richness for consumers only. Squares, circles, and triangles are 30 m, 120 m and 480 m isolation treatments respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey whereas the ones with a gray border are from the third survey.

Second Survey



Third Survey

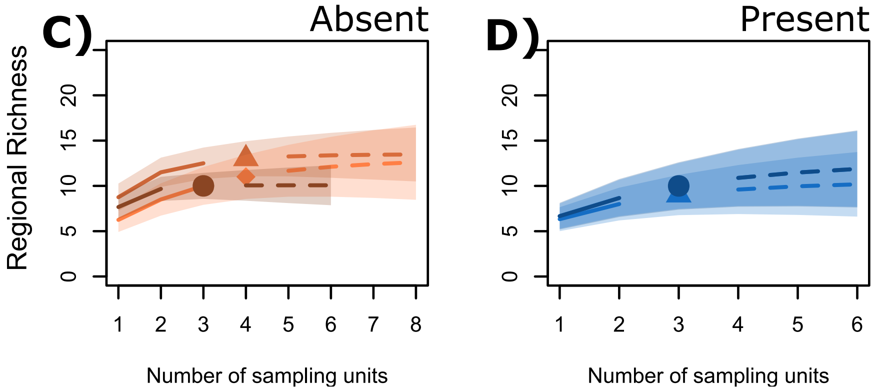
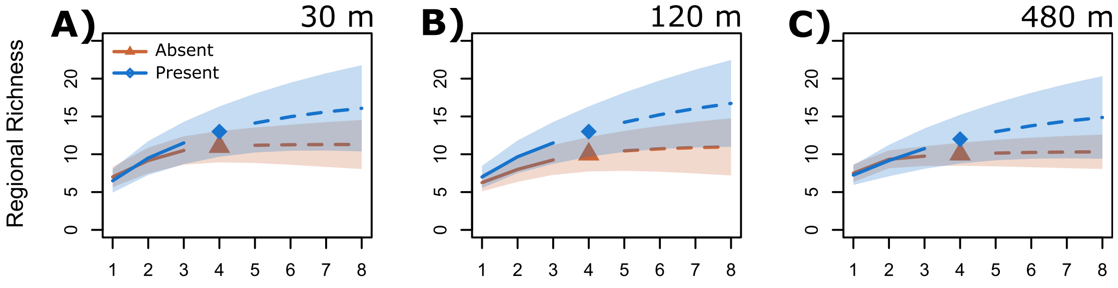


Figure S7.7. Rarefaction curves of regional richness of consumers in fish and fishless ponds in each isolation treatment, for both second and third surveys separately. Shaded areas are 95% confidence intervals for gamma diversity. Not superposed confidence intervals indicate significant differences. Dotted lines represent extrapolated and solid lines interpolated gamma diversity along increasing number of sample units. Diamonds, triangles and circles are the actual observed regional richness in each treatment.

Second Survey



Third Survey

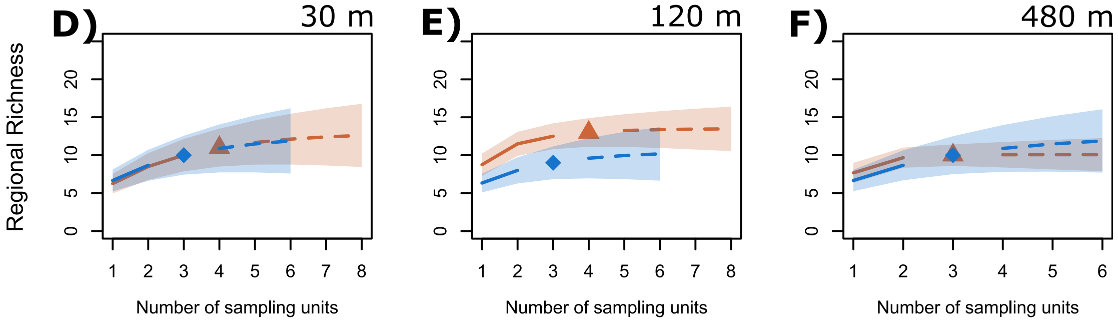


Figure S7.8. Same as figure 7 but now comparing fish and fishless treatments in different isolation treatments.

Table S7.3. Anova table of linear mixed models for values of observed, expected beta-diversity and beta-deviation.

		Observed Beta Diversity		**Expected Beta Diversity**		**Beta-Deviation**	
	Df	F	p	F	p	F	p
Predatory Insects							
Fish	1	1.127	0.304	**10.915**	**0.004**	3.4	0.083
Isolation	2	1.132	0.346	1.76	0.203	1.381	0.279
Sampling Survey	1	5.325	0.034	2.057	0.17	0.908	0.354
Fish : Isolation	2	2.943	0.08	1.974	0.17	1.02	0.382
Fish : Sampling Survey	1	**9.072**	**0.008**	2.724	0.118	**11.275**	**0.004**
Isolation : Sampling Survey	2	3.483	0.055	2.999	0.077	0.118	0.89
Fish : Isolation : Sampling Survey	2	1.747	0.205	2.71	0.096	2.755	0.093
Consumers							
Fish	1	3.577	0.076	0.366	0.554	**6.402**	**0.022**
Isolation	2	1.597	0.232	**6.189**	**0.01**	**4.163**	**0.034**
Sampling Survey	1	3.115	0.096	3.916	0.065	0.271	0.61
Fish : Isolation	2	**6.984**	**0.006**	2.74	0.094	3.235	0.065
Fish : Sampling Survey	1	0.372	0.55	1.389	0.255	0.278	0.605
Isolation : Sampling Survey	2	**5.643**	**0.014**	**6.905**	**0.007**	1.758	0.203
Fish : Isolation : Sampling Survey	2	2.5	0.113	0.623	0.548	**6.322**	**0.009**

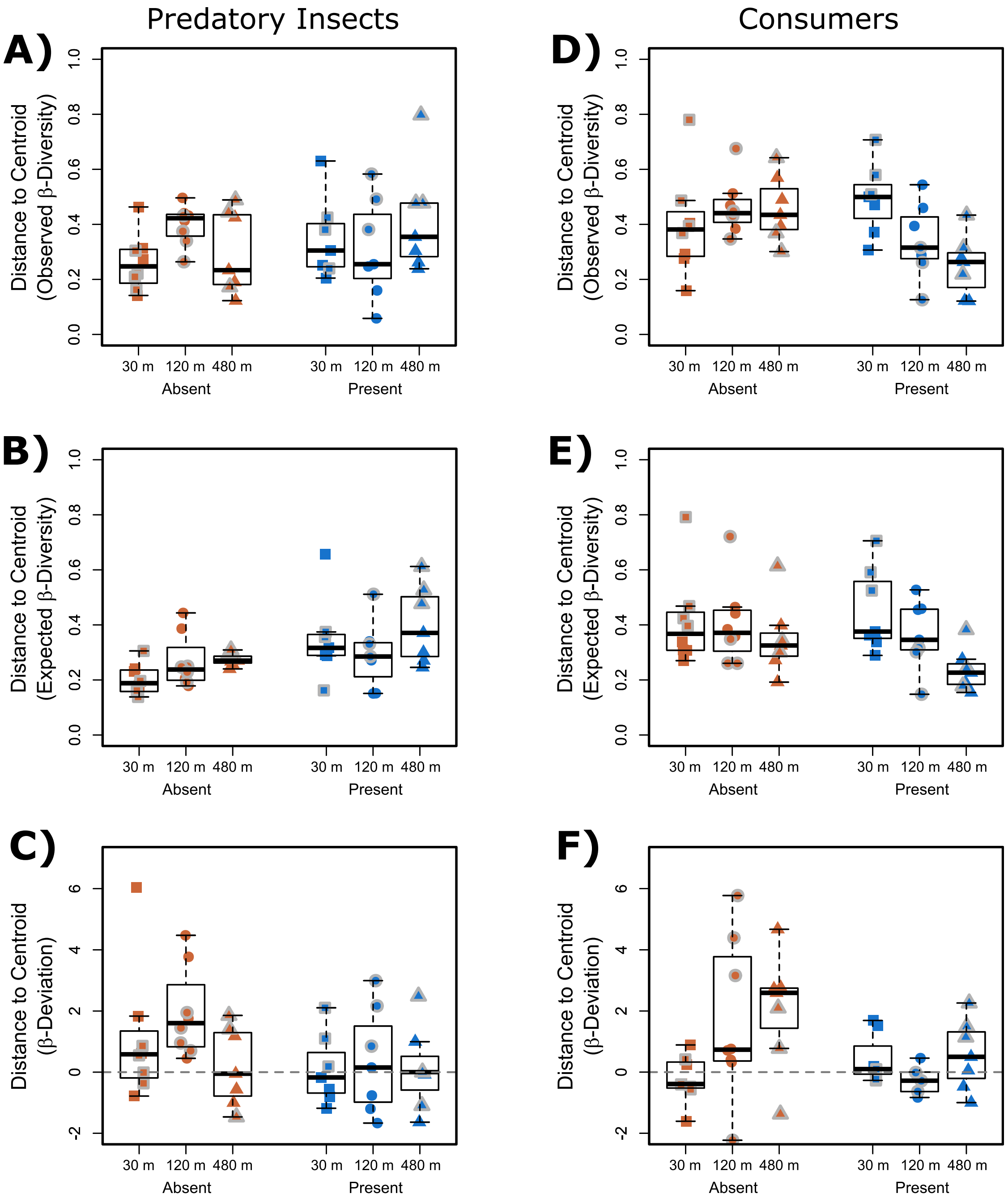


Figure S7.9. Box plots of values of distance to the centroid of observed dissimilarity values based on Bray-Curtis distance (A), expected distance according to 1000 simulations of null communities (B), and distance to centroid based on beta-deviation measures (C) for predatory insects only (A, B and C) and consumers only (D, E and F). Squares, circles, and triangles are 30 m, 120 m and 480 m isolation treatments respectively. Orange squares, circles, and triangles are fishless ponds, whereas blue are ponds with fish. Squares, circles, and triangles without a grey border are from the second survey whereas the ones with a gray border are from the third survey. Pairwise comparisons of observed beta-diversity and beta-deviation for predatory showed that both were higher in the last survey, but only for ponds with fish. For consumers, pairwise comparisons of observed beta-diversity showed that 30 m ponds were different from 480 m ponds, but only for ponds with fish. For beta-deviation pairwise comparisons showed no significant difference among treatments.

General Conclusion

SPECIFIC CONCLUSIONS

In this Thesis, we generally found that spatial isolation can favor species with higher dispersal rates, irrespective of their trophic level. In macroinvertebrate communities, consumers generally have higher dispersal rates than predatory insects because of their greater abundances and shorter generation times, resulting in more events of dispersal (Shulman & Chase 2007; Chase & Shulman 2009; Hein & Gillooly 2011). Therefore, they show patterns of increased abundance in more isolated habitats due to trophic cascades (*i.e.,* lower predation pressure). However, a few predatory insects, including species of dragonflies, have high dispersal ability, being able to disperse across long distances (McCauley 2006). In our experiments, two dragonflies even showed patterns of increased abundance with distance, likely because of a reduction in the abundance of other predatory insects (*i.e.,* possible competitors). Additionally, we found that, surprisingly, generalist amphibians did not show evidence of dispersal limitation in their abundance pattern across our isolation gradient. We attribute this pattern to the fact that the savanna landscape where we conducted our experiments might have been a suitable terrestrial habitat for generalist adult amphibians with adaptations to living in dryer habitats (Haddad and Prado 2005, Vasconcelos et al. 2014).

The stocking of fish had, as expected (Diehl 1992; Wellborn *et al.* 1996), a strong negative effect on predatory insects, especially on the large-bodied ones (*i.e.*, dragonflies and beetles). However, because fish used in aquaculture, such as the Tilapias, are often generalist omnivores, they also prey upon consumers when sufficiently abundant. Therefore, the negative effects of generalist predatory fish do not necessarily implicate in an increase in the abundance of consumers due to a trophic cascade. Rather, the net effects we observe result from the direct negative effects of the tilapias on insect consumers overriding the indirect positive effect of the tilapias on insect consumers via a reduction of predatory insects.

Shifting from impacts of aquaculture to agriculture, we observed that fertilization has little effect on temporary pond communities, only slightly increasing the abundance of two predatory insects in our study system, likely via *bottom-up* effects. We believe that nutrient enrichment might have had a less relevant effect on community structure because small temporary ponds in both savannah and agricultural landscapes are already in the extreme portion of the eutrophication gradient (*i.e.,* hypertrophic). Thus, fertilization might represent a relatively small fraction of the nutrient load in these habitats.

We also observed that the insecticide Regent ® 800WG (active ingredient fipronil) can strongly and negatively affect insect populations, including both consumers and predatory insects. Such effects are followed by an increase in the abundance of the few generalist amphibian species colonizing our mesocosms. However, when the concentration of the insecticide in water decreases, insect populations rapidly recover, and, by the end of the reproductive season, both insect and amphibian abundance return to numbers observed in mesocosms receiving fertilizer applications only. Even though such effects are temporary, we argue that they could be relevant for biodiversity in temporary ponds because insecticide application is synchronized with the peak of the reproductive season of many semi-aquatic insects and amphibians. Importantly, a variety of life-history strategies are represented among species colonizing temporary ponds, including species ranging from multiple to single reproductive bouts, with an evident wide range of demographic responses to such applications.

The potential of the effects of land management (*i.e.,* stocking of fish, pasture, and sugarcane) to interact with the effects of spatial isolation was highly dependent on the type of land management. The effects of spatial isolation changed drastically when predatory fish was present. For instance, the two predatory insects that are positively affected by isolation in fishless ponds, *Pantala* and *Orthemis* dragonflies, were negatively affected by it in ponds with fish. We believe that, because other suitable prey become rarer with isolation, and these dragonflies are not dispersal limited, fish likely increased predation pressure over these dragonflies in more isolated habitats. Similarly, because generalist fish also prey upon consumers when they are abundant, it decreased the indirect positive effects that spatial isolation would have on them.

Different from when we manipulated exotic fish, both the effects of fertilization and pesticide pulses do not change with spatial isolation. We believe that this result is due to three main reasons: First, fish affected different taxa differently, depending on their abundance patterns, which in turn is affected by isolation. The insecticide, by contrast, had the same acute effect on all invertebrate taxa regardless of isolation. Second, different from insecticide pulses, the presence of fish represents a constant environmental pressure.

Therefore, its direct and indirect effects might only be reinforced with time. Third, most of the responses to agrochemicals in our experiment came from taxa that were not dispersal limited: amphibians, and two of their most important dragonfly predators, thus reducing the potential of both fertilization and the insecticides to differently affect communities in different isolation treatments. Another possibility is that amphibians were absent in the experiment where we manipulated generalist predatory fish, likely because we started this experiment late in the rainy season. However, we do not believe it would drastically change our results. Both amphibians and insect consumers were equally affected by isolation in the second experiment. Thus, they would have probably followed the same patterns in the first one, at least in fishless ponds. The main expected difference would be that amphibians, different from insect consumers, would likely have had a strong reduction in their abundance in ponds with fish, mostly because of pre-colonization habitat selection (Resetarits & Wilbur 1991; Resetarits 2005), thus having little effect on the effect of fish on other taxa.

We also found that communities that undergo the same local environmental pressures can have very different patterns of site-to-site variability (*i.e.*, beta-diversity within treatments) depending on the spatial context. We verified in fishless ponds that, as spatial isolation increases, community variability also tends to increase, and such effects cannot be attributed to the increasing effects of ecological drift. Rather, there appears to be some deterministic process driving communities to different structures in more isolated habitats, likely historical contingency leading communities to different structures. However, the simple presence of a generalist predatory fish can override these effects. In this case, communities become more similar to each other as isolation increases, probably because the abundance of consumers increases with isolation (*i.e.*, trophic cascade), thus increasing community size, which decreases the importance of ecological drift on community structure (Myers *et al.* 2015).

GENERAL CONCLUSION

Classic metacommunity theory is based on communities that are horizontally structured by competition, where all species are from one single trophic level and have similar dispersal rates (Leibold *et al.* 2004; Vellend 2016; Leibold & Chase 2018). Clearly, this framework is not sufficient to account for all the patterns we found here. If we were to consider predictions for competition-based metacommunities - where species have equal dispersal rates - both fish and agrochemicals would have stronger effects on community structure in intermediate isolation because dispersal would not be high enough to override effects of niche selection, nor low enough to increase the consequences of ecological drift (Leibold *et al.* 2004; Vellend 2010; Winegardner *et al.* 2012). Considering the same assumptions, if our local environmental filters were strong enough, we would have observed an increase in the effect of niche selection with isolation because it would have entirely overridden the effects of ecological drift (Howeth & Leibold 2008). However, we did not observe any of those patterns.

We found that the effect of fish on community structure not only increases with isolation but actually changes by having different net effects on different taxa in different spatial contexts. In the same first experiment, ecological drift decreased community variability along the isolation gradient, instead of increasing it, likely because spatial isolation increases community size by benefiting consumers through trophic cascades. In our second experiment, the use of agrochemicals did not change its effects along the gradient of spatial isolation. This is because, even though our manipulated distances represented a strong dispersal barrier to some predatory insects (Wilcox 2001; Trekels *et al.* 2011), the taxa that were most affected by the agrochemical treatments were not affected by isolation at all (*i.e.,* dragonflies and amphibians). Therefore, most, if not all, of the inconsistencies of our results with predictions from classic metacommunity theory comes from (1) the different dispersal rates of freshwater insects and amphibians, and (2) the multi-trophic nature of the communities in our model system. (Vellend *et al.* 2014). We, therefore, claim that if we seek to understand how different ecological processes act together to structure biological communities, we must include both interspecific variation in dispersal rates and multiple trophic levels into the new metacommunity frameworks, which are only starting to be developed (*e.g.,* Vellend *et al.* 2014; Guzman *et al.* 2019).

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