Predator phylogenetic diversity decreases predation rate via antagonistic interactions

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## Introduction

Predators can have strong top-down effects, both on community structure and ecosystem processes (Estes et al. 2011); however the combined effects of different predator species are not well understood. The combined effect of predator species on communities is often stronger or weaker than that predicted from a study of those same species in isolation (Sih et al. 1998; Ives et al. 2005). These non-additive effects of predators occur when predators interact with each other directly, or via the prey species. For example, predators can feed directly on each other (intra-guild predation), consume the same prey (resource competition) or modify the behaviour of prey or the other predator species (Sih et al. 1998; Nyström et al. 2001; Griswold and Lounibos 2006). These non-additive effects can be positive or negative. For example, prey may have an induced defense against one predator which increases (negative non-additive effect) or decreases (positive non-additive effect) the likelihood of consumption by a second predator. While there are many possible mechanisms underlying the effect of predator composition, we lack a means of predicting *a priori* the strength and direction of this effect on community structure and ecosystem function.

Phylogenetic diversity could provide a framework for making these predictions about predators. Phylogenetic diversity incorporates the evolutionary relationships of species into measures of diversity. This approach was first used to interpret observations of community structure, as ecologists interpreted nonrandom phylogenetic structure (i.e. under- or over dispersion) as evidence for the processes (i.e., habitat filtering or competition) which structure communities (Webb et al. 2002; Cavender-Bares et al. 2009). Recently, this approach has been applied to manipulative experiments. For example, the phylogenetic diversity of plant communities is a better predictor of productivity than either species richness or diversity (Cadotte et al. 2008; e.g. Cadotte et al. 2009; Godoy et al. 2014). In all cases, an implicit assumption is that increased phylogenetic distance is associated with increased ecological dissimilarity -- either in the form of differences in species niches, interactions, or functional traits. When this is true, high phylogenetic diversity should lead to complementarity in resource use between species, resulting in increased ecosystem functioning (Srivastava et al. 2012). Despite the prevalence of phylogenetic community ecology and the importance of predators to natural systems, the phylogenetic diversity of local predator assemblages has rarely been measured (Bersier and Kehrli 2008; Naisbit et al. 2011). Many studies of phylogeny and predator traits focus on whole clades, rather than local assemblages (e.g. *Anolis* lizards (Knouft et al. 2006), warblers (Böhning-Gaese et al. 2003), tree boas (Henderson et al. 2013) and wasps (Budriene and Budrys 2004)) making it difficult to connect these results to predator effects at the scale of a local community. These clade specific studies often find weak evidence for phylogenetic signal in ecologically relevant traits. In contrast, studies at the level of the whole biosphere (Bersier and Kehrli 2008; Gómez et al. 2010) demonstrate that related organisms often have similar interspecific interactions, i.e. related predators often consume similar prey. At the local scale, only a few studies have examined how phylogeny may shape food webs (Rezende et al. 2009; Cagnolo et al. 2011); these observational studies found that models containing both relatedness (either phylogenetic or taxonomic) and body size were better at predicting which predator-prey interactions occurred than models with body size alone. As observational studies, however, they cannot isolate if it is differences in predator distribution or diet that leads to a phylogenetic signal in predator-prey interactions, nor how these interactions affect the whole community.

Within a local community, the effect of predator species diversity will depend on three factors: how predators are distributed among habitats, how they interact with prey, and how they interact with each other. To the extent that phylogenetic relationships are correlated with these three factors, phylogeny may be able to predict the impact of predator diversity on communities. For instance, phylogeny could constrain predator species co-occurrence if more distant phylogenetic relatives have more distinct fundamental niches, while close relatives are too similar to co-exist (Webb et al. 2002; Emerson and Gillespie 2008). When predators do co-occur, phylogeny may correlate with their feeding behavior, such that closely related predators consume similar prey. For example, diet overlap (shared prey species between predators) will depend on the feeding traits and nutritional requirements of predators -- both of which may be phylogenetically conserved. If this is the case, then predator assemblages with higher phylogenetic diversity will show a greater range of prey consumed and therefore stronger top- down effects (Finke and Snyder 2008). In some cases, predator diets may extend to include other predators, leading to direct negative interactions such as intraguild predation, which may also have a phylogenetic signal (Pfennig 2000).

We examined the consequences of phylogenetic diversity using a natural mesocosm: the community of macroinvertebrates living within bromeliads. Bromeliads (Bromeliaceae) are flowering plants abundant in the Neotropics. Many bromeliad species contain water and trapped, terrestrial detritus; the decomposition of this detritus supplies nutrients for the bromeliad (Reich et al. 2003). The small size of these habitats permits direct manipulations of entire food webs, manipulations which would be difficult in most natural systems. Within this aquatic food web, damselfly larvae (e.g. *Leptagrion* spp., Odonata:Coenagrionidae) are important predators, dramatically reducing insect colonization (Hammill et al. 2015) and emergence (Starzomski et al. 2010), and increasing nutrient cycling (Ngai and Srivastava 2006). However, other predators are also found in bromeliads, including large predaceous fly larvae (Diptera: Tabanidae) and predatory leeches (Hirudinae:Arhynchobdellida) (see Frank et al. (2009)). Predators have been shown to have large top-down effects on ecosystem functions in bromelaids, including nitrogen uptake by the plant (Ngai and Srivastava 2006), detrital decomposition and CO2 flux (Atwood et al. 2013; Atwood et al. 2014).

We used a series of observations, lab feeding trials, and manipulative field experiments to measure how the phylogenetic diversity of the predator assemblage predicts community composition and ecosystem function. We test three related hypotheses concerning similarity in distribution, diet and top- down ecosystem effects of predators:

1. *Distributional similarity*: Closely-related predators may occur together more frequently than less-related predators if there is a strong phylogenetic signal to habitat requirements. Alternatively, very closely related species may never co-occur because high overlap in ecological niches results in competitive exclusion.
2. *Diet similarity*: Closely-related predators will eat similar prey at similar rates. Alternatively, closely related species may have evolved different diets to facilitate coexistence.
3. *Ecosystem-level effects*: We performed experiments at the scale at which organisms interact (i.e. within a single bromeliad) to test hypotheses about direct and indirect effects of predator combinations (on prey consumption, bromeliad nitrogen uptake and detrital decomposition), predicting:
   1. Closely-related predators will have similar effects on the community. This will occur if related predators have similar trophic interactions (e.g. predation rate, diet similarity). Our single-species treatments allow us to assess the effect of each predator both on prey survival and on ecosystem functions.
   2. Predator assemblages with higher phylogenetic diversity will have synergistic (greater than additive) effects on prey consumption and associated ecosystem functions. This will occur if phylogenetic distance correlates with increasing trait difference, and if this trait difference in turn results in niche complementarity. However, at the extreme, different predators may consume each other, thus creating antagonistic (less than additive) effects on prey consumption. By comparing treatments with pairs of predators to treatments that received each predator alone, we are able to estimate additive and non-additive effects.

## Methods

### Study Design

We collected three datasets to address each of the questions posed in the Introduction. We examined distributional similarity among predator species (Question 1) by sampling bromeliads for invertebrates. We examined diet similarity (Question 2) with a series of no-choice feeding trials. Finally, we examined the effects of predators on invertebrate communities (Question 3) with a field experiment, in which predators were added to bromeliads containing standardized communities of prey. This experiment included both single species treatments and two species treatments; the latter were chosen to create the widest possible range of phylogenetic diversity.

In each dataset, we related the results to published phylogenies accessed from "timetree.org", an online database of published molecular time estimates (Hedges et al. 2006). Node age data was available for all but the youngest nodes, where either a lack of taxonomic information (e.g. Tabanidae) or a lack of phylogenetic study (e.g. *Leptagrion*) prevented more information from being included. These branches were left unresolved and were all assigned identical, arbitrary and short branch lengths (15 Mya).

We conducted all three experiments in Parque Estadual da Ilha do Cardoso (25o 03' S, 47o 53' W), a 22.5 ha island off the south coast of São Paulo state, Brazil. We worked in a coastal forest (*restinga*) with an understory dominated by *Quesnelia arvensis* Mez. (Bromeliaceae). *Q. arvensis* is a large terrestrial bromeliad that catches and holds rainwater (phytotelmata), accumulating up to 2.8 L of rainwater in a single plant. Our observational survey found more than 47 species of macroinvertebrates in these aquatic communities (Romero and Srivastava 2010), in 25 bromeliads of various sizes. This diversity encompasses multiple trophic and functional groups. Filter feeders were entirely mosquito larvae (Diptera:Culicidae); detritivores include shredders (Diptera:Tipulidae, Trichoptera:Calamoceratidae), scrapers (Coleoptera:Scirtidae), and collectors (All Diptera:Chironomidae, Syrphidae, Psychodidae). All these species are prey for a diverse predator assemblage dominated by at least three species of damselfly larvae (*Leptagrion* spp., Odonata:Coenagrionidae), two species of Horse Fly larvae (Diptera:Tabanidae), and two species of leech (Arhynchobdellida). A lower percentage of predator biomass was composed of Dytiscid larvae (Coleoptera), midge larvae (Diptera: Ceratopogonidae) and chironomid larvae (Diptera: Tanypodinae).

## Data collection

### Question 1: Distributional similarity

To examine distributional similarity among predator species, we used a detailed survey of bromeliad communities. In 2008, each bromeliad was dissected and washed to remove invertebrates and the resulting water was filtered through two sieves (150 and 850 µm), which removed particulate organic matter without losing any invertebrates. All invertebrates were counted and identified to the lowest taxonomic level possible. The body length of all individuals was measured when possible for small and medium-sized taxa (< 1cm final instar) and always for large-bodied taxa (> 1 cm final instar).

### Question 2: Diet Similarity

To examine diet similarity among predator species, we fed prey species to predators in laboratory feeding trials. We conducted 314 feeding trials of 10 predator taxa and 14 prey taxa between March and April 2011. We included all potential predator-prey pairs present in the experiment (described below), and attempted to perform all other combinations whenever possible. However, due to the rarity of some taxa, many predator-prey pairs were not possible; we tested 56 pairwise combinations. Most trials were replicated at least five times, but the number of replicates ranged from 1 to 11. To conduct the trials, we placed predators together with prey in a 50ml vial, with a stick for substrate. The only exception was the tabanid larvae, which we placed between two vertical surfaces to imitate the narrow space found in bromeliad leaf axils (their preferred microhabitat, necessary for successful feeding). Generally our trials contained a single predator and a single prey individual, except in the case of very small prey (*Elpidium* sp.) or predators (*Monopelopia* sp.) in which case we increased the density. We recorded whether prey was consumed after 24 hours.

### Question 3: Community effect experiment

Our third question had two parts: (a) how do predator species differ in their effects on the invertebrate community and ecosystem processes and (b) do predator combinations show non- additive effects on community and ecosystem processes, and do these non-additive effects increase or decrease with phylogenetic distance? We measured five community and ecosystem response variables: production of fine particulate organic matter (FPOM), decomposition of coarse detritus, bromeliad growth, uptake of detrital nitrogen into bromeliad tissue, and survival of invertebrate prey (emerged adults + surviving larvae)

#### Experimental design

In this experiment we focused on the four most abundant large predators found in the community: *Leptagrion andromache* and *Leptagrion elongatum* (Odonata: Coenagrionidae), a predatory Tabanid fly (Diptera:Tabanidae:*Stibasoma* sp.) and a predatory leech. We combined these species in eight treatments: predator-free control (no predators), each of the four predator species alone (3a) and pairs of predator species chosen to maximize the range of phylogenetic distance (3b). Specifically, these pairs were: two congeneric damselflies (*Leptagrion andromache* and *Leptagrion elongatum*), two insects (*L. elongatum* and *Stibasoma*), and two invertebrates (*L. elongatum* and a predatory leech). We used five replicate bromeliads for each of these 8 treatments (8 treatments, n=5). This experiment, therefore, allows the estimation of the effect of each predator species (single-species treatments), as well as the detection of non-additive effects in predator combinations.

To evaluate the effect of these predators on natural bromeliad systems, we created bromeliad communities that were as similar as possible to each other, and also to the average composition of a bromeliad. In February 2011 we collected bromeliads with a volume between 90 and 200ml, thoroughly washed the plants to remove organisms and detritus, and soaked them for 12 hours in a tub of water. We then hung all bromeliads for 48 hours to dry. This procedure was intended to remove all existing insects; one bromeliad dissected afterwards contained no insects (a similar technique was used by Romero and Srivastava (2010)). We simulated natural detritus inputs from the canopy by adding a standard mass of dried leaves of the species *Plinia cauliflora* (Jabuticaba, Myrtaceae; a common Brazilian tree; 1.5g bromeliad -1 ± 0.02, mean ± sd). In order to track the effects of detrital decomposition on bromeliad N cycling, we enriched these leaves with 15N by fertilizing five plants with 40ml pot-1 day-1 of 5g L-1 ammonium sulphate containing 10% atom excess of 15N. After 21 days we then collected *P. cauliflora* leaves, air-dried until constant weight, and then soaked them for three days. This procedure removes excess nutrients from the artificial fertilization. Because some of our prey species consume fine detritus, not coarse, we also added a standard amount of dried fine detritus to our bromeliads (0.23g bromeliad -1 ± 0.02). We separated coarse and fine detritus by passing water from bromeliads through two sieves (as above for observational work, 150 and 850 µm). We defined "coarse detritus" as anything retained on the 850 µm sieve, and "fine detritus" as anything found on the 150 µm sieve.

Each bromeliad was stocked with a representative insect community (See supplementary material). The densities of each prey taxon were calculated from the observational dataset (Question 1), using data from bromeliads of similar size to those in our experiment. We ran this experiment in two temporal blocks for logistical reasons: three complete replicates of all treatments were set up on 20 February 2011, and two on 08 March 2011. We first placed the prey species into the bromeliad, allowed two days for the prey to adjust, then added predators. After 26 days from the beginning of each block, we added the same prey community a second time to simulate the continuous oviposition that characterizes the system. We concluded the experiment 43 days from the first addition of prey (20 April 2011). Throughout the experiment, all bromeliads were enclosed with a mesh cage topped with a malaise trap and checked daily for emergence of adults. At the end of the experiment we completely dissected our bromeliads, collecting all invertebrates and detritus remaining inside.

We used a substitutive design which maintained the same predator metabolic capacity in all replicates (see below). Substitutive experiments often hold total abundance constant, but when species differ substantially in body size - as in this experiment - allometric effects of body size on feeding rate can confound detection of effects based on trophic traits or species interactions, and standardizing to community metabolic capacity is preferred (Srivastava and Bell 2009). Integrating the allometric relationship between body size and feeding rate (Brown et al. 2004; Wilby et al. 2005) over all individuals of a species allows estimates of "metabolic capacity", or the potential energy requirements of a species (Srivastava and Bell 2009). Metabolic capacity is equal to individual body mass raised to the power of 0.69 (an invertebrate-specific exponent determined by Peters (1986) for invertebrates and confirmed by Chown et al, (2007)); this reflects the nonlinear relationship between feeding rate and body size across many invertebrate taxa.

At the end of the experiment, to quantify the effect of predators on ecosystem function, we measured five community and ecosystem response variables: decomposition of coarse detritus, production of fine particulate organic matter (FPOM), bromeliad growth, uptake of detrital nitrogen into bromeliad tissue, and survival of invertebrate prey (emerged adults + surviving larvae). We measured decomposition by once again passing the bromeliad water through a 850 µm sieve, collecting the retained detritus and determining the mass of this detritus after oven-drying it at approximately 70C. We measured the production of FPOM by taking the remaining liquid and filtering it on pre-weighed coffee filters, which were then dried and reweighed. We measured bromeliad growth as the average increase in length of five leaves per plant. We tracked the uptake of labeled detrital nitrogen by analyzing three innermost (closest to meristem) bromeliad leaves at the end of the experiment. Finally, we quantified the species composition and survivorship of invertebrate prey by combining counts of emerging adult insects and surviving larvae.

We measured decomposition by collecting all *Plinia* leaves from bromeliads; these were oven-dried at 70C before their mass was determined. At the end of experiment, we sampled three new bromeliad leaves for isotopic (15N) and nitrogen concentration analyses. These analyses were performed at the Stable Isotope Facility laboratory (UC Davis, CA, USA) using continuous flow isotope ratio mass spectrometer (20–20 mass spectrometer; PDZ Europa, Sandbach, England) after sample combustion to N2 at 1000C by an on-line elemental analyzer (PDZ Europa ANCA-GSL).

## Data analysis

We quantified the effect of phylogenetic distance on each of distributional (Question 1) and diet (Question 2) similarity. First, we calculated phylogenetic distance between each pair of species, then fit several functions to the relationship between phylogenetic diversity an either distributional or diet similarity. We used linear, constant, and several appropriate nonlinear functions (nonlinear, because our measures of similarity are bounded by 0 and 1; see below). We compared these models using AIC and generated confidence intervals as appropriate (parametric or bootstrap for linear and nonlinear, respectively). We evaluated both distributional and diet similarity between predators using Pianka's index of niche overlap (Pianka 1974):

For each pair of predators, and represent the preference of predator or for resource or habitat . The value represents similarity (in our case, in either distribution or diet) and ranges from 0 (complete dissimilarity) to 1 (complete similarity). The resources represent the different habitats surveyed for Question 1 (distributional similarity), or the different prey species assayed for Question 2 (diet similarity). Preference () represents the proportion of a predator's total metabolic capacity found in a particular bromeliad (Question 1); or the proportion of feeding trials in which it ate a particular prey (Question 2).

We tested effects of both single and multiple predator species on community responses with a manipulative experiment where identical prey communities were exposed to treatments of either a single predator, or pairs of predators representing increasing phylogenetic diversity. We divided the analysis of this experiment into three parts: quantifying the effect of phylogenetic distance on prey community similarity, on community and ecosystem responses responses, and on non-additive effects. First, we compared the four treatments with single predator species by calculating the similarity in species composition (Pianka's index) between surviving prey communities and relating this to the phylogenetic distance between predators. If predator feeding choices are phylogenetically conserved, that diet similarity will decline with increasing phylogenetic distance.

Second, we examined how predators affected the five community and ecosystem responses measured in this experiment, testing in turn the effect of predator presence, number, species identity, and finally phylogenetic diversity. To test for an effect of predator presence, we compared the control treatment (predators absent) with the mean responses of all seven treatments that did contain predators. To test for an effect of predator species number (one or two predators), we compared the means of all single-species treatments with the means of all two-species treatments. To test for an effect of predator identity, we compared all four single-species treatments. Finally, to test for an effect of predator combinations we compared all two-species treatments (3 pairs total). We analyzed each of these of these orthogonal comparisons with one-way ANOVA.

In our third and final analysis we quantified the non-additive effect of predator species. We calculated this effect as the difference between the response in bromeliads with both predator species (n=5) and the mean response in bromeliads with either one of these two predator species (n=5 for each predator species). We generated bootstrap confidence intervals for these non-additive effects; confidence intervals which do not overlap zero indicate a significant non-additive effect of a predator combination. We used R version 3.2.0 (R Core Team 2015) for most calculations, and two packages: picante (Kembel et al. 2010) for calculation phylogenetic distances matrices, and vegan (Oksanen et al. 2015) for distance metrics.

## Results

### Question 1 similarity in distribution

We did not find any significant relationship between predator co-occurrence in bromeliads (measured as Pianka's index of niche overlap) and the phylogenetic distance between them (Figure 1a, F1,89=2.39, p=0.13). This indicates that all 14 predator species have roughly similar habitat distributions at the level of the bromeliad. Indeed we often found multiple predator species co-occurring in the same bromeliads (mean 4.4 ± 2.9 predator species per plant). We were able to sample a wide range of phylogenetic relatedness, including two groups of congenerics -- two species of *Bezzia* sp. (Diptera:Ceratopogonidae) and three species of *Leptagrion* sp. (Odonata:Coenagrionidae). There were also two groups of confamilials -- three species of Tabanidae and two species of Empididae, all Diptera. Deeper divisions were also present: three families of Diptera are represented by a single predator species each (Dolichopodidae, Corethrellidae and Chironomidae) and the deepest taxonomic divide is between all insects present and the predatory leeches (Arhynchobdellida:Hirudinidae).

### Question 2: Similarity in diet

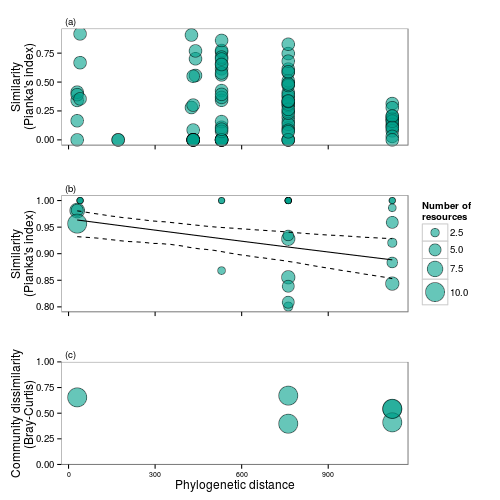
Phylogenetically distant predators differed in their preference of prey species, as measured by the niche overlap index (Fig 1b, regression weighted by the number of prey assayed, F1,26=5.98, p=0.022). Despite the decline in diet similarity with phylogenetic distance, the variation in predator feeding behaviour did not translate into a significant difference in the composition of prey species surviving the manipulative experiment (Fig 1c, F1,4=1.46, p=0.29, distance measured as Bray-Curtis dissimilarity)

### Question 3: similarity in top-down effects

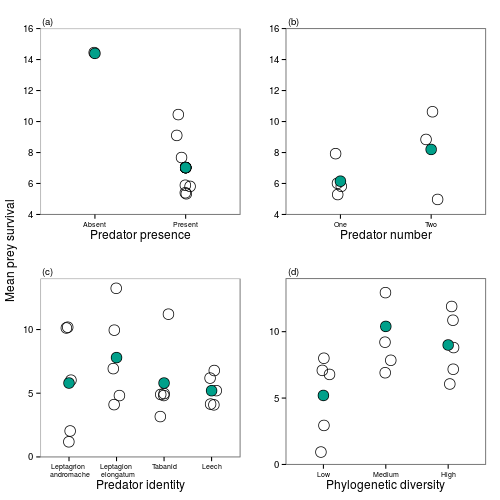
We analyzed our five univariate response variables from the manipulative experiment by dividing them into four separate and orthogonal tests: predator presence, predator number, predator species identity and, increasing predator phylogenetic diversity. Across all four tests, we saw the strongest responses for total prey survivorship (Table 1). Prey survivorship was halved when predators were present (Figure 2a, Table 1). Although individually predator species had similar effects on survivorship (Figure 2c, Table 1), combinations of predators with higher phylogenetic diversity showed a significant increase in total prey survivorship (Fig 2d). That is, more phylogenetically diverse predator combinations caused less prey mortality. Interestingly, these effects on prey surviorship did not result in a change in the processing of detritus (measured either as reduction in coarse detritus or production of fine detritus), bromeliad growth or nitrogen cycling (Table 1).

In order to further understand the effects of predator combinations, we tested for non-additive effects of predator phylogenetic diversity with a randomization test. When we compared the actual effects of predator combination with those expected from the mean of each single-species treatment, we found that predator pairs with the greatest phylogenetic diversity had the highest prey survival. While effects of *L. andromache* and *L. elongatum* in combination were quite similar to the effect of either alone, when *L. elongatum* was placed in the same plant as either a *Stibasoma* larva or leeches, on average five more prey individuals (18% of total prey community) survived till the end of the experiment (Fig 3; Tabanid randomization p = 0.01, Leech randomization p = 0.032). Once again, this effect on invertebrate density did not in turn create a significant difference in other response variables.

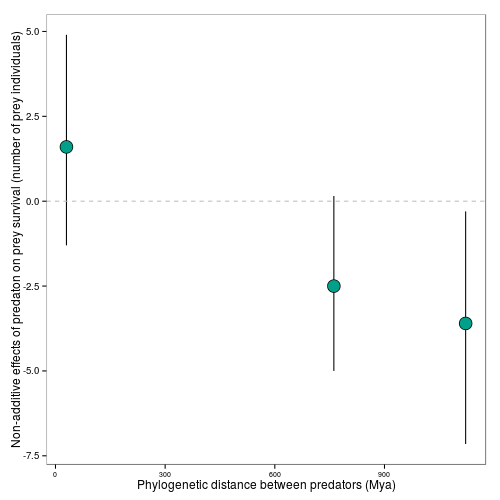
### Figures



**Figure 1**: Phylogenetic distance between predators as a predictor of niche overlap among predators and impacts on prey composition. Our measures of niche overlap were: (a) distribution among bromeliads and (b) diet preferences. We also show the effect of phylogenetic distance between predators on (c) community dissimilarity of surviving prey (Bray-Curtis dissimilarity). We measured distributional similarity (a) by counting all predators in 25 bromeliads, estimating their total metabolic capacity, and calculating niche overlap (Pianka's index) among all pairs of species. We measured diet preferences (b) for a subset of these predators by offering them various prey in no-choice trials, and again calculated niche overlap among them. Finally, we measured community composition of surviving prey (c) at the end of an experiment in which predators were placed in bromeliads with standardized prey communities. For (a) and (b) we used Pianka's index of niche overlap (1 = complete niche overlap) and tested various nonlinear and linear models (see Appendix) of the relationship between this index and phylogenetic distance. Solid lines show significant model fit, and dashed lines show bootstrap 95% quantiles.



**Figure 2**: Orthogonal comparisons of the effect of predators on prey survival. We show the effects of predator presence (a), and then within predator present treatments the effects of predator species number (b). Within treatments with one predator species, we show effects of predator identity (c). Within treatments with two predator species, we show the effect of increasing phylogenetic diversity (d, arranged in order of increasing phylogenetic distance: Low = *L. andromache* + *L elongatum*, Medium = *L elongatum* + tabanid, High = *L elongatum* + leech). Shaded dots represent grand means for each group; unshaded dots are either treatment means (2a and 2b, n = 5) or individual bromeliads (2c and 2d). Points are jittered horizontally slightly to reveal all datapoints.



**Figure 3**: Non-additive effects of predator combinations on prey decrease with increasing phylogenetic distance between predators. A difference of 0 indicates that two-predator treatments resulted in no more prey mortality than would be expected from simply averaging single-predator treatments. Error bars represent bootstrap 95% confidence intervals.

**Table 1** Predator diversity effects on community and ecosystem variables. We measured five community-level variables: total prey survival (both emerged adults and surviving larvae; see Figure 1), the breakdown of coarse detritus (decomposition), the production of fine particulate organic matter (FPOM), the cycling of nitrogen, and the growth of the bromeliad itself. We contrast treatments in our experimental design in four ways: comparing treatments with predators to those without (" Predator Presence"), contrasting predator species ("Identity"), comparing predator communities of 1 or 2 species ("Richness"), and considering the effects of phylogenetic distance between predators ("Pairwise PD"). \* = p < 0.05.wic

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| --- | --- | --- | --- | --- |
| Response | Predator Presence | Identity | Richness | Pairwise PD |
| Total prey survival | F1,10 = 9.07\* | F3,16 = 0.60 | F1,5 = 1.96 | F1,13 = 7.64\* |
| Decomposition (g) | F1,10 = 0.47 | F3,15 = 1.29 | F1,5 = 0.21 | F1,13 = 0.40 |
| FPOM (g) | F1,10 = 0.46 | F3,15 = 0.28 | F1,5 = 6.19 | F1,13 = 1.45 |
| Bromeliad growth | F1,10 = 0.51 | F3,16 = 0.96 | F1,5 = 0.49 | F1,12 = 1.29 |
| Nitrogen cycling | F1,10 = 2.00 | F3,16 = 1.84 | F1,5 = 0.50 | F1,13 = 0.15 |

## Discussion

We found that in our bromeliad system the phylogenetic distance between predators had variable importance. The phylogenetic distance between them predators was unrelated to co-occurrence (Question 1). However, as phylogenetic distance between predator species increased, diet overlap decreased by 20% (Question 2). Interestingly, these apparent diet preferences in the lab did not generate a difference in composition of surviving species in the field (Question 3a). Phylogenetic diversity caused an increase in prey survival (i.e. a decrease in predation); phylogenetically distant pairs of predators that co-occurred in bromeliads had less impact on prey than expected from their performance in isolation (Question 3b).

### Phylogenetic distance and similarity in distribution

We found no relationship between the phylogenetic distance between predator species and the overlap in their habitat distribution. This similarity in distribution could be caused by two processes: low habitat variability among bromeliads, or low variability in preference of predators for different habitats. A concurrent observational study (AAMMD, unpublished) showed that bromeliads vary widely in abiotic conditions, size, detritus amount and prey community; therefore it seems unlikely that low patch variation explains the lack of pattern. It appears instead that predators do not possess any strong phylogenetically-conserved preferences for different habitat characteristics, showing instead very generalist habitat preferences. This is not surprising, given that these organisms live in small, fluctuation-prone habitats. As a group, predatory invertebrates in bromeliads do not show more sensitivity to bromeliad size or drought than other invertebrates (Amundrud and Srivastava 2015). The co-occurrence of predator species within bromeliads suggests that antagonistic interactions among predators do not limit species distributions. Additionally, it appears that predator species are able to co-occur in many different combinations, creating a range of phylogenetic diversities found within bromeliads. This suggests that the range of phylogenetic diversity we tested in our experiment was realistic.

### Phylogenetic distance and similarity in diet

We observed a negative relationship between phylogenetic distance and overlap in diet as measured by laboratory feeding trials. This suggests that there is a phylogenetic signal to predator feeding traits. For example, damselflies are visual predators which engulf prey whole using specialized mouthparts; they are gape-limited and cannot eat prey that are too large. Leeches, in contrast, lack eyes but are able to pierce prey and consume them without swallowing. Damselflies showed a much stronger preference for culicid larvae than did leeches, while leeches were slightly more able to kill and consume scirtids. Culicid larvae are free swimming in the water column, and are therefore easily captured by engulfing predators, whereas scirtid larvae crawl on surfaces and are difficult to remove. Although in this study such feeding traits are phylogenetically structures, in other studies functional traits can be more important than phylogeny *per se* to a predator's diet: Moody (1993) found that unrelated decapod species which were morphologically similar were also functionally similar. Similarly, Rezende et al. (2009) found that both body size and phylogeny determined the food web "compartment" (shared predator-prey interactions) of predators in a marine foodweb.

### Phylogenetic distance and non-additive effects

We found that the presence of predators reduced prey survival, but that this reduction was less for phylogenetically-diverse combinations of predators. This was contrary to our hypothesis that more distant predators would show an increase in prey capture via complementarity. *L. andromache* did not produce an antagonistic (i.e. less than additive) effect in combination with *L. elongatum*, while the two more phylogenetically diverse combinations (*L. elongatum* with the Tabanid or leech) did. *Leptagrion* species may not distinguish between conspecifics and congenerics. In predicting a synergistic non-additive effect of predators, we were imagining an outcome much like those reported by Nilsson et al. (2006). They found that stoneflies caused prey to move into habitats where fish predators could consume them, increasing total predation (a synergistic effect, caused by a phylogenetically distinct predator). Our results are more consistent with those of Finke and Denno (2005), who found that combinations with two insect predators had a higher per-capita effect on leafhopper prey than combinations with an insect and a spider. That is, more phylogenetically diverse combinations of predators showed more predation on lower trophic levels.

In our experiment we did not see any effect of predator presence, nor of increasing predator phylogenetic diversity, on ecosystem function. This was contrary to our predictions based on the results of Ngai and Srivastava (2006), who found that adding predators to a community increased the rates of nitrogen fixation. While we did observe differences in prey consumption, the resulting changes in detritivore density did not cause differences in decomposition of detritus. This difference may be due to leaf traits of the *restinga* vegetation. In *restinga* vegetation, leaves are generally extremely tough and waxy, while in rainforests (e.g. Northwest Costa Rica, the site of Ngai and Srivastava (2006)), leaves tend to be softer -- with the result that, in *restinga*, invertebrates are unable to consume leaves directly while in rainforests they are. Several lines of evidence support this assertion. Romero and Srivastava (2010) studied the effects of the spider *Corinna* gr. *rubripes* (Corinnidae) on bromeliad ecosystems. This spider has no effect on the composition of detritivore communities, nor on decomposition rates, but increases nitrogen content in bromeliads, probably by depositing feces or the carcasses of terrestrial prey. This indicates that *restinga* bromeliads may derive less of their nitrogen from terrestrial detritus, but may benefit more from terrestrial inputs. Additionally, detritus breakdown rates are lower in *restinga* than in rainforest, and less dependent on invertebrates (R. Lecraw, pers comm). A separate experiment (GQ Romero, pers comm) supports the hypothesis that lower decomposition in *restinga* is due to plant traits. This second experiment contrasted decomposition caused by invertebrates and bacteria with that caused by bacteria alone (by comparing bagged detritus enclosed in coarse vs fine mesh). The experiment used two species of detritus: leaves from a rainforest tree, and leaves from a *restinga* tree. Invertebrates only caused an increase in decomposition for the rainforest tree, not the *restinga* tree.

When *L. elongatum* occurred with more distantly related predators, prey survivorship was greater than expected. This non-additive effect may have been due to a reduction in predation by odonates in the presence of non-odonate predators. Odonates have been shown to be sensitive to chemical cues (Barry and Roberts 2014) or tactile cues (Atwood et al. 2014) of potential predators, which causes a decrease in feeding rate. For example, a different species of bromeliad damselfly -- *Mecistogaster modesta* Selys -- reduces predation when it is housed with Dytiscid adults (Atwood et al. 2014). If there is a phylogenetic signal to the chemical cues released by predators, individuals of one species might be unable to distinguish close relatives (congenerics in our case) from conspecifics. One limitation of our approach is that all phylogenetic diversity treatments contained one species in common, *Leptagrion elongatum*. It is possible that this species is more sensitive to the presence of other predators, and therefore shows a larger effect, than would other species in this community. However, this is the most common predator in this community and our results indicate that its top-down effects are likely to be frequently reduced by the presence of other predators. These differences in survival did not create differences in decomposition, nor subsequent nitrogen uptake by bromeliads. This contrasts with the increase in N uptake found by Ngai and Srivastava (2006) in Costa Rican bromeliads.

In most natural communities, diverse predator species co-occur and often simultaneously affect prey species. By combining an observational study, laboratory trials and a field experiment we have shown that phylogenetic relatedness of species can help predict some aspects of lower trophic level responses. An approach based on phylogenetic diversity offers an organizing framework around which to compare diverse datasets on the distribution, trophic interactions and combined effect of multiple predator species, to predict the top-down effect of diverse predator assemblages.

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