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). The effects of all predators in a community (the predator assemblage) is often dependent on species composition, and can be more or less than the sum of effect sizes of individual predators (Sih, Englund, and Wooster 1998; Ives, Cardinale, and Snyder 2005). Detailed studies manipulating predator diversity have identified many direct and indirect mechanisms of predator interaction. For example, predators may differ in their preference for microhabitats resulting in spatial or temporal partitioning between predator species (Schmitz 2007). Even when predators occupy the same patch, they may feed on different prey species and so have additive top-down effects (Schmitz 2007). Non-additive effects of predator diversity occur when predators interact directly with each other, or indirectly via prey species, in a way that increases or decreases total predation. For example, antagonistic predator-predator interactions can reduce the top-down effects of a diverse consumer assemblage, e.g. when predators feed directly on each other (intra-guild predation) or modify the behaviour of predators or prey via non- consumptive (i.e. trait-mediated) interac-

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tions (Sih, Englund, and Wooster 1998; Griswold and Lounibos 2006; Nyström et al. 2001). While there are many mechanisms potentially underlying predator compositional effects, we lack a means of predicting *a priori* which combinations are likely to have antagonistic versus synergistic effects on ecosystem functions.

The measurement of phylogenetic diversity has become a popular means for ecologists to make inferences about ecological and evolutionary mechanisms behind observed patterns in natural communities (Cavender-Bares et al. 2009). For example, phylogenetic diversity of plant communities is better correlated with productivity than is species richness or diversity (e.g. M. W. Cadotte et al. 2009; M. W. Cadotte, Cardinale, and Oakley 2008; Godoy, Kraft, and Levine 2014). Central to most ecological applications of phylogenetic diversity is the assumption that increased phylogenetic distance implies increased ecological dissimilarity – either in the form of differences in species niches, interactions, or functional traits. When these relationships occur, the resulting variation in ecologically-relevant traits and interactions could increase ecosystem function (D. S. 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 (L. Bersier and Kehrli 2008; Naisbit et al. 2011). Many studies of phylogenetic signal in predator traits focus on whole clades, rather than local assemblages (e.g. *Anolis* lizards (Knouft et al. 2006), warblers (Böhning-Gaese, Schuda, and Helbig 2003), treeboas (Henderson, Pauers, and Colston 2013) and wasps (Budriene and Budrys 2004)) making it difficult to connect these results to top- down effects at the scale of a local community. While these clade-specific studies often find weak evidence for phylogenetic signal in ecologically- relevant traits, stud-

ies at the level of the whole biosphere (Gómez, Verdú, and Perfectti 2010; L. Bersier and Kehrli 2008) demonstrate that related organisms often have similar interspecific interactions – i.e. related predators often consume similar prey. At the level of a community, the effect of a predator assemblage will depend on both the spatial distribution and trophic interactions of all predator species. Phylogeny may constrain species distributions when distant relatives have distinct fundamental niches, while close relatives are too similar to co-exist (Webb et al. 2002; Emerson and Gillespie 2008). The effects of other predators can also be be important: during dispersal, predators may use cues of prey, competitors, or intra-guild predators when selecting a habitat patch (Chase and Shulman 2009; McCauley et al. 2008; D. S. Srivastava et al. 2008). After colonization, patterns of co-occurrence may be further modified by intraguild predation (L. P. Lounibos et al. 2008; Juliano 2009) or competition (Fincke, Yanoviak, and Hanschu 1997).

When predators do co-occur, their direct effects on each other and on their prey is determined by their diet breadth. Diet breadth (the diversity of prey species which predators consume) will depend on their feeding traits and nutritional – all of which may be phylogenetically conserved. If this is the case, then predator assemblages with higher phylogenetic diversity will show greater complementarity and a stronger top-down effect on ecosystem function (Finke and Snyder 2008). For example, some predators are gape-limited, and can swallow any prey smaller than their mouth; these predators typically broaden their diet as they grow (e.g., larvae of Odonata) (E. Werner and Gilliam 1984). Such ontogenetic changes in diet breadth can radically influence food web structure (Moya-Laraño 2011). Other predators (e.g. Leeches, or Diptera:Tabanidae) are "piercing-sucking" predators (Bay 1974) and may

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63 be more general consumers at all life stages, since they can feed on larger prey individuals

64 even at very early predator life stages. In some cases, predator diets may extend to include

65 other predators, leading to direct negative interactions such as intraguild predation which

66 may also have a phylogenetic signal (Pfennig 2000).

67 Indirect interactions among predators are common in many communities, and can lead to

68 nonlinearities which prevent the inference of predator effect from the additive combination

69 of individual predators (Sih, Englund, and Wooster 1998). For example, decreasing predator

70 richness has been shown to increase herbivory in a three-level kelp food web (Byrnes et al.

71 2006). The complex traits that underlie such species interactions ("higher-order" interactions,

72 Sih *et al.* (1998)) may also correlate with phylogeny; if so, then phylogenetic diversity

73 will be a better predictor of ecosystem function than richness alone (M. W. Cadotte et al.

74 2009). For example, phylogenetically diverse assemblages of mycorrhizae increased primary

75 productivity more than equally speciose treatments from the same lineage (Maherali and

76 Klironomos 2007). The effect of predators on prey may also be non-additive, the result of

77 indirect interactions either with prey species or other predators: prey species may respond to

78 the presence of one predator by a behavioural shift that increases the predation by another

79 predator population (Carey and Wahl 2010). These trait-mediated indirect effects (TMII, (E.

80 Werner and Peacor 2003)) can be difficult to predict; however phylogenetic relatedness has

81 been suggested as a possible means of predicting the combined effect of predators (Naisbit

82 et al. 2011)

83 We used a series of observations, lab feeding trials, and manipulative field experiments to

84 measure how the phylogenetic diversity of the predator assemblage predicts community com-

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position and ecosystem function. We test three related hypotheses concerning co-occurance patterns, diet similarity and top-down ecosystem effects of diverse predators, using a natural mesocosm: the community of invertebrates living within bromeliads. Bromeliads (Bromeliaceae) are epiphytic plants native to the Neotropics. Many bromeliad species contain water, detritus and a complex insect food web within their leaves; the decomposition of this detritus supplies nutrients for the bromeliad (Benzing 2000). 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; their presence dramatically alters community dynamics (e.g. decreases insect emergence (Starzomski, Suen, and Srivastava 2010) and increases nutrient cycling (Ngai and Srivastava 2006)).

1. *species co-occurrence*: 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*: similarity in diet (as measured by feeding trials) decreases with phylogenetic distance if diet is phylogenetically conserved. Alternatively, closely related species may have evolved different diets to allow coexistence.

3. *ecosystem-level effects*: Our experiments at the level of the whole habitat patch (i.e. a single bromeliad) allows us to examine direct and indirect effects of predator combinations.

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a) monoculture treatments allow us to assess the effect of each predator on a complete prey community and ecosystem function. Phylogenetic similarity among predators is predicted to correlate with similarity in single-species effects if related predators share similar trophic interactions (e.g. predation rate, diet breadth)

b) By comparing treatments with pairs of predators to treatments which received a monoculture of each predator, we are able to estimate additive and nonadditive effects. If distance between species correlates with increasing difference, we will see an increase in these non-additive effects with phylogenetic distance. At the extreme, differences between predators may lead to IGP among predators.

Methods

Site and Species

We conducted all observations and experiments in Parque Estadual da Ilha do Cardoso (25°03′ S, 47°53′ W), a 22.5 ha island off the south coast of São Paulo state, Brazil. We worked in a closed coastal forest (*restinga*) the understory of which is mostly covered by *Quesnelia arvensis* Mez. (Bromeliaceae), a large terrestrial bromeliad that accumulates up to 2.8 L of rainwater in tanks formed by individual leaves. We used two datasets: an observational survey of insect diversity from 2008, and a manipulative experiment in 2010. Our observational survey found more than 47 species of macroinvertebrates in these aquatic communities (Romero and Srivastava 2010). This diversity encompasses multiple trophic and functional groups: filter feeders (Diptera:Culicidae); detritivores including shred-

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ders (Diptera:Tipulidae, Trichoptera), scrapers (Coleoptera:Scirtidae), and collectors (All Diptera:Chironomidae, Syrphidae, Psychodidae). These species are the main prey for a diverse predator assemblage of at least three species of damselfly larvae (*Leptagrion* spp., Zygoptera:Coenagrionidae), two species of predatory Horse Fly larvae (Diptera:Tabanidae), and 2 species of leech (Hirudinidae). Other, smaller-bodied predators present include predatory midge larvae (Diptera:Chironomidae:Tanypodinae) and less abundant species such as Dytiscid beetles.

Phylogenetic distance, metabolic capacity and niche overlap

In 2008, we counted and measured macroinvertebrates in an observational study of 25 bromeliads. We identified 14 species as predators, including two groups of congeneric taxa – *Bezzia* sp. (Diptera:Ceratopogonidae) with two species and *Leptagrion* sp. (Odonata:Coenagrionidae) with three – and two of confamilial taxa – three species of Tabanidae and two 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 a species of predatory leech (Annelida:Hirudinidae).

We obtained node age estimates for all seven internal nodes of the tree, using `www.timetree.org`, an online database of published molecular time estimates (Hedges, Dudley, and Kumar 2006). Most dates came from only a single study; where multiple dates were found we used the median estimate: Insecta–Hirudina (543 to 700 Mya, n=5 studies), Odonata–Tabanidae (151 to 543 Mya, n= studies) and Tabanidae–Diptera (106.2 to 474 Mya, n=7 studies).

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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 as polytomies, and were all assigned identical, arbitrary and short branch lengths (15 Mya).

Species co-occurrence is often measured in terms of non-random patterns of species presence/absence or abundance, but such measures will only be poorly related to the functional effects of species when species differ substantially in body size. Integrating the allometric relationship between body size and feeding rate (Brown et al. 2004; Wilby, Villareal, and Lan 2005) over all individuals of a species allows estimates of "metabolic capacity", or the potential energy requirements of a species (D. S. Srivastava and Bell 2009). Metabolic capacity is equal to individual body mass raised to the power of 0.69 (an insect-specific exponent determined by Peters 19xx, (Chown et al. 2007)); this reflects the nonlinearity of feeding rate on body size across many invertebrate taxa. We used metabolic capacity to inform both our observational results and our experimental design (details below), with the exception of our feeding trial data. This is because the feeding trials were intended to measure which prey our predators ate, rather than their feeding rate (only the latter should scale with metabolic capacity).

Questions 1 and 2 above focus on similarity ("niche overlap") between predator species. Question 1 hypothesizes that if habitat preference is phylogenetically conserved and competition between predators unimportant, then distributional similarity will decline with increased phylogenetic distance between a pair of predators. Question 2 hypothesizes that if diet is conserved, then diet similarity declines with increased phylogenetic distance between a pair

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of predators. We evaluated both distributional and diet similarity between predators using

Pianka's index of niche overlap (Pianka 1974):

$$O_{kl} = \frac{\sum_i^n p_{il} p_{ik}}{\sqrt{\sum_i^n p_{il}^2 \sum_i^n p_{ik}^2}}$$

For each pair of predators, p_{ik} and p_{il} represent the preference of predator k (or l) for

resource or habitat i. In terms of predator co-occurrence, $n=25$ bromeliads surveyed in the

observational dataset described below. In terms of diet similarity, the number of resources (n)

is defined as the total number of prey species assayed with both predator taxa, and preference

is defined as the proportion of diet trials (see below) for each predator-prey combination that

resulted in prey mortality.

We quantified the effect of phylogenetic distance on each of distributional and diet similarity.

First, we calculated phylogenetic distance between each pair of species, then fit several

functions to this relationship (linear, constant, and several appropriate nonlinear functions).

We compared these models using AIC, selected the best model, and generated confidence

intervals as appropriate (parametric or bootstrap for linear and nonlinear, respectively).

Distributional Similarity

We used the 2008 survey to examine patterns of co-occurrence among predator taxa. Each

bromeliad was dissected and washed to remove invertebrates. The resulting water was filtered

through two sieves (250 and 850 µm), which removed particulate organic matter without los-

ing 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

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190 instar).

191 We calculated total metabolic capacity of each predator species in each bromeliad by sum-

192 ming estimates of biomass for all individuals of the same species within a plant. We used

193 these values to calculate niche overlap as measured by habitat occupancy. Overlap was

194 calculated for each pair of predators across all bromeliads, even if neither species occurred

195 there.

Diet Similarity

197 We conducted 314 feeding trials of 10 predator taxa fed 14 prey taxa between March and

198 April 2011. We covered all potential predator-prey pairs present in the experiment (de-

199 scribed below), and attempted to perform all other trials whenever possible. However, due

200 to the rarity of some taxa many predator-prey pairs were not possible; we tested 56 pairwise

201 combinations. Most trials were replicated at least five times, but the number of replicates for

202 various combinations ranged from 1 to 11. We placed predators together with prey in a 50ml

203 vial, with a leaf or stick for substrate. The only exception was the tabanid larvae, which

204 we placed between two vertical surfaces to imitate the narrow space found in bromeliad leaf

205 axils, their preferred microhabitat. Generally our trials contained a single predator and a

206 single prey individual, except in the case of very small prey (*Elpidium* sp.) or predators

207 (*Monopelopia* sp.) in which case we increased the density. We replicated each combination

208 up to five times where possible, and allowed one day for predation to occur.

209 Again, we estimated overlap between different predators using Pianka's index, using the pro-

210 portion of trials which resulted in predation as our measure of predator preference. Because

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not every predator/prey pair was equally replicated, we weighted our analysis by the number of trials conducted.

Community effect experiment

Our experiment was created with two goals in mind: first, to measure the major effects of each of these predators on their prey and ecosystem function and second, to estimate the non- additive effects of pairwise predator combinations. The strength of these non-additive effects could then be related to the phylogenetic distance between a pair of predator species.

We selected the four most common predators in this system, which also created a range of relatedness: two congeneric damselflies (*Leptagrion andromache* and *Leptagrion elongatum*), two insects (*L. elongatum* and a Tabanid predatory fly), and two invertebrates (*L. elongatum* and leeches). We used each of the pairs of predators just described, as well as each species in monoculture, and a predator-free control (8 treatments, n=5). Combinations were substitutive, maintaining the same amount of total predator metabolic capacity to isolate the effects of predator behaviour and traits. 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 (D. S. Srivastava and Bell 2009). This experiment allows the estimation of the effect of each predator species (monoculture treatments), as well as the detection of non- additive effects in predator combinations.

In Feburary 2011, bromeliads between 90 and 200ml in capacity were collected, thoroughly

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washed to remove organisms and detritus and soaked for 12 hours in a tub of water. They were then hung for 48 hours to dry. One bromeliad dissected after this procedure contained no insects.

Each bromeliad was supplied with dried leaves, simulating natural detritus inputs from the canopy. We enriched these leaves with ^{15}N by fertilizing five (Jabuticaba, *Plinia cauliflora*) plants with 40ml pot^{-1} day^{-1} of 5g L^{-1} ammonium sulphate containing 10% atom excess of ^{15}N over 21 days. Whole leaves were then picked from plants and air-dried until constant weight, and then soaked for three days and the water discarded. About 1.5 g of leaves were placed in each bromeliad (1.5006g $\pm$ 0.0248).

Each bromeliad was stocked with a representative insect community. The densities of each prey taxon were calculated from the 2008 observational dataset, using data from bromeliads of similar size to those in our experiment. All densities used were within the range of these calculated abundances, and all experimental bromeliads received the same insect community. Halfway through the experiment, insects were added to bromeliads a second time to simulate the continuous oviposition that characterizes the system. Throughout the experiment, all bromeliads were enclosed with a mesh cage topped with a malaise trap and checked daily for emergence of adults.

In this experiment we measured five response variables: production of fine particulate organic matter (FPOM), decomposition of coarse detritus, bromeliad growth, uptakle of detrital nitrogen into bromeliad tissue, and survival of invertebrate prey (emerged adults + surviving larvae). We analyzed each of these responses with ANOVA. For each pair of predator species and each response type, we calculated the non-additive 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 nonadditive effects; confidence intervals which do not overlap zero indicate a significant non-additive effect of a predator combination.

Results

Distributional similarity

Across all bromeliads, predator species differed widely in metabolic capacity, from 4.5 $\times$ 10-4 for a species of *Monopelopia* to 0.15 for large predatory flies in the family Tabanidae. Predators often co-occurred in bromeliads (4.4 $\pm$ 2.9 predator species per plant). However, the niche overlap between the total metabolic capacity of pairs of predators did not show any relationship with phylogenetic distance between them ($F_{1,89}$=2.3918, p=0.13).

diet similarity

Among the most common predator taxa (i.e. those used in our experiment, described below) the damselflies (*Leptagrion andromache* and *Leptagrion elongatum*) showed the highest rates of prey consumption (prey consumed in 94.3% and 67.7% of trials, respectively). All predators showed a very generalist diet breadth, consuming nearly all species offered to them. However, more phylogenetically distant predators differed in their preference of prey species, as measured by the niche overlap index ($F_{1, 26}$=5.98, p=0.022, regression weighted by the number of prey species assayed.)

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Ecosystem-level effects and phylogenetic distance

In our manipulative experiment, we placed a standardized prey community into bromeliads and measured five response variables. Predators had a large effect on prey survivorship: on average all predator treatments showed 51% lower prey emerging or surviving as larvae relative to the predator-free control. Nitrogen transport to bromeliad leaves was slightly decreased in bromeliads with predators relative to predator-free controls (-11%), and was only higher than the control in treatments including Tabanid predators. We found a similar pattern for plant growth: on average, predators had a -18% effect on growth of bromeliad leaves (leaf elongation in mm), though Tabanids seemed to create a slight increase. The decomposition of coarse detritus and production of fine organic matter showed no obvious pattern related to the presence of predators.

Predator combinations tended to have a non-additive effect on our response variables, even though we held total metabolic capacity constant amongst all treatments. Approximately 14% more prey survived in polyculture, on average, compared to all monocultures. Nitrogen uptake increased by (8%) and bromeliad growth by (11%). Production of fine particulate organic matter increased by 29% more when predators were present in combination.

Our experimental design allows us to estimate the non-additive effect of predator species pairs on whole communities of prey, and the functioning of the bromeliad ecosystem. We used randomization tests to test the hypothesis that increased phylogenetic distance between members of a predator pair results in a greater magnitude of nonadditive effect. We contrasted the differences of the mean individual predator treatments from the control with the mean difference of their pairwise combination from the control.

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We found the greatest effect for prey survival: while effects of *L. andromache* and *L. elon-gatum* in combination were quite similar to the effect of either alone, when *L. elongatum* was placed in the same plant as either a Tabanid larva or leeches, on average five more prey individuals (18% of total prey community) survived till the end of the experiment (Fig 3). This effect was smaller among the other variables, most of which showed confidence intervals from the randomization test which overlap zero.

Figures
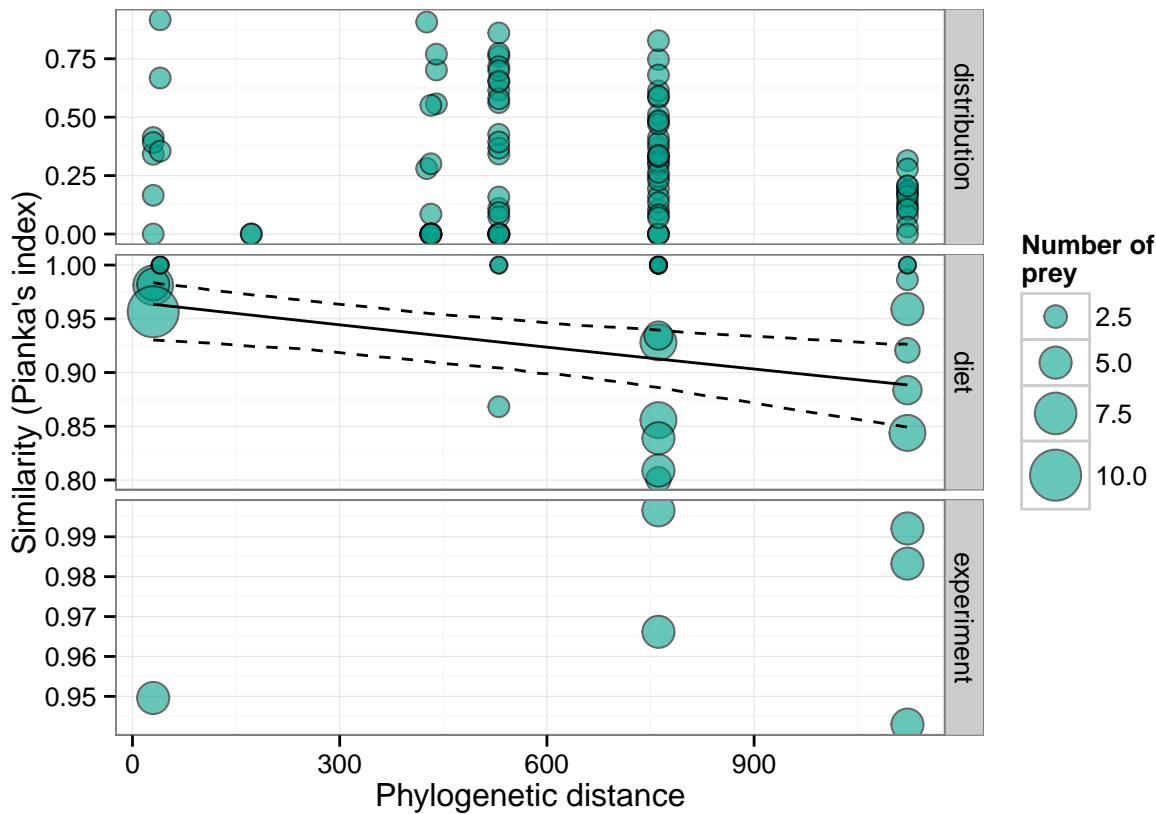
Figure 1: Phylogenetic distance between predator pairs and their ecology. Each point represents a pair of predator species. Distributional similarity was quantified from the total metabolic capacity for each predator species in each bromeliad. Diet similarity is calculated from a series of feeding trials, and experimental similarity as the surviving prey community

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emerging from bromeliads. Similarity between each pair of predators was calculated using

Pianka's index. Solid line shows the best fit non-linear model (see Appendix) and dashed

lines show bootstrap 95% quantiles.
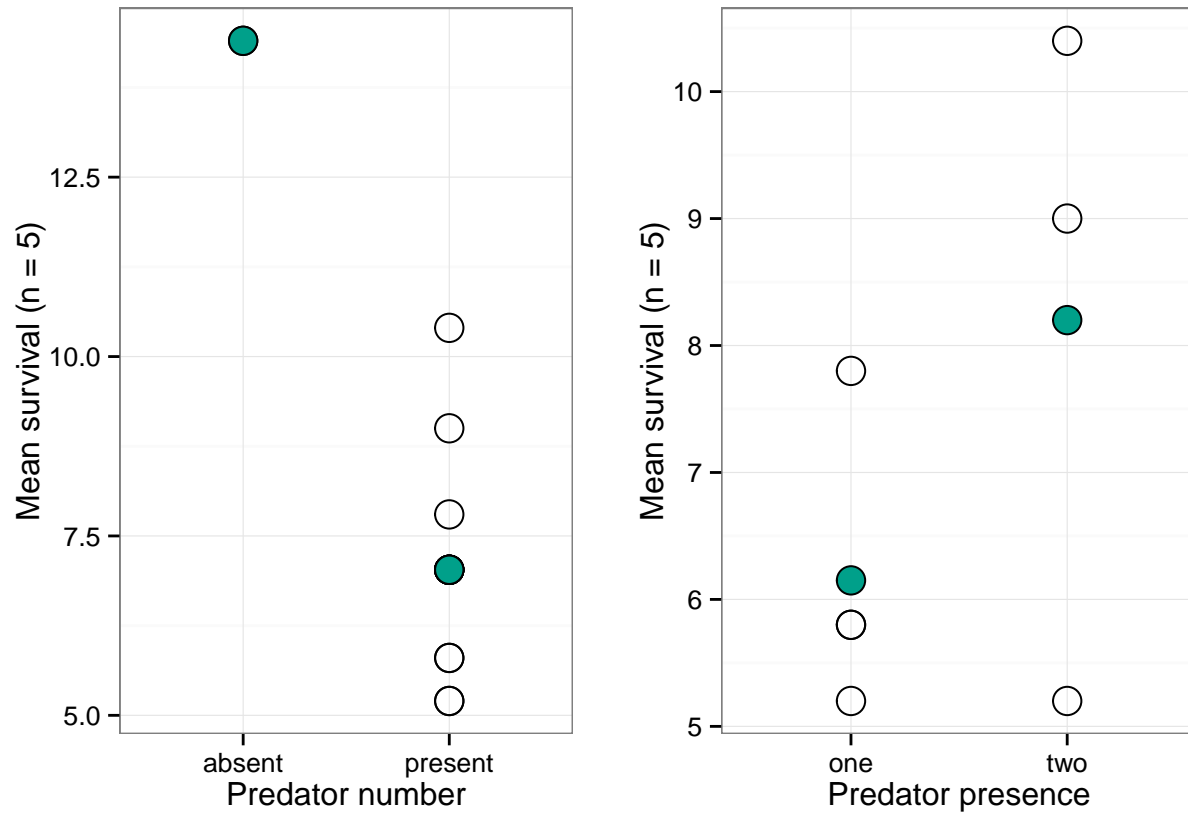
Figure 2: Adding predators reduces insect emergence, probably because they eat the prey. However, increasing predator number raises survival again. This is because the predators are interfering with each other.
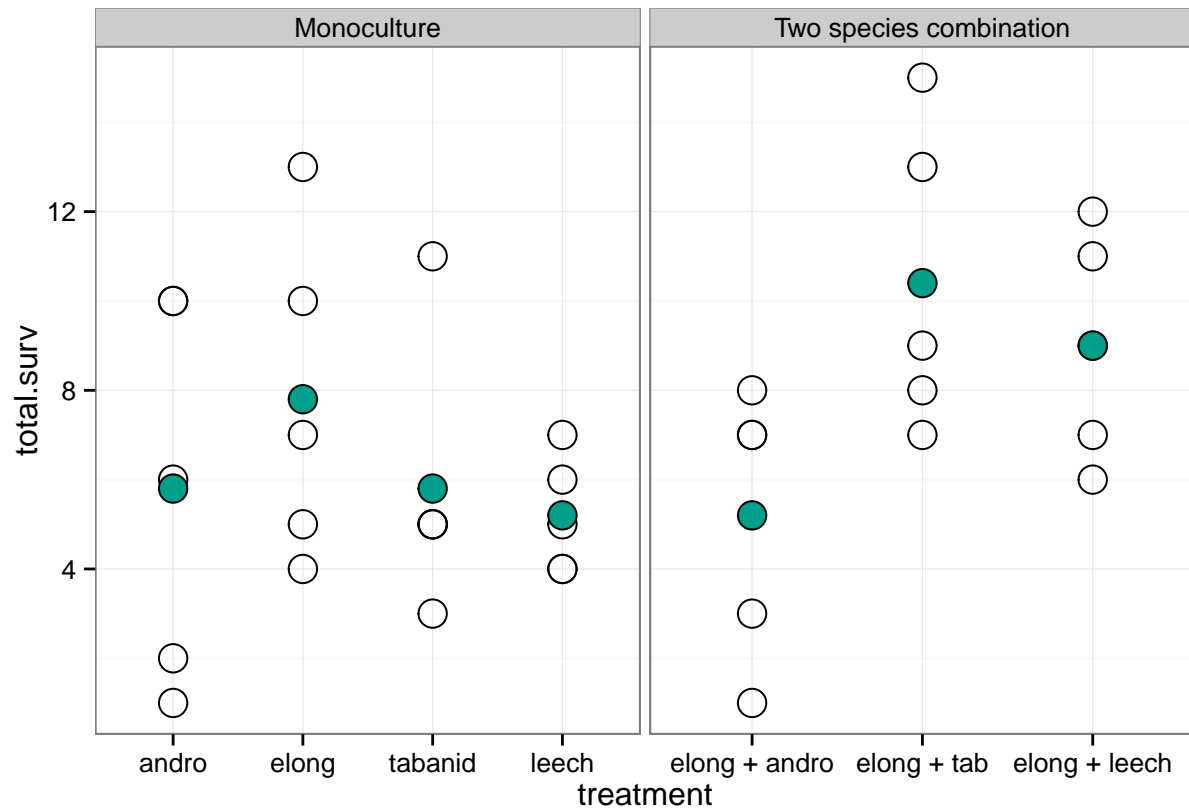
Figure 3: Predator community compositon, in monoculture and polyculture, and its effects on prey survival. Prey emergence is lowest when damselflies are paired together, but increases when damselflies are combined with other species.

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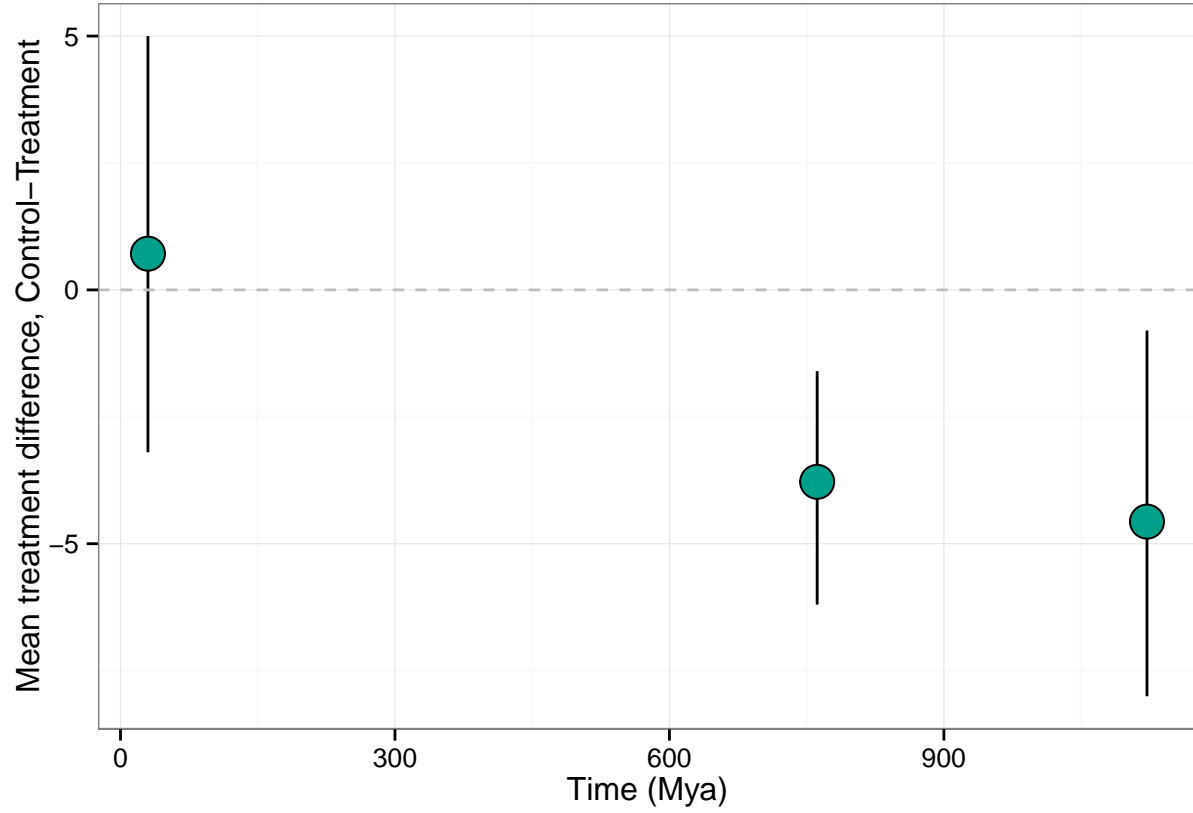
Figure 4: Combinations of predators beyond congenerics show a negative non-additive effect on predation rate. Points represent the mean difference between the means of two monocultures compared to the mean of a polycultures. The dashed line represents differences $= 0$, i.e. no nonadditive effect. These values are presented as differences from (predator free) controls; therefore zero indicates no significant non-additive effect. Relative to control (no predator) plants, bromeliads containing two predators which were not congeneric showed less predation

Table 1

Response	Predator Presence	Identity	Richness	Pairwise PD
Total prey survival	$F_{1,10} = \mathbf{9.07}$	$F_{3,16} = 0.6$	$F_{1,5} = 1.96$	$F_{1,13} = \mathbf{7.64}$

Response	Predator Presence	Identity	Richness	Pairwise PD
FPOM (g)	$F_{1,10} = 0.92$	$F_{3,16} = 0.42$	$F_{1,5} = 6.47$	$F_{1,13} = 1.35$
Decomposition (g)	$F_{1,10} = 0.47$	$F_{3,15} = 1.29$	$F_{1,5} = 0.21$	$F_{1,13} = 0.4$
Bromeliad growth	$F_{1,10} = 0.51$	$F_{3,16} = 0.96$	$F_{1,5} = 0.49$	$F_{1,12} = 1.29$

Discussion

Summary

We tested whether related predators are similar or dissimilar in their habitat distribution (Question 1). We found only weak support: There is no evidence for close relatives being either more or less likely to be found in association. Very distant relatives may be slightly likely to co-occur, as shown by the decelerating curve in Fig 1.

We also tested whether diet similarity decreased with predator phylogenetic distance (Question 2), and found a stronger pattern: diet similarity declined more rapidly than distributional similarity with PD, in a nearly linear relationship.

Question 3 has two parts. Question 3a hypothesized that differences among predator responses would correlate with their phylogenetic divergence, and we found no support for this pattern. We did find support for hypothesis 3b for one of our responses: increased phylogenetic diversity of the predator assemblage was associated with a negative non-additive effect on predation (increased prey survival).

Do related organisms occur in different bromeliads?

Predator pairs showed only a weak relationship between phylogenetic distance and differences in distribution.

This may reflect a lack of variation among bromeliads, either in environmental variables or prey community composition, at least over the lifespans of the predators.

It may also indicate that predator species in this system are habitat generalists. Organisms which live in small habitats, which are prone to large fluctuations, may develop a wide physiological tolerance as they integrate over variation in prey community composition, temperature, etc.

We observed little predator mortality in our experiment; this indicates that perhaps even negative interactions among predators would not necessarily lead to differences in their distribution.

The trend in Figure 1 is largely caused by the distribution of leeches among bromeliads. The leeches are both distantly related to the other predator taxa (all holometabolous insect species) and most different from them in distribution. It is not clear why this should be, but we can speculate that we are seeing the result of different dispersal modes: of all the large predators, only leeches disperse passively.

This indicates that a range of phylogenetic diversity is present in nature, with some bromeliads containing close relatives and others more distant. It also supports our experimental design by suggesting that our predator combinations – even the most disparate – are common in nature.

There are several potential reasons why the negative intraspecific effects that we observed in our experiment do not appear to limit predator distribution. First of all, the probability of interaction in real bromeliads could be quite low, especially in larger bromeliads than those used in our experiment. The negative effects could still be occurring, but not resulting in mortality. Indeed, if animals are actually able to adjust their development times and feeding rates when conditions are unfavourable (and if the presence of a competing predator creates such unfavourable conditions), then we would expect to observe more frequent, not less frequent, co-occurances of predators. Negative interactions need not result in dissimilar distributions or "checkerboard units".

Do related organisms have similar diets?

Predators consumed very similar prey, except at the most extreme differences in the phylogeny.

Leeches and tabanids showed different patterns of consumption compared with *Leptagrion* predators: they consumed less frequently, and notably consumed prey in less than half of trials with *Culex* (leech) or *Scirtes* (Tabanid). This may be because these predators lack the strong jaws and "mask" of odonata, and these two prey species are particularly active (in the case of culicids) or difficult to handle (scirtids). Tabanids in particular differ in microhabitat use from odonates; living deep within leaf axils, where culicids are rarely found.

One of our morphospecies, *Leptagrion* "tan", showed a greater preference for harder-bodied prey species (i.e. Ostracoda, Scirtes and *Phylloicus*, which is a caddisfly.) All of these animals are consumed more frequently by this animal than by the other species of Leptagrion, while

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the other damselflies more frequently consumed softer *Culex* and *Polypedilum* prey.

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 a predator in a marine foodweb.

Our experimental results are consistent with high similarity among predator diets: all predator species had comparable effects on all response variables, including prey species survival.

Phylogenetic diversity and non-additive effects

Predator pairs which were more phylogenetically distant had a larger negative nonadditive effect on prey capture. This is contrary to one of our first predictions, which suggested that more distant predators would show an increase in prey capture.

It is intriguing that the congener *L. andromache* did not produce a trait-mediated effect in *L. elongatum*, while more phylogenetically distinct predators did. It may be that these odonates have behavioural traits that reduce the probability of their interaction, for example each nymph occupying a single leaf-well (Srivastava, CR). If this is the case, each damselfly may not experience many cues indicating the presence of other predators, resulting in no non-additive interaction.

Odonates can be sensitive to chemical cues of potential predators, which causes a decrease in feeding rate (Barry and Roberts 2014). However other research in bromeliads has demonstrated that it is physical contact with other organisms that reduces damselfly predation

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rate (Trish). If this is the case for *Leptagrion*, then when combined with leeches they may be responding to frequent contact with those very active predators. Tabanids, however, are rarely observed outside of a deep leaf axil – in this case, it may be chemical cues which are responsible.

In feeding trials with leeches, actual predation was rarely observed – except in one instance, where the damselfly ate the leech. However, trait-mediated indirect effects may not always reflect realized probability of predation: an animal may change behaviour when exposed to a "predator" poses little threat (REF).

One limitation of our approach is the focus on a single focal predator, the odonate *Leptagrion elongatum*, which was common in all of our predator treatments. It is possible that this species is more sensitive to the presence of other predators, and therefore shows a larger trait-mediated indirect effect, than would other species in this community. However, this is the most common species in this community and our results indicate that its top-down effects are likely to be frequently reduced by the presence of other predators.

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