

Food web structure shaped by habitat size and climate across a latitudinal gradient

GUSTAVO Q. ROMERO,^{1,3,4} GUSTAVO C. O. PICCOLI,¹ PAULA M. DE OMENA,¹ AND THIAGO GONÇALVES-SOUZA²

¹Laboratory of Multitrophic Interactions and Biodiversity (LIMBIO), Department of Animal Biology, Institute of Biology, University of Campinas (UNICAMP), CP 6109, Campinas, SP 13083-970 Brazil

²Laboratory of Phylogenetic and Functional Ecology (ECOFFUN), Department of Biology, Area of Ecology, Federal Rural University of Pernambuco (UFRPE), R. Dom Manoel de Medeiros s/n, Recife, PE 52171-900 Brazil

³Brazilian Research Network on Climate Change (Rede Clima)

Abstract. Habitat size and climate are known to affect the trophic structure and dynamics of communities, but their interactive effects are poorly understood. Organisms from different trophic levels vary in terms of metabolic requirements and heat dissipation. Indeed, larger species such as keystone predators require more stable climatic conditions than their prey. Likewise, habitat size disproportionately affects large-sized predators, which require larger home ranges and are thus restricted to larger habitats. Therefore, food web structure in patchy ecosystems is expected to be shaped by habitat size and climate variations. Here we investigate this prediction using natural aquatic microcosm (bromeliad phytotelmata) food webs composed of litter resources (mainly detritus), detritivores, mesopredators, and top predators (damselflies). We surveyed 240 bromeliads of varying sizes (water retention capacity) across 12 open *restingas* in SE Brazil spread across a wide range of tropical latitudes (-12.6° to -27.6° , ca. 2,000 km) and climates (Δ mean annual temperature = 5.3°C). We found a strong increase in predator-to-detritivore mass ratio with habitat size, which was representative of a typical inverted trophic pyramid in larger ecosystems. However, this relationship was contingent among the *restingas*; slopes of linear models were steeper in more stable and favorable climates, leading to inverted trophic pyramids (and top-down control) being more pronounced in environments with more favorable climatic conditions. By contrast, detritivore-resource and mesopredator-detritivore mass ratios were not affected by habitat size or climate variations across latitudes. Our results highlight that the combined effects of habitat size, climate and predator composition are pivotal to understanding the impacts of multiple environmental factors on food web structure and dynamics.

Key words: *Brazilian restingas; bromeliad food webs; climatic stability; freshwater ecology; global changes; habitat size; inverted trophic pyramids; keystone predators; latitudinal gradient; mesopredators.*

INTRODUCTION

Climate and habitat size are known to each affect food web structure and dynamics (Ledger et al. 2013, Petermann et al. 2015), but their interactive effects are poorly understood. Organisms from different trophic levels vary in terms of metabolic requirements and capacity to dissipate heat, resulting in different responses to climate, with potential changes in the trophic structure (e.g., Voigt et al. 2003, Daufresne et al. 2009, Brose et al. 2012, Dossena et al. 2012, Forster et al. 2012, Ledger et al. 2013, Jonsson et al. 2014). Indeed, it is known that top predators require more stable climatic conditions than their prey (e.g., Daufresne et al. 2009, Brose et al. 2012, Dossena et al. 2012, Ledger et al. 2013). In addition, since extreme temperatures increase organism metabolic rates (Brown et al. 2004), differential consumption rates among trophic levels could cause the food web structure to collapse (Winkler et al. 2002, Dobson et al. 2006).

Likewise, habitat size is also known to disproportionately affect organisms from different trophic levels, such as large-sized keystone predators, which require larger home ranges, being restricted to larger habitats (Dobson et al. 2006, Srivastava et al. 2008, Brose et al. 2012). Therefore, the combined effects of climate and habitat size are essential to understanding the impacts of multiple environmental factors on food web structure and dynamics at a global level.

The biomass structure of food webs integrates functional characteristics of communities, such as energy flow and turnover (Odum 1971, Brown et al. 2004). Elton (1927) was the first to propose that the biomass of each consumer trophic level should always be less than that of the trophic level immediately below (the traditional bottom-heavy pyramid). Nowadays, it is well known that biomass pyramids might be bottom-heavy, columnar, or top-heavy (i.e., inverted) (Hatton et al. 2015, Petermann et al. 2015). Such variation depends on the relative rates at which biomass and energy move between different trophic levels (Brown et al. 2004). Biomass pyramids change with habitat coupling (Tunney et al. 2012), and across

Manuscript received 26 February 2016; revised 9 May 2016; accepted 31 May 2016. Corresponding Editor: L. P. Lounibos.

⁴E-mail: gqromero@unicamp.br

environmental gradients, including warming (Kratina et al. 2012, Shurin et al. 2012), and habitat size (Petermann et al. 2015). For instance, warming produces top-heavy food webs by consistently enhancing primary production via increased turnover rates of autotrophs (Kratina et al. 2012, Shurin et al. 2012). In addition, habitat size gradients affect pyramid shape by changing standing-stock biomass ratios between trophic levels (detritivore:resource and predator:detritivore mass ratios) when larger predators are present in the regional pool (Petermann et al. 2015). These authors showed that large-bodied top predators (Odonata) are restricted to larger habitats (bromeliads with greater water-holding capacity); thus, in larger habitats keystone predator:detritivore mass ratios ($PDMR_{top\ predators}$) increase to the point where trophic pyramids become inverted (i.e., $PDMR_{top\ predators} > 1$, representing top-heavy pyramids). By contrast, detritivore:resource mass ratios (DRMR) showed a negative relationship with habitat size, most likely because of stronger top-down control in larger habitats. Although variation in biomass pyramid shape has been related to climate (warming) and habitat size, to date no study has investigated combined effects of climate and habitat size on pyramid shape.

Aquatic communities subsidized by allochthonous resources, such as phytotelmata (e.g., tank-bromeliads), are ideally suited to studies on trophic structure along environmental gradients. These systems have a clear trophic division between detritivores, meso-, and top predators (Kitching 2000). Furthermore, tank-bromeliads have easily quantifiable communities and gradients of detrital concentration, varying habitat sizes (bromeliad water-holding capacity), and are widely distributed over the Neotropical region. The tanks formed by bromeliad leaves can impound water, leaf litter, and other organic detritus, and host aquatic macroinvertebrate communities dominated by larval insects (Kitching 2000). Detritus present in the tanks constitutes the main source of nutrients for the aquatic food web (Kitching 2000), although bromeliads in full sunlight can also contain a substantial amount of algae that might account for autochthonous resource-based food webs (Brouard et al. 2011, Marino et al. 2011, Farjalla et al. 2016). The detritus is processed in a facilitative chain of interactions promoted by different functional groups of microorganisms, detritivorous, and saprophagous aquatic macroinvertebrates (Kitching 2000). In bromeliad phytotelmata, damselfly larvae (Odonata, Zygoptera) are one of the most important top predators, preying exclusively on aquatic invertebrates.

We investigated generalities and site-specific contingencies in the relationships of DRMR and PDMR to habitat size (i.e., bromeliad water retention capacity) along a latitudinal gradient (Fig. 1A). As site-specific contingencies are detected, we took advantage of natural variations in climate along a latitudinal gradient to investigate to what extent the relationships of PDMR and DRMR to habitat size at a local scale are shaped by environmental conditions at a regional scale. We predict that populations of top predators (damselflies) could be larger

(and/or their larvae heavier) in more stable and favorable climatic environments. Since these predators with long larval stages (over six months) seem to avoid small, drought-prone bromeliads (Srivastava et al. 2008), we expect a high concentration of top predators in larger bromeliads, and consequently, steeper slopes of $PDMR_{top\ predators}$ against habitat size under more stable climatic conditions (prediction 1a; Fig. 1B). We thus expect a positive relationship between a climatic gradient across latitudes (from unstable or severe to stable or favorable) and the slopes of $PDMR_{top\ predators}$ against habitat size at a regional scale (Fig. 1D). On the other hand, regardless of population size, individual female damselflies could be able to choose smaller habitats to lay eggs under more climatically stable environments (e.g., to minimize intraspecific competition in larger bromeliads), but search for more stable habitats (i.e., larger bromeliads) and/or have their offspring killed by drought in small bromeliads under harsh environments, thus producing an opposite trend, i.e., a negative relationship between a climatic gradient and the slopes of PDMR against habitat size (prediction 1b; Fig. 1C–D). However, none of these patterns is expected for mesopredators and detritivores, which are small organisms with shorter larval stages, and might therefore, live in a broader range of habitat sizes regardless of climate conditions (prediction 2a; Fig. 1E, G). Alternatively, as a consequence of climate-induced changes in top-down responses at the top predator level (predictions 1a–b), we would even expect opposite patterns on the biomass of smaller organisms (and their biomass ratios and slopes) to habitat size at local and regional scales (prediction 2b or c; Fig. 1F, G).

MATERIAL AND METHODS

Study areas and system

This study was carried out in 12 Brazilian coastal sites along a latitudinal gradient ranging from 12°34' to 27°37' S (ca. 2,040 km), with altitudes varying from 3 to 91 m asl. The average distance between neighboring sites was 199 km (max 566.3 km, min 14.7 km; Gonçalves-Souza et al. 2014, 2015). To minimize the influence of environmental heterogeneity (e.g., light incidence, resource availability etc.), we concentrated on open areas called *restingas*, which are sandy soil communities dominated by cacti, bromeliads, herbs, and small shrubs under high light incidence (Lacerda et al. 1984). Bromeliads at these sites are typically distributed in patches along the margins of shrubby vegetation. We have summarized the climate regimes from each *restingas* in Appendix S1.

Bromeliads in open *restingas* are inhabited by a diverse community comprising aquatic invertebrates (mostly insect larvae) of several functional groups, including keystone (top) predators (Zygoptera), mesopredators (Corethrellidae, Tanypodinae, Hydrophyliidae larvae, Ceratopogonidae), filter feeders (most Culicidae),

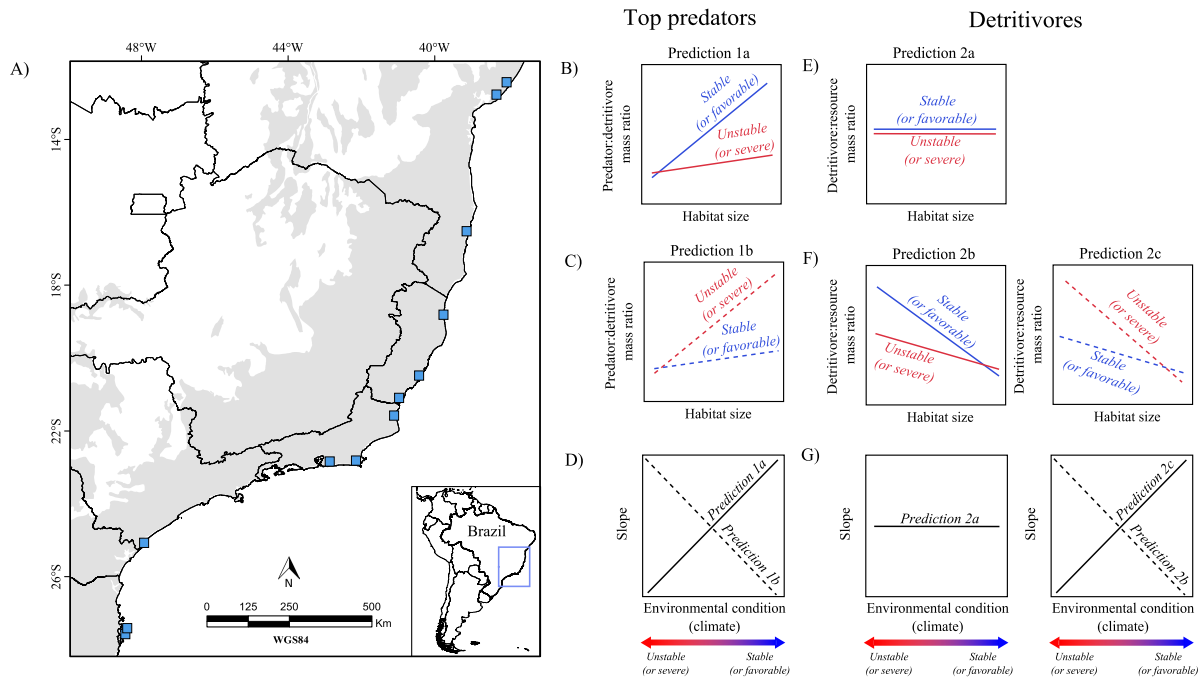


FIG. 1. (A) Map of the study sites (*restingas*). Two hundred and forty bromeliads of selected sizes were surveyed across twelve open *restingas* (20 bromeliads/site) in five Brazilian states, spread along a wide range of tropical latitudes (12.6 °S to 27.6 °S). The grey area represents the Atlantic Rainforest biome, where the open *restingas* are embedded. (B–D) We predict that the structure of trophic pyramids (i.e., top predator:detritivore mass ratios, $PDMR_{top\ predator}$) is shaped by environmental conditions; higher values of slopes between habitat size and $PDMR_{top\ predator}$ ($b > 0$) are expected under more stable and favorable climatic conditions, where predators are more abundant and/or larger (prediction 1a). (D) Thus, a positive relationship between a climatic gradient, from unstable (or severe) to stable (or favorable), and slopes is expected. Opposite relationships are expected for prediction 1b ($b < 0$, see text). (E–G) Small organisms (mesopredators, detritivores) have shorter larval stages, thus they live in a broader range of habitat sizes regardless of climate conditions; therefore, (E, G) we expect slopes of their biomass ratios $PDMR_{mesopredators-DRMR}$ to be zero ($b = 0$, prediction 2a). Alternatively, (F, G) as a consequence of top-down control (predictions 1a–b), we would expect opposite patterns on the biomass of smaller organisms (and their biomass ratios and slopes) to habitat size ($0 < b < 0$, predictions 2b or c). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

shredders (Limoniidae, Trichoptera), scrapers (Scirtidae), and collectors (Psychodidae, Chironomidae, Syrphidae, Ceratopogonidae). This fauna also includes small non-insect invertebrates, such as Oligochaeta, Ostracoda (both detritivores), Hirudinea, Turbellaria (predator), and aquatic Acari (multiple trophic levels) (Romero and Srivastava 2010).

Sampling design

We sampled 240 bromeliads in the 12 *restingas*. At each site (*restingas*) we sampled 20 bromeliads of varying sizes within five plots of 40 × 100 m each, totaling 20,000 m² of surveyed area. Within each plot, we sampled four tank-bromeliads located 5–15 m apart from each other, belonging to different size classes (according to tank capacity): the largest, the smallest, a large-to-medium sized one, and a small-to-medium sized one. The average maximum capacity of the 20 bromeliads ranged from 348.7 to 830.6 mL among sites, and size variations (coefficient of variation, CV) were not significant among sites (Table 1). All bromeliads had

their actual and maximum volume estimated according to Romero and Srivastava (2010). We used the maximum water-holding capacity of bromeliads (volume in mL) as a measure of habitat size; bromeliad capacity ranged from 32 to 2,616 mL at our study sites.

Invertebrates were surveyed at each of the 12 sites from September to November 2009. Since some of these *restingas* belong to protected reserves (Table 1; Gonçalves-Souza et al. 2015), we decided not to use destructive methods. Instead, we used a shaking and washing method, which consisted of removing every rooted bromeliad from the ground and placing it upside down on a big white tray. These bromeliads were shaken in this position and gently beaten five times against the tray, and then their internal parts (tanks, leaf axils) were washed carefully to remove all accumulated detritus and macroinvertebrates with the help of tweezers. Each bromeliad was washed three times using clean water, and detritus (fine and coarse) were rinsed in the field to survey the invertebrates that were collected and fixed in hydrated alcohol (80%). Detritus was oven dried for 24 h at 60°C and then weighed to get dry mass (g). Detrital dry mass was used as a measure of

TABLE 1. Characteristics of the study sites (twelve *restingas*).

Sites (<i>restingas</i>)	Geographical information			Species	Bromeliad traits		
	Latitude (°S)	Longitude (°W)	Altitude (m, asl)		Mean max Volume (mL)	Volume Variation (CV)	% with Zygoptera
Praia do Forte	12.569444	38.002333	28	<i>Aechmea cf. aquilega</i>	588.7	0.87	50
Salvador	12.918139	38.320722	17	<i>Hohenbergia litorallis</i>	348.7	1.16	30
Trancoso	16.655944	39.099528	20	<i>Aechmea blanchetiana</i>	734.5	0.98	15
Barra Nova	18.95725	39.738667	6	Bromeliaceae sp.1	715.5	0.7	35
Setiba	20.605417	40.416389	3	<i>Aechmea lingulata</i>	708.7	0.57	10
Praia das Neves	21.236444	40.974111	5	<i>Aechmea lingulata</i>	673.7	0.5	0
Iquipari	21.729278	41.032083	4	<i>Neoregelia cruenta</i>	732.2	0.64	0
Arraial do Cabo	22.946389	42.130722	3	<i>Neoregelia cruenta</i>	830.1	0.78	60
Maricá	22.96075	42.847889	3	<i>Neoregelia cruenta</i>	755.9	0.66	70
Ilha do Cardoso	25.067806	47.912778	6	<i>Quesnelia arvensis</i>	624.1	0.64	45
Rio Vermelho	27.4945	48.403972	27	<i>Aechmea</i> sp.	477.4	0.56	20
Lagoa da Conceição	27.619167	48.45875	91	<i>Aechmea</i> sp.	830.6	0.54	70

resource availability for bromeliad food webs, and represented the basal compartment of trophic pyramids in these ecosystems (see Petermann et al. 2015). The method used for sampling invertebrates has been previously used successfully (e.g., Céréghino et al. 2011), and can capture ca. 94% of aquatic individuals from bromeliads (Romero and Srivastava 2010).

Macroinvertebrate communities

We recorded the size and abundance of all aquatic invertebrates visible to the naked eye (>0.5 mm); they were later identified to the lowest possible taxonomic level by specialized taxonomists. The trophic position of each taxon was determined according to literature (Merritt and Cummins 1996, Kitching 2000), as well as through data from feeding trials in Cardoso Island (G. C. Piccoli, *unpublished data*). All organisms that feed on detritus (scrapers, shredders, and collectors) and filter feeders were considered as primary consumers and are henceforward designated as “detritivores” (prey). Mesopredators and top predators were defined as those organisms that feed on other macroscopic invertebrates (engulfers and piercers), and were classified according to their size (mesopredators are typically much smaller than top predators) and taxon (as noted previously). Although there is some intraguild predation in bromeliad invertebrate food webs (The authors, P. M. de Omena, G. C. O. Piccoli, and G. Q. Romero *unpublished data*), this is usually incidental to the main prey of detritivorous invertebrates (Petermann et al. 2015). Most of the top predators were damselflies of the genus *Leptagrion*; since the results including and excluding other few top predators (e.g., leeches and dytiscid beetles) were similar (results not shown), our analysis is presented only with damselflies as top predators. Invertebrate biomass was estimated from previously developed allometric equations between body length and dry mass of

bromeliad invertebrates, or from mean dry mass for very small organisms (D. S. Srivastava, G. Q. Romero, G. C. Piccoli, P. M. de Omena, *unpublished data*).

Macroclimatic variables

We obtained the macroclimatic variables from WorldClim (<http://www.worldclim.org/bioclimate>) at a resolution of 1 km² (Hijmans et al. 2005). We used 10 bioclimatic variables (out of 19 available ones) related to temperature and precipitation derived from monthly values of: (1) annual mean temperature, (2) mean diurnal range (monthly mean temperature [maximum temperature – minimum temperature]), (3) isothermality (bio2/bio7 × 100), (4) temperature seasonality (standard deviation × 100), (5) maximum temperature of the warmest month, (6) mean temperature of the driest quarter, (7) mean temperature of the warmest quarter, (8) mean temperature of the coldest quarter, (9) annual precipitation, (10) precipitation seasonality (coefficient of variation, CV) (Appendix S1). Variables 1 and 5 to 9 denote climatic extremes, and variables 2–4, and 10 denote climatic seasonality. The higher the value of variables 2–4 and 10, the more unstable is the climate. We used these variables as predictors of trophic structure at the regional scale.

Statistical analyses

We tested for contingencies of the relationships of detritivore-to-resource, and predator-to-detritivore mass ratios (including total predator biomass and biomass of keystone and mesopredators) with habitat size (bromeliad capacity, mL) among sites (*restingas*) using generalized linear models (GLMs), with site as fixed effect. Additive and interactive models were analyzed using type II and III sums of squares (SS), respectively. We used likelihood ratio tests (LRT) in the GLMs to compute

P-values. Contingencies emerge from significant interactions between habitat size and site, and warrant further analyses of regional patterns (i.e., influence of latitude and its macroclimatic components on response variables). According to the nature and distribution of the data, we used different families (Gaussian, Poisson), and their respective link functions. Overdispersion was evaluated and corrected when necessary.

Once contingencies were detected, we developed regional analyses using standing-stock biomass of resources, detritivores, mesopredators, and top predators, as well as slopes (*b*) of linear models by regressing detritivore-to-resource and predator-to-detritivore mass ratios (including total predator biomass, and biomass of keystone predators and mesopredators) against habitat size. Slopes were obtained from log-log transformations after adding 1 to the response variables ($\log[n] + 1$) to include bromeliads that lack predators or detritivores. Some sites (e.g., Setiba, Praia das Neves, and Iquipari) suffered from recent impacts of extreme droughts (2009–2011), leading to a severe collapse of bromeliad-dwelling aquatic communities (Azevedo 2013, Machado 2013, R. M. Machado, T. Gonçalves-Souza, D. S. Srivastava, and G. Q. Romero, *in preparation*). This may have decreased damselfly populations in these areas (see Table 1). Thus, to enable slope analyses considering only top predators, and to minimize unwanted influences of unbalanced datasets, outliers and sampling effects, only sites with more than 20% (i.e., four out of 20) of the bromeliads occupied by damselflies (Table 1) were considered. The predictors used were absolute latitude and macroclimatic components of latitude, obtained from WorldClim (see previous). To minimize the effects of multicollinearity among macroclimatic variables, we conducted a Principal Component Analysis (PCA) and extracted the first three orthogonal axes (cumulative proportion of ca. 90%) to use as macroclimatic predictor variables

(Gonçalves-Souza et al. 2014). Significances, effect magnitudes, and directions of the contribution from each climatic variable to the axes (scores) were evaluated using linear models. Linear models were used to test the relationships between latitude, PCA axes of macroclimatic variables, and slopes. Since these relationships showed non-normal distributions, we log-transformed the slopes for the analyses and graphic presentations.

All the analyses were performed using the language environment R version 3.2.2 (R Developmental Core Team, 2015). The established significance level was $\alpha = 0.05$. We checked variance heterogeneity, normality, and outliers through graphic inspections (e.g., qq-plots, Cook’s *d*, and influence). We ran PCA using the functions *rda* and *prcomp* from packages *vegan* and *stats*, respectively, implemented in R version 3.2.2.

RESULTS

Generalities and contingencies at a local scale

Most of the analyses performed for biomass ratios, overall biomass of detritivores and predators were contingent, i.e., their relationships to habitat size (volume) were not independent of site (size vs. site interactions; Table 2; Appendix S2). Whereas $PDMR_{top\ predators}$ generally increased with habitat size, $DRMR$ was not related to habitat size (Table 2). Although $PDMR_{mesopredators}$ was negatively related to habitat size in some *restingas* (Appendix S2), we detected a strong interaction between habitat size and site in these relationships (Table 2; Appendix S2). Shape of $DRMR$ and $PDMR_{mesopredators}$ were not affected by top predator biomass ($P > 0.05$).

Standing stocks of total resource and of all trophic levels (detritivore, mesopredator, top predator) were contingent (size vs. site interactions; Table 2; Appendix S2). Although

TABLE 2. Results of generalized linear model (GLM) analyses of the effects of habitat size (bromeliad volume), site, and interactions on biomass ratios (pyramid shape) and total biomass (standing stock) of resource (detritus), detritivores, predators (all), mesopredators and top predators.

Sources of variation	Volume (V)		Site (S)		V × S	
	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
Biomass ratios						
Detritivore:Resource	0.13	0.722	48.0	<0.001	–	–
Predator:Detritivore (all predators)	23.4	<0.001	29.0	0.002	39.1	<0.001
Predator:Detritivore (mesopredators)	0.39	0.53	74.0	<0.001	64.5	<0.001
Predator:Detritivore (top predators)	42.0	<0.001	16.1	0.013	–	–
Biomass (standing stock per bromeliad)						
Resources	16.0	<0.001	19.8	0.048	19.7	0.048
Detritivores*	0.06	0.81	41.3	<0.001	58.1	<0.001
Predators (all)	13.2	<0.001	37.0	<0.001	46.5	<0.001
Mesopredators**	0.03	0.86	47.6	<0.001	33.6	<0.001
Top predators	8.7	0.003	13.9	0.031	15.5	0.017

Note: Probabilities were calculated using likelihood ratio tests (LRT, χ^2). Statistically significant results are shown in bold.

*Other predictors in the model: resource ($\chi^2 = 0.61, P = 0.43$) and total predator dry mass ($\chi^2 = 0.01, P = 0.92$)

**Other predictors in the model: resource ($\chi^2 = 0.30, P = 0.58$) and top predator dry mass ($\chi^2 = 0.002, P = 0.96$)

slopes of top predator and resource biomass against habitat size were much higher than those of detritivores and mesopredators (Appendix S2), we did not detect an influence of top predator biomass (and top predator presence; $P > 0.05$) on the standing stock of detritivores and mesopredators (Table 2).

Influence of latitude and macroclimatic variables at a regional scale

Temperature seasonality contributed with positive values to the first PCA axis, and annual mean temperature, isothermality, maximum temperature of the warmest month, mean temperature of the driest quarter, mean temperature of the warmest quarter, and mean temperature of the coldest quarter contributed with negative values to this axis (Table 3). Therefore, the higher values of PC1 (i.e., more positive), the more climatically favorable (less severe) and more stable are the environments (*restingas*). Of the 10 climatic variables used, eight were related to temperature, and two were related to rainfall; whereas seven out of eight variables associated with temperature contributed to the first axis of the PCA, none of those related to rainfall contributed to this axis. The values of PC1 were negatively correlated with latitude (negative values representing south latitudes; $R^2_{\text{adj}} = 0.76$, $b = -4.5$, $t = 29.8$, $P < 0.001$), meaning that more southerly latitudes (southern Brazil) present more stable and favorable climatic conditions.

The slopes of DRMR and $\text{PDMR}_{\text{all predators}}$ against habitat size did not vary with latitude and with PCA scores representing macroclimatic variables ($P > 0.05$; Table 4). Slope of $\text{PDMR}_{\text{mesopredators}}$ against habitat size increased with latitude ($R^2 = 0.30$, $b = -0.08$, $t = -2.41$,

$P = 0.036$), but becomes nonsignificant after removing an outlier (Table 4). On the other hand, slopes of $\text{PDMR}_{\text{top predators}}$ against habitat size increased with latitude and with climatic stability (Fig. 2, Table 4), but were not affected by the second and third PCA axes ($P > 0.05$). This relationship becomes nonsignificant when areas with low frequency of top predators (Table 1; most of them affected by recent severe drought) are included ($n = 12$, $R^2 = 0.11$, $P = 0.16$). Slopes of standing-stock biomass of resource, detritivores, mesopredators and all predators pooled against habitat size did not vary with latitude and PCA axes ($P > 0.05$), either. On the other hand, slopes of standing-stock biomass of top predators against habitat size increased with increasing climate stability (Fig. 3, Table 4).

DISCUSSION

Our results show that top predator-to-detritivore mass ratio (PDMR) increases with habitat size, thus representing a typical inverted trophic pyramid in larger habitats. However, we found site-specific contingencies on the slopes of the relationship between top PDMR and habitat size, which varied with latitude and macroclimatic variables. This means that although trophic pyramids are inverted in large ecosystems, the dependency of this inversion on habitat size can be driven by climate components, and they might be even more top-heavy (i.e., steeper PDMR slopes) under more favorable climatic conditions. Since predator:prey mass ratio is a proxy of interaction strength, steeper slopes may represent stronger top-down control under more favorable and stable climates. On the other hand, detritivore-resource and mesopredator-detritivore mass ratios were not affected by habitat size or climate variations across latitudes. Our findings add to a growing consensus that the composition of predators (mesopredators vs. top predators), climate, and habitat size can shape food web structure and potentially affect its dynamics.

It is known that inverted trophic pyramids in bromeliad aquatic ecosystems seem to occur because keystone predators, e.g., damselflies, require larger habitats to survive (Srivastava et al. 2008, Petermann et al. 2015). These predators are much heavier than their prey (standing-stock and individual mass), and thus they change the pyramid to a top-heavy shape in larger ecosystems. A relevant question, but still unanswered for bromeliad aquatic ecosystems, is how these inverted trophic pyramids can be supported. Several mechanisms have been hypothesized to account for observed variations in the shape of pyramids (Brown et al. 2004, Trebilco et al. 2013). A favored mechanism invokes the productivity-biomass ratio (turnover rate), which predicts that biomass turnover rate is constant among the trophic levels in bottom-heavy pyramids. It also predicts that top-heavy pyramids (inverted pyramids) result from the decrease in turnover rates as trophic rank increases (Brown et al. 2004, Hatton et al. 2015). We recognize that

TABLE 3. Bioclimatic variables extracted from WorldClim (as in Hijmans et al. 2005) and their loadings in a principal component analysis (PCA) of the sampled *restingas*.

	PC1 (60.5%)	PC2 (20%)	PC3 (9.1%)
Bioclimatic variables			
bio1 *	-0.404	-0.02	0.056
bio2	-0.069	-0.582	-0.185
bio3	-0.383	0.139	0.014
bio4	0.361	-0.295	0.091
bio5 *	-0.315	-0.399	0.077
bio9 *	-0.378	0.184	0.222
bio10 *	-0.366	-0.227	0.084
bio11 *	-0.403	0.078	-0.006
bio12 *	0.089	-0.379	0.769
bio15	-0.092	-0.4	-0.548

Note: Percentages in parentheses indicate the variance explained by each PCA axis. Loadings in bold represent the highest and significant values (linear regression) of the first three principal components. Asterisks (*) in front of the bioclimatic variables indicate climate severity, and the remaining variables denote climatic variability ([in]stability). See text (*Materials and Methods*) or Appendix S1: Table S1 for definitions of variables.

TABLE 4. Results of linear regressions of latitude and the first axis of a principal component analyses (PCA) of bioclimatic components from WorldClim (see Table 3 and Appendix S1) against the slopes of detritivore:resource and predator:detritivore mass ratios, and average resource (detritus), detritivore and predator biomass per bromeliad in the pool, against habitat size (bromeliad capacity, mL). Statistically significant results are shown in bold.

	Latitude			PC1		
	r^2	t	P	r^2	t	P
Slopes (mass ratio vs. habitat size)						
Detritivore:Resource	0.03	1.16	0.27	-0.09	-0.34	0.74
Predator:Detritivore (mesopredators only)	-0.00	-0.97	0.35	0.08	1.42	1.19
Predator:Detritivore (all predators)	0.15	-1.72	0.12	0.15	1.69	0.12
Predator:Detritivore (top predators only)	0.64	-3.43	0.019	0.77	4.6	0.006
Slopes (standing stock biomass vs. habitat size)						
Detritus	-0.00	-0.39	0.70	-0.02	-0.32	0.75
Detritivore	-0.09	0.12	0.91	-0.12	-0.6	0.58
Mesopredators only	0.44	1.97	0.08	0.47	-0.95	0.36
All predators	0.09	-1.47	0.17	0.25	2.16	0.056
Top predators only	0.26	-1.76	0.14	0.62	3.32	0.021

top-heavy pyramids may occur in larger bromeliads as keystone predators in these ecosystems have long life-cycles, whereas their prey (e.g., culicids, chironomids) have a very short life-cycle and colonize bromeliads at high rates.

Our results revealed that slopes of top predator-to-detritivore mass ratio against habitat size were steeper at higher latitudes and under more stable and favorable climatic conditions (southern Brazil), meaning that the inverted shape of trophic pyramids is more pronounced in environments with more favorable climate, which thus supports our prediction 1a (Fig. 1). This prediction is based on the assumption, tested and confirmed by our results, that top predators are more abundant, heavier, and have higher standing-stock biomass in more stable, climatically favorable environments. This information pertains to the aquatic larvae in bromeliad tanks, but also likely applies to their terrestrial adults being favored by climatic conditions. Since damselfly adults seem to oviposit preferentially in larger bromeliads and avoid small, drought-prone ones (Srivastava et al. 2008), we found a higher concentration of top predators in larger bromeliads under more stable climatic conditions at a local scale, which explains the steeper slopes. But how do bromeliads from these favorable environments support larger individuals and populations of top predators? It is likely that biomass turnover rates of prey are higher under more stable and favorable climatic conditions, and could also contribute to support more predators in these ecosystems. In fact, experimental mesocosms showed that climatic components (temperature) can change biomass turnover rates of lower trophic levels and thus produce top-heavy pyramids (Shurin et al. 2012). Moreover, climatic instability and extremes can decrease insect population sizes and delay insect development and survivorship (Jonsson et al. 2014), thus decreasing biomass turnovers in these harsh ecosystems.

The standing-stock biomass of allochthonous basal resource (detritus) increased with habitat size, meaning that larger habitats (bromeliads) are more productive. Although we did not measure autochthonous resources in our samples, algae have been shown to be more abundant in larger bromeliads (Brouard et al. 2011, Marino et al. 2011), and thus could also contribute to improving productivity of larger ecosystems. Moreover, the biomass of basal resources (detritus) has increased more sharply than those of detritivores with habitat size; nevertheless, we found no relationship between detritivore:resource mass ratio (DRMR) and a gradient of bromeliad size (Table 2). Similarly, the standing-stock biomass of mesopredators and its PDMRs did not vary with habitat size, either. One could interpret these results as a signature of top-down control. However, we found no influence of top predators (biomass and presence) on the standing stocks of mesopredators and detritivores. These results suggest that mesopredators and detritivores seem to be less sensitive to habitat size and climatic variations compared to top predators, being able to inhabit different bromeliad sizes, thus producing lower (or flat) slopes of biomass ratios to habitat size irrespective of climatic conditions. These results support our prediction 2a for smaller organisms (Fig. 1), and indicate that, at lower trophic levels (resource, detritivores, and mesopredators), pyramid shape is bottom-heavy, and apparently is not strongly driven by habitat size and climate, as observed for top predators. However, our results for the detritivores differ slightly from those reported by Petermann et al. (2015), who found a decrease in DRMR with increasing habitat size, meaning that pyramids became more bottom-heavy along the habitat size gradient. These differences can be explained by the fact that the systems studied by Petermann et al. (2015) are more productive, i.e., they are located mostly in forested environments and thus accumulate an enormous amount of

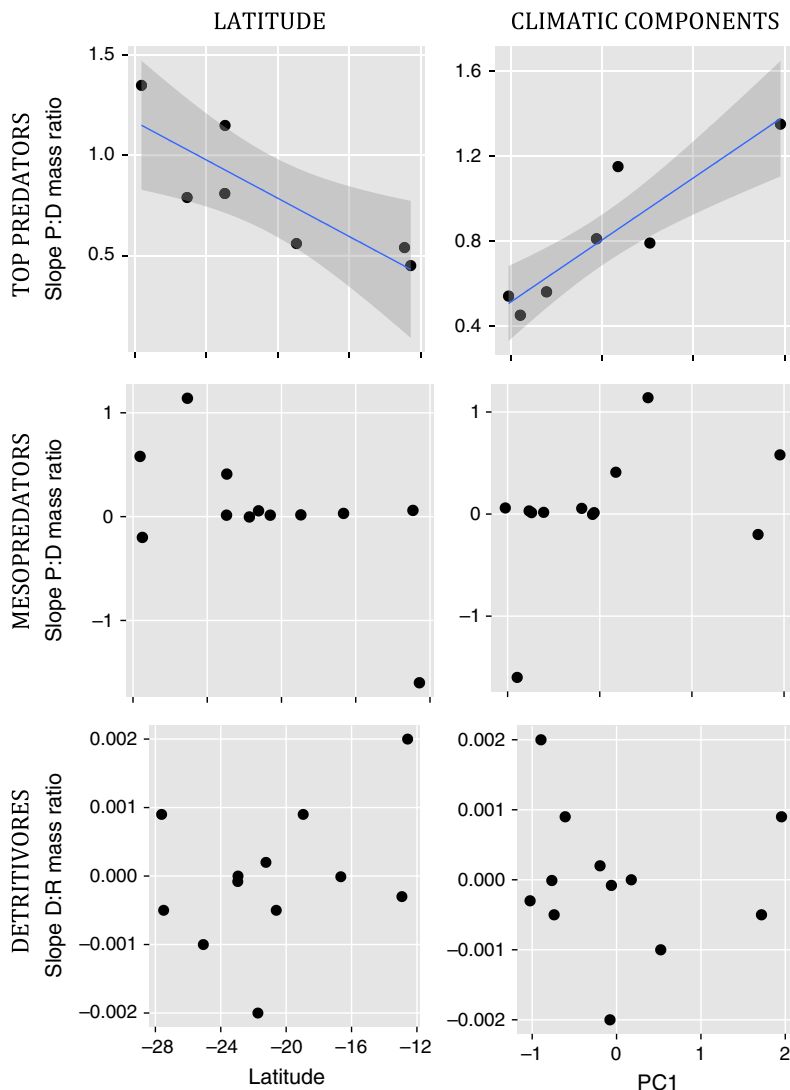


FIG. 2. Relationship between absolute latitude ($^{\circ}$ S) and the first axis of a principal component analysis (PCA) for bioclimatic components at each site (see Tables 3, 4 and *Materials and methods*) and the slopes of predator:detritivore and detritivore:resource mass ratios against habitat size. Linear regression lines in blue, with shaded 95% confidence regions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

leaf litter (detritus, basal resource) in larger habitats. On the other hand, the systems studied here occur at open sites (*restingas*) where accumulations of allochthonous resources are much smaller. Therefore, the shape and width of pyramid bottoms might be driven by variations in type of productivity (autochthonous vs. allochthonous), which may vary with the type of environment (open vs. forested).

There is a growing consensus, from independent studies, that reduced habitat size and climate changes can affect larger organisms, especially keystone predators. Since keystone predators require larger home ranges, they are restricted to larger habitats. Furthermore, since large-bodied organisms have greater difficulty dissipating heat (Daufresne et al. 2009), which is more intense for aquatic organisms because of decreased dissolved oxygen

(Forster et al. 2012), they are more vulnerable to climatic variations and extremes. However, the relative influence of macroclimatic variables and habitat size has been poorly explored for aquatic food web structure at broad biogeographic scales (Baiser et al. 2012). We here took advantage of two natural gradients, habitat size and macroclimatic variables along a latitudinal gradient, and demonstrated that habitat size was most influential in producing typical top-heavy pyramids, whereas climate conditions influenced the degree to which these pyramids are inverted.

Although our latitudinal gradient had a wide range of mean annual temperature (5.3°C), similar to predicted levels for global warming in the next decades (IPCC 2007) and close to those manipulated in experimental warming (e.g., Kratina et al. 2012, Shurin et al. 2012,

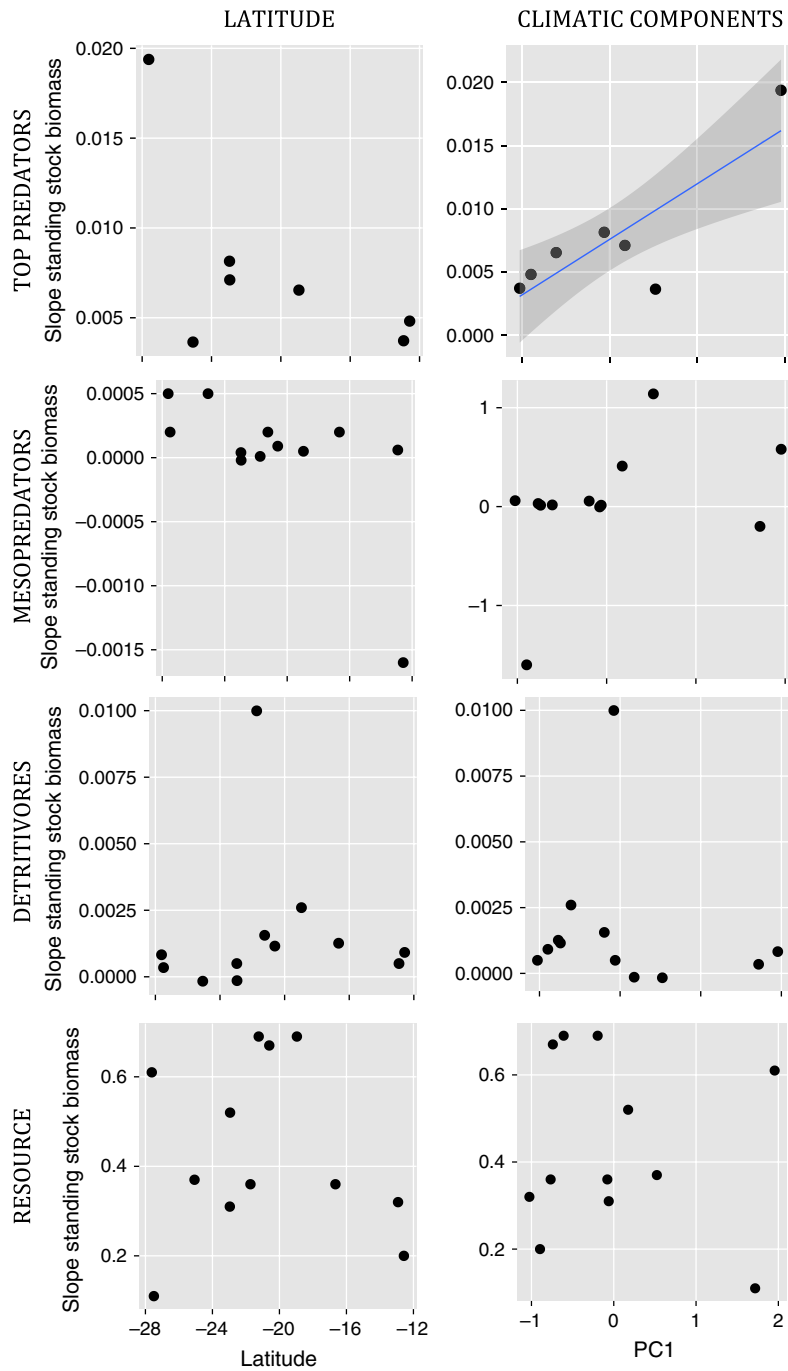


FIG. 3. Relationship between absolute latitude ($^{\circ}$ S) and the first axis of a principal component analysis (PCA) for bioclimatic components at each site (see Tables 3, 4 and *Materials and methods*) and the slopes of standing stock biomass of predators, detritivores and resource against habitat size. Linear regression lines in blue, with shaded 95% confidence regions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Jonsson et al. 2014, Lefort et al. 2015), our results slightly differ from those manipulating temperature. For instance, we did not find communities of larger organisms (key-stone predators) being replaced by smaller species (mesopredators, detritivores) in hotter ecosystems, as reported earlier (e.g., Brose et al. 2012). Maybe top predators are

locally adapted to climate conditions. Advantages in using latitudinal gradients as natural experiments to investigate climate changes are threefold. First, latitudinal gradients allow us to understand how climate change might affect organisms that are naturally adapted to a set of natural variations in climate. Second,

latitudinal gradients provide a methodological set-up to overcome the drawbacks of other observational and experimental warming methods (De Frenne et al. 2013). Third, by studying natural climatic variations, it is feasible to measure responses of larger organisms facing climate changes, with long generations, instead of limiting our knowledge to adaptive responses of microorganisms, with short generations. Despite that, latitudinal gradients have been used much less than other types (e.g., altitude, De Frenne et al. [2013]) for methodological reasons as natural laboratories to assess responses of terrestrial organisms to climate warming.

Some studies have shown that climatic variations (e.g., warming, drought) and extremes caused by global changes can affect food web structure and dynamics (Kratina et al. 2012, Shurin et al. 2012, Ledger et al. 2013, Hong and Shurin 2015). In addition, other studies have pointed out that warming can produce communities dominated by smaller organisms (Brose et al. 2012, Dossena et al. 2012, Lurgi et al. 2012, Lefort et al. 2015), with keystone predators being replaced by mesopredators (e.g., Prugh et al. 2009, Brose et al. 2012). Although we have not detected trophic ranking replacements, we showed that mesopredators and detritivores seem to be less sensitive to habitat size and climatic variations. In contrast, standing stocks of top predators (not of mesopredators) can configure top-heavy pyramids in these ecosystems, and these inverted pyramids are steeper (i.e., larger PDMR slopes) under milder, more stable and favorable climatic conditions. Since it has been suggested that predator:prey mass ratios are proxies of interaction strength (Berlow et al. 2008), we should expect these ecosystems to be less top-down controlled under more unstable and harsh climatic conditions, predicted for a near future.

In conclusion, whereas habitat size shapes the top-heavy pyramids, as reported elsewhere (Petermann et al. 2015), climate components caused variations in the shape of inverted pyramids, i.e., inverted pyramids were steeper (i.e., larger PDMR slopes) under more stable and favorable climatic conditions. Components of temperature and their variations seemed to drive food web structure here, although the effects of droughts, forecasted for the next decades, might also have strong impacts on food web structure and on the functioning of freshwater ecosystems (Ledger et al. 2013). In contrast, smaller organisms (detritivores and mesopredators), as well as their biomass ratios and slopes (i.e., pyramid shape), seem to be less vulnerable to habitat size and climate conditions compared to top predators (damselflies). Our study adds new knowledge and insights on the importance of considering natural gradients of habitat size and climate (latitude) as natural experiments to study the combined impacts of habitat size and climate on food web structure, and to search for generalities and contingencies in species interactions.

ACKNOWLEDGMENTS

The authors thank three anonymous reviewers for the valuable comments on the first edition of this report. A. L. Mendonça

helped with invertebrate surveys, and identifications were conducted by L. C. de Pinho (Chironomidae), A. L. Mendonça (Culicidae), M. M. Ronderos (Ceratopogonidae), and A. Gessner (Scirtidae). The maps in Fig. 1 were drawn by M. F. Araújo. This study was supported by a São Paulo Research Foundation (FAPESP) grant, coordinated by G. Q. Romero. G. Q. Romero was also supported by a CNPq-Brazil research grant. This paper is a contribution of the Brazilian Network on Global Climate Change Research funded by CNPq (grant #550022/2014-7) and FINEP (grant #01.13.0353.00).

LITERATURE CITED

- Azevedo, F. D. 2013. Effect of the number of islands in an archipelago on the structure of macroinvertebrate community in tank bromeliads. Dissertation. Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.
- Baiser, B., N. J. Gotelli, H. L. Buckley, T. E. Miller, and A. M. Ellison. 2012. Geographic variation in network structure of a nearctic aquatic food web. *Global Ecology and Biogeography* 21:579–591.
- Berlow, E. L., U. Brose, and N. D. Martinez. 2008. The 'Goldilocks factor' in food webs. *Proceedings of the National Academy of Sciences of the United States of America* 105:4079–4080.
- Brose, U., J. A. Dunne, J. M. Montoya, O. L. Petchey, F. D. Schneider, and U. Jacob. 2012. Climate change in size-structured ecosystems. *Philosophical Transactions of the Royal Society B* 367:2903–2912.
- Brouard, O., A.-H. Le Jeune, C. Leroy, R. Céréghino, O. Roux, L. Pelozuelo, A. Dejean, B. Corbara, and J.-F. Carrias. 2011. Are algae relevant to the detritus-based food web in tank-bromeliads? *PLoS One* 6:e20129.
- Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Towards a metabolic theory of ecology. *Ecology* 85:1771–1789.
- Céréghino, R., C. Leroy, J. F. Carrias, L. Pelozuelo, C. Ségura, C. Bosc, A. Dejean, and B. Corbara. 2011. Ant-plant mutualisms promote functional diversity in phytotelm communities. *Functional Ecology* 25:954–963.
- Daufresne, M., K. Lengfellner, and U. Sommer. 2009. Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* 106:12788–12793.
- De Frenne, P., et al. 2013. Latitudinal gradients as natural laboratories to infer species' responses to temperature. *Journal of Ecology* 101:784–795.
- Dobson, A., et al. 2006. Habitat loss, trophic collapse, and the decline of ecosystem services. *Ecology* 87:1915–1924.
- Dossena, M., G. Yvon-Durocher, J. Grey, J. M. Montoya, D. M. Perkins, M. Trimmer, and G. Woodward. 2012. Warming alters community size structure and ecosystem functioning. *Proceedings of the Royal Society B* 279:3011–3019.
- Elton, C. 1927. *Animal ecology*. Macmillan, New York, New York, USA.
- Farjalla, V. F., A. L. González, R. Céréghino, O. Dézerald, N. A. C. Marino, G. C. O. Piccoli, B. A. Richardson, M. J. Richardson, G. Q. Romero, and D. S. Srivastava. 2016. Terrestrial support of aquatic food webs depends on light inputs: a geographically-replicated test using tank bromeliads. *Ecology*. <http://dx.doi.org/10.1002/ecy.1432>
- Forster, J., A. G. Hirst, and D. Atkinson. 2012. Warming-induced reductions in body size are greater in aquatic than terrestrial species. *Proceedings of the National Academy of Sciences of the United States of America* 109:19310–19314.

- Gonçalves-Souza, T., G. Q. Romero, and K. Cottenie. 2014. Metacommunity versus biogeography: a case study of two groups of neotropical vegetation-dwelling arthropods. *PLoS One* 9:e115137.
- Gonçalves-Souza, T., A. J. Santos, G. Q. Romero, and T. M. Lewinsohn. 2015. Conservation along a hotspot rim: spiders in Brazilian coastal *restingas*. *Biodiversity and Conservation* 24:1131–1146.
- Hatton, I. A., K. S. McCann, J. M. Fryxell, T. Jonathan Davies, M. Smerlak, A. R. E. Sinclair, and M. Loreau. 2015. The predator-prey power law: biomass scaling across terrestrial and aquatic biomes. *Science* 349:aac6284.
- Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25:1965–1978.
- Hong, B. C., and J. B. Shurin. 2015. Latitudinal variation in the response of tidepool copepods to mean and daily range in temperature. *Ecology* 96:2348–2359.
- IPCC. 2007. *Climate change 2007: the physical science basis*. Pages 1–18 in S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Contributions of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK and New York, New York, USA.
- Jonsson, M., P. Hedström, K. Stenroth, E. R. Hotchkiss, F. R. Vasconcelos, J. Karlsson, and P. Byström. 2014. Climate change modifies the size structure of assemblages of emerging aquatic insects. *Freshwater Biology* 60:78–88.
- Kitching, R. L. 2000. *Food webs and container habitats: the natural history and ecology of phytotelmata*. Cambridge University Press, Cambridge, UK.
- Kratina, P., H. S. Greig, P. L. Thompson, T. S. A. Carvalho-Pereira, and J. B. Shurin. 2012. Warming modifies trophic cascades and eutrophication in experimental freshwater communities. *Ecology* 93:1421–1430.
- Lacerda, L. D., D. S. D. Araújo, R. Cerqueira, and B. Turq. 1984. *Restingas: origem, estrutura e processos*. Universidade Federal Fluminense, CEUFF, Niterói, Brazil.
- Ledger, M. E., L. E. Brown, F. K. Edwards, A. M. Milner, and G. Woodward. 2013. Drought alters the structure and functioning of complex food webs. *Nature Climate Change* 3:223–227.
- Lefort, S., O. Aumont, L. Bopp, T. Arsouze, M. Gehlen, and O. Mauray. 2015. Spatial and body-size dependent response of marine pelagic communities to projected global climate change. *Global Change Biology* 21:154–164.
- Lurgi, M., B. C. López, and J. M. Montoya. 2012. Novel communities from climate change. *Philosophical Transactions of the Royal Society B* 367:2913–2922.
- Machado, R. M. 2013. *Abiotic stress reduces the strength of the top-down effects of a terrestrial predator on bromeliad invertebrate communities*. Dissertation. State University of São Paulo, São José do Rio Preto, Brazil.
- Marino, N. A. C., R. D. Guariento, V. Dib, F. D. Azevedo, and V. F. Farjalla. 2011. Habitat size determine algae biomass in tank-bromeliads. *Hydrobiologia* 678:191–199.
- Merritt, R. W., and K. W. Cummins. 1996. *An introduction to the aquatic insects of North America*. Kendall/Hunt, Dubuque, Iowa, USA.
- Odum, E. P. 1971. *Fundamentals of ecology*. W. B. Saunders, Philadelphia, Pennsylvania, USA.
- Petermann, J. S., et al. 2015. Dominant predators mediate the impact of habitat size on trophic structure in bromeliad invertebrate communities. *Ecology* 96:428–439.
- Prugh, L. R., C. J. Stoner, C. W. Epps, W. T. Bean, W. J. Ripple, A. S. Laliberte, and J. S. Brashares. 2009. The rise of the mesopredator. *BioScience* 9:779–790.
- R Core Team. 2015. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.Rproject.org/>
- Romero, G. Q., and D. S. Srivastava. 2010. Food-web composition affects cross-ecosystem interactions and subsidies. *Journal of Animal Ecology* 79:1122–1131.
- Shurin, J. B., J. L. Clasen, H. S. Greig, P. Kratina, and P. L. Thompson. 2012. Warming shifts top-down and bottom-up control of pond food web structure and function. *Philosophical Transactions of the Royal Society B* 367:3008–3017.
- Srivastava, D. S., M. K. Trzcinski, B. A. Richardson, and B. Gilbert. 2008. Why are predators more sensitive to habitat size than their prey? Insights from bromeliad insect food webs. *American Naturalist* 172:761–771.
- Trebilco, R., J. K. Baum, A. K. Salomon, and N. K. Dulvy. 2013. Ecosystem ecology: size-based constraints on the pyramids of life. *Trends in Ecology and Evolution* 28:423–431.
- Tunney, T. D., K. S. McCann, N. P. Lester, and B. J. Shuter. 2012. Food web expansion and contraction in response to changing environmental conditions. *Nature Communications* 3:1105.
- Voigt, W., et al. 2003. Trophic levels are differentially sensitive to climate. *Ecology* 84:2444–2453.
- Winkler, D. W., P. O. Dunn, and C. E. McCulloch. 2002. Predicting the effects of climate change on avian life-history traits. *Proceedings of the National Academy of Sciences of the United States of America* 99:13595–13599.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.1496/supinfo>