

# Predator kairomones change food web structure and function, regardless of cues from consumed prey

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Predation risk in aquatic systems is often assessed by prey through chemical cues, either those released by prey or by the predator itself. Many studies on predation risk focus on simple pairwise interactions, with only a few studies examining community-level and ecosystem responses to predation risk in species-rich food webs. Further, of these few community-level studies, most assume that prey primarily assess predation risk through chemical cues from consumed prey, even heterospecific prey, rather than just those released by the predator. Here, we compared the effects of different predation cues (predator presence with or without consumed prey) on the structure and functioning of a speciose aquatic food web housed in tropical bromeliads. We found that the mere presence of the top predator (a damselfly) had a strong cascading effect on the food web, propagating down to nutrient cycling. This predation risk cue had no effect on the identity of colonizing species, but strongly reduced the abundance and biomass of the macroinvertebrate colonists. As a result, bacterial biomass and nitrogen cycling doubled, with a concomitant decrease in bacterial production, but CO<sub>2</sub> flux was unaffected. These community and ecosystem effects of predator presence cues were not amplified by the addition of chemical cues from consumed prey. Our results show that some of the consequences of predation risk observed in controlled experiments with simplified food webs may be observed in a natural, species-rich food web.

Predators can play a large role in community dynamics and ecosystem functioning (Vonesh et al. 2009, Atwood et al. 2013). These top-down effects of predators have often been attributed to consumption of prey, but a substantial body of evidence suggests that such effects could also be mediated by non-consumptive effects (NCEs) of predators on prey (Schmitz et al. 2004). Such NCEs usually involve changes in prey traits and behaviour (e.g. altered prey foraging activity: Schmitz et al. 1997, Paterson et al. 2013, Hammill et al. 2015b, and habitat use: Rieger et al. 2004, Wesner et al. 2012), which may result, for instance, in changes in prey development (Relyea 2007, McCauley et al. 2011) and body nutrient composition (Hawlena and Schmitz 2010). These NCEs can be at least as important as consumptive effects (CEs) in mediating the impact of predators on lower trophic levels (Preisser et al. 2005), and may have important effects on community assembly (Vonesh et al. 2009, Wesner et al. 2012) and ecosystem function (Hawlena et al. 2012, Hammill et al. 2015b).

The occurrence of NCEs requires prey to be able to perceive and respond to predation risk. Predation risk is often transmitted to prey through predation cues released into the environment (Ferrari et al. 2010). There are usually two sources of predation cues: those originating from predators (chemical cues called kairomones) and prey alarm cues (Ferrari et al. 2010). Prey alarm cues can also

be divided into cues from injured prey and cues released as a by-product of consumption of prey by predators. Cues from injured prey may induce only weak prey responses when compared to chemical cues from consumed prey (Schoeppner and Relyea 2005, 2009). Similarly, prey consistently respond to conspecific alarm cues (Schoeppner and Relyea 2005, Schoeppner et al. 2009), but there is mixed evidence for the importance of heterospecific alarm cues. For instance, prey may be more likely to respond to alarm cues from phylogenetically-related taxa than distantly-related taxa (Schoeppner et al. 2009), but may also respond to alarm cues from distantly-related, coexisting heterospecifics (Mathis and Smith 1993, Fraker 2009) or show no responses to heterospecific alarm cues (Mathis and Smith 1993, Nilsson and Bengtsson 2004). Evidence for the importance of predator kairomones is also controversial: compared to prey alarm cues, kairomones from starved predators may induce no response from prey (Nilsson and Bengtsson 2004, Schoeppner et al. 2009, Schoeppner and Relyea 2009, Davenport et al. 2014), may be as effective as prey alarm cues (Paterson et al. 2013, Davenport et al. 2014) or may induce stronger responses than prey alarm cues (Bell et al. 2006). These studies illustrate the potential for a diversity of prey responses that may be found within communities, but are not extensive enough yet to allow for generalizations.

Predation risk may affect colonization dynamics and community assembly in aquatic habitats (Resatarits and Binckley 2009, Vonesh et al. 2009, Wesner et al. 2012, Hammill et al. 2015b). A common procedure to test the effect of predation risk is to use caged predators, which allows the presence of the predator to be manipulated while restricting its consumption of prey. Predators are usually fed inside the cages with some prey individuals, from one or a few prey species found in the community, potentially producing prey alarm cues (Vonesh et al. 2009, Wesner et al. 2012, Hammill et al. 2015b). However, feeding predators with a few prey species has the implicit assumption that all coexisting prey are able to detect and respond to heterospecific alarm cues. Moreover, the assumption that the detection of predation risk relies on the production of prey alarm cues ignores the importance of predator kairomones, which are produced even when predators are starved (Bell et al. 2006, Ferrari et al. 2010). Furthermore, once prey are consumed by predators, nutrients from prey carcasses may act as a subsidy for the food web (Costa and Vonesh 2013), which creates additional complications for understanding the effect of predation risk on food web structure and ecosystem function. Therefore, to better understand the consequences of predation risk for multispecies prey communities in natural systems, the role of predator kairomones and prey alarm cues needs to be clarified.

Here, we report on a study where we examine how the addition of prey alarm cues to predator cues can affect the structure and function of a natural and species-rich food web. The miniature aquatic ecosystem found within tank bromeliads hosts a very diverse detritus-based food web (Srivastava and Bell 2009). This food web is composed of aquatic insect larvae, other small invertebrates (e.g. microcrustaceans and annelids) and microorganisms (e.g. bacteria). Sit-and-wait, predatory damselfly larvae usually occupy the top of the food web throughout their range (Petermann et al. 2015). These damselfly larvae prey upon an intermediate consumer level, mostly macroinvertebrates (insect larvae, annelids and ostracods) in different detritivore functional feeding groups (e.g. filter feeders, collectors, piercers). The basal resources of the food web include detritus and the microorganisms that condition it (Srivastava and Bell 2009). Bromeliad size has a strong positive effect on macroinvertebrate richness, abundance and biomass (Marino et al. 2013, Petermann et al. 2015). In contrast, the presence of the damselfly has a strong negative effect on prey abundance and biomass (Petermann et al. 2015), mediated through direct consumption (Srivastava and Bell 2009, Starzomski et al. 2010) as well as predation risk (Hammill et al. 2015b). The effect of the damselfly larvae on prey induces a trophic cascade that can reduce detrital breakdown (Srivastava 2006), increase nutrient cycling (Ngai and Srivastava 2006), and either decrease or increase CO<sub>2</sub> flux to the atmosphere (Atwood et al. 2013, Hammill et al. 2015b). Recent research has shown that caged damselflies fed mosquito prey reduces oviposition by a variety of invertebrate species (Hammill et al. 2015b). However, there are still some questions that need to be addressed. For instance, it is not clear if the effects of predation risk on bromeliad food web structure are dependent on prey alarm cues or if the mere presence of the damselfly can produce these same patterns, given that a mixture of both cues were

used in Hammill et al. (2015a). Similarly, while consumption of prey by the damselfly may affect nutrient cycling (Ngai and Srivastava 2006), it is not clear whether predation risk may also affect nutrient cycling. Finally, it is still not clear how the presence of the damselfly affects the CO<sub>2</sub> flux, given opposing results from previous studies (Atwood et al. 2013, Hammill et al. 2015b). The importance of predation risk on ecosystem function has been demonstrated in controlled experiments with simplified food webs in other systems (Stief et al. 2006, Hawlena and Schmitz 2010, Hawlena et al. 2012, Sitvarin and Rypstra 2014), but the possibility of such effect to occur in a natural, species-rich food web has only recently been demonstrated (Hammill et al. 2015b) and deserves further attention.

We performed a field experiment to test if adding prey alarm cues to predator kairomones are necessary to affect food web structure (macroinvertebrate community abundance, biomass, richness and composition, bacterial biomass) and ecosystem function (bacterial production, nutrient cycling and ecosystem respiration) or whether predator kairomones alone could produce the same effect. We experimentally manipulated predation risk through the presence or absence of caged damselfly larvae. Information on predation risk was provided to lower trophic levels both through the presence of predators (predator kairomones alone) and in combination with chemical cues from consumed prey (predator kairomones and consumption cues). This experimental manipulation allowed us to test for the non-consumptive presence of the top predator while controlling for the amount of information on predation risk available to prey. We asked: 1) whether the mere presence of predators, in the absence of consumed prey, can affect the bromeliad food web structure and function; and, 2) if an additional source of predation cue (alarm cues from consumed prey) increases the responsiveness to predation risk. We did not consider the effects of cues from consumed prey in the absence of predator cues, as this combination would not occur in nature. We predict that the ability of prey to detect and respond to predation risk increases as additional types of predation cues become available, generating larger responses across all trophic levels and ecosystem processes.

## Methods

### Study area and the local bromeliad food web

This study was conducted in a closed *restinga* forest at Parque Nacional da Restinga de Jurubatiba (22°12'S, 41°29'W), northeast of Rio de Janeiro state, Brazil. *Restinga* forest, found on the sandy plains of the Atlantic rainforest bordering the ocean, is composed of small patches of shrub vegetation and often has abundant tank bromeliads. The most abundant tank bromeliad in our site is *Neoregelia cruenta*, a terrestrial tank bromeliad that can accumulate up to 1 l of rainwater (Marino et al. 2013). *Neoregelia cruenta* hosts a very diverse aquatic macroinvertebrate community, composed of the sit-and-wait top predator *Leptagrion andromache* (Odonata: Coenagrionidae), and several species of intermediate predators (Tabanidae, Tanypodinae, Syrphidae, Ceratopogonidae and Corethrellidae) and prey species (Marino et al. 2013).

Prey species belong to different functional feeding guilds: filter feeders (several species of mosquito larvae from the genus *Microculex*), detrital shredders (Tipulidae), detrital scrapers (Scirtidae), collectors (including Psychodidae and several species of Chironomidae) and other small detritivorous invertebrates (such as the oligochaete *Dero superterrenus* and the ostracod *Elpidium bromeliarum*).

## Experimental design

To determine the food web and ecosystem consequences of predation risk, and to understand the roles of predator kairomones and prey alarm cues, we manipulated damselfly presence and alarm cues from consumed prey in a field experiment. Replicates without the damselflies were used to establish community and ecosystem responses in the absence of either type of cue or consumptive effects of damselflies. When predators were present, we always placed them in cages to restrict their effects to purely non-consumptive, and we either fed the damselfly or let it to starve throughout the experiment. This resulted in three treatments with five replicates each ( $n = 15$ ): damselfly absent (control), damselfly caged and fed (damselfly presence and consumption cues) and damselfly caged and starved (damselfly presence only). Therefore, note that we do not included treatments with uncaged damselflies, as the purpose of our experiment was to establish the presence and mechanisms of the non-consumptive effect, not its contribution to the net effect of predators (Hammill et al. 2015b).

To set up the experiment, we collected 15 bromeliads of *N. cruenta* from our study site. We washed all bromeliads and retained the contents within a large bucket. The bromeliads were hung above the ground, upside down, for seven days to eliminate any aquatic macroinvertebrate and any residual predation cues prior to use in the experiment. Therefore, any difference in community structure or ecosystem function found among treatments would likely be caused by our manipulation, and not by previous conditions. We collected all *L. andromache* larvae used for this experiment from the bromeliad contents retained in the bucket. We stored the damselflies individually in plastic containers, where they were left unfed for seven days until the beginning of the experiment. We washed the detritus contained in the bucket through 1 mm (coarse detritus, mostly dead leaves) and 53  $\mu\text{m}$  (fine particulate detritus) soil sieves and dried both size fractions (60°C for 12 h) to kill all eggs before using it in the experiment. After seven days, we took the bromeliads to the field and planted them in pots in the sandy soil of the *restinga* (mean distance  $\pm$  SD between bromeliads of  $6.0 \pm 3.4$  m; minimum distance = 1 m; maximum distance = 13.8 m). We placed  $12.66 \pm 2.96$  g (mean  $\pm$  SD) of coarse detritus ( $> 1$  mm) and  $0.73 \pm 0.15$  g of fine particulate detritus ( $< 1$  mm and  $> 53 \mu\text{m}$ ) in each bromeliad, filled them to maximum capacity with commercial mineral water and then randomly assigned them to one of the three treatments.

We manipulated top-predator presence by caging *L. andromache* in 50 ml plastic centrifuge tubes. In each plastic centrifuge tube, we made a hole near the bottom and in one side of the plastic tube (9 cm<sup>2</sup>) and covered it with 350  $\mu\text{m}$  mesh, allowing the exchange of water and chemical compounds produced in the tube with the bromeliad, while

preventing direct contact of the damselfly with the macroinvertebrates inside the bromeliad. We also covered the top opening of the plastic centrifuge tubes with 500  $\mu\text{m}$  mesh to prevent oviposition of terrestrial adults inside the tubes and the escape of the caged predator. We used mesh rather than a solid lid to allow for gas exchange between the tube interior and the outer atmosphere. While sorting *L. andromache* from the washed bromeliads, we observed that every bromeliad contained at least two *L. andromache* larvae. We assumed that two larvae was the mean abundance of *L. andromache* in the study site and, accordingly, incubated two plastic centrifuge tubes on opposite leaves in each bromeliad: control treatments received two empty tubes, while damselfly present treatments (damselfly presence and consumption cues and damselfly presence only) received two tubes each with one late-instar (3rd or 4th instar) larva of *L. andromache*. The *L. andromache* larvae from the damselfly presence and consumption cues treatment were fed every three days with two *Microculex* mosquito larvae, while *L. andromache* from the damselfly presence only treatment were never fed. We watered bromeliads daily with commercial mineral water to keep water levels constant and avoid any effect of large water level fluctuations in our response variables. We also checked the plastic centrifuge tubes daily for any dead/emerging *L. andromache* or for other macroinvertebrates that could have entered the tubes. Any dead or emerging *L. andromache* larvae were promptly replaced. We performed similar manipulations with tubes from all bromeliads, to control for the effect of our presence in the surroundings of each experimental bromeliad. The experiment ran for 42 days, after which we sampled each bromeliad, as described below.

## Response of the aquatic communities to predation risk cues

We examined the responses of aquatic macroinvertebrates and bacteria to the presence of the damselfly. For each bromeliad, we combined water from multiple tanks into a single sample to assess bacterial biomass. Water routinely flows between tanks in a bromeliad, especially during rainfall, so the appropriate level of analysis is bromeliad rather than tank. We filtered the samples through 0.2  $\mu\text{m}$  pore size filters (25 mm, polycarbonate plain black filters), stained with DAPI (4', 6-diamidino-2-phenylindole) and counted bacteria using epifluorescence microscopy. We converted total bacteria counts into bacterial biomass using a conversion factor of  $2 \times 10^{-14}$  g C per cell for freshwater bacteria (Ducklow 2000).

We dissected and washed each bromeliad, leaf by leaf, for collection of aquatic macroinvertebrates. We identified aquatic macroinvertebrates to the lowest possible taxonomic level, recorded their abundance and measured the body length of each individual. We estimated aquatic macroinvertebrate biomass from body length using taxon-specific allometric equations developed at the field site ( $r^2$  from 0.708 to 0.942). Data available from the Zenodo Digital Repository: <http://dx.doi.org/10.5281/zenodo.21539> (Marino 2015).

## Ecosystem level responses to predation risk cues

We examined three important ecosystem processes in response to our manipulation: bacterial production, nutrient

cycling and ecosystem respiration. In each case, responses were measured from several tanks of the same bromeliad pooled together, as tanks are non-independent: detritivores and water bearing chemical cues move between tanks.

Bacterial production (i.e. bacterial growth rate) was measured through the  $\text{H}^3$ -leucine incorporation method (Kirchman et al. 1985) and trichloroacetic acid (TCA) protein extraction (Smith and Azam 1992; modified by Miranda et al. 2007). Note that bacterial production is a rate, whereas bacterial biomass is a stock, and so these measures are distinct from each other. For each bromeliad, we combined water from multiple tanks into a single sample. Samples were incubated in the dark for 45 min with 20 nM of  $\text{H}^3$ -leucine (specific activity 150 Ci mmol $^{-1}$ ). We set up negative controls by adding 90  $\mu\text{l}$  of TCA before starting the incubations. After the incubation,  $\text{H}^3$ -leucine incorporation was stopped by the addition of 90  $\mu\text{l}$  TCA. Bacterial protein was extracted by washing with 5% TCA and 80% ethanol. After protein extraction, we added a liquid scintillation cocktail to each sample, which were then radio-assayed by liquid scintillation counting. We converted the resulting disintegration per minute (DPM) to bacterial carbon production using a 0.86 protein-to-carbon conversion factor (Wetzel and Likens 1991).

We measured nitrogen flux from the detritus to the bromeliad tissue as a relevant measure of nutrient cycling (Ngai and Srivastava 2006, Romero and Srivastava 2010). Small shrubs of *Eugenia uniflora* (Myrtaceae) were watered daily, for 30 days, with 50 ml of a labelled solution (5g l $^{-1}$  day $^{-1}$ ) of ammonium sulphate ( $\text{NH}_4^{15}\text{SO}_4$  (10% atom excess of  $^{15}\text{N}$ ). The enriched *E. uniflora* leaves were collected and dried. After the first week of the experiment, a total of  $2.03 \pm 0.02$  g dry mass of labelled  $^{15}\text{N}$  leaves of *E. uniflora* were distributed into several tanks of each bromeliad. At the end of the experiment, we clipped two new bromeliad leaves from the innermost node of each bromeliad for isotopic ( $^{15}\text{N}$ ) analysis.

For determination of ecosystem respiration, we measured dissolved carbon dioxide ( $\text{pCO}_2$ ) concentration from water samples taken from several tanks of each bromeliad. Samples were taken between 08:00 a.m. and 09:00 a.m. using 20 ml syringes, sealed in vacuntainers and equilibrated with 10 ml of atmospheric air. We made all readings within 2 h after sample extraction using an infrared gas analyzer. We calculated  $\text{CO}_2$  flux (mg C m $^{-2}$  day $^{-1}$ ) to the atmosphere from  $\text{pCO}_2$  readings, using the method presented in Teodoru et al. (2009), with a  $\text{CO}_2$  exchange velocity coefficient of 0.48 m day $^{-1}$  (Atwood et al. 2013).

## Statistical analysis

Our experiment was designed to test the community and ecosystem effects of predation risk as moderated by the type of predation cue. Therefore, after establishing an overall effect of our three treatments, we performed two orthogonal contrasts to determine: 1) if predation risk affected the system (contrast 1: damselfly absent versus presence [damselfly presence and consumption cues + damselfly presence only]) and 2) if prey alarm cues have any additional effect on the system to that induced by predator kairomones (contrast 2: damselfly presence and consumption cues versus damselfly

presence only). For all analyses of the macroinvertebrate community we considered only the fully aquatic organisms that have their larval stage (mostly insects) or entire life cycle within the bromeliad (such as *D. superterrenus* and *E. bromeliarum*). We acknowledge that some terrestrial invertebrates may also play an important role in community structure and ecosystem function (e.g. spiders; Romero and Srivastava 2010), but the large spiders known to have important effects in a closed restinga at another site were absent from the open restinga at the experimental site.

We used generalized linear models (GLMs) to test for the effects of predation risk cues. Metrics that correlate with bromeliad size (e.g. plant volume, number of leaves and detritus content; Marino et al. 2013) did not differ among treatments (Table 1; MANOVA, Pillai–Bartlett statistic = 0.124,  $p = 0.956$ , DF = 2, residual DF = 12), but we decided to include a measure of bromeliad size as a covariate in our models, as bromeliad size may have a large impact on macroinvertebrate communities (Petermann et al. 2015). The size covariate was quantified as the first axis of a principal component analysis (PCA; 74.7%, explained variance; Supplementary material Appendix 1 Fig. A1; site scores multiplied by  $-1$  to ensure that higher scores correspond to larger bromeliads). We tested for the main effects of predation risk cues and bromeliad size, and their interaction in our models. If the interaction term was not significant and decreased model fit to the data, we dropped it from the final model and used a type II sum of squares to calculate the main effects of each term. We used a GLM with a gamma distribution to analyse effects of the treatments on response variables at the macroinvertebrate trophic level (species richness, abundance and biomass; log link), bacterial biomass (log link), and nitrogen flux and  $\text{CO}_2$  flux (identity link, log-transformed data). A GLM with Gaussian distribution was used to analyse bacterial production (identity link, log-transformed data). We chose these distributions and link functions as they provided the best fit to the data, as determined by normality of residuals, homogeneity of variances and absence of outliers with standard diagnostic plots and metrics. We also ran these analyses using the non-parametric Kruskal–Wallis test, but it did not affect the interpretation of our results. Moreover, the Kruskal–Wallis test does not allow the use of covariates, which could ignore the ecological importance of bromeliad size on our response variables. Therefore, we present the results based on the GLM. We carried out all analyses using the statistical language R, ver. 3.1.2 (< [www.r-project.org](http://www.r-project.org) >) and used the package *car* (Fox and Weisberg 2011) to calculate type II sum of squares for GLM.

We also evaluated changes in aquatic macroinvertebrate community composition in response to our manipulations. To test if community composition differed among treatments we used a permutational multivariate analysis of variance (PERMANOVA, 999 permutations). We then used a permutational multivariate analysis of group dispersions (PERMDISP, Raup–Crick dissimilarity, 999 permutations) to assess the degree of variance in community composition within each treatment. To perform both analyses, we used Bray–Curtis and Jaccard dissimilarity indexes to describe changes on community composition in terms of species abundance and species occurrence, respectively. Results for analyses on community composition and dispersion



Table 1. Bromeliad morphological characteristics measured for the bromeliads of each of the three predation risk cue treatments. Values are the mean  $\pm$  SE ( $n = 5$  per treatment) for each characteristic and values in parentheses are the minimum and maximum. Plant volume was measured by slowly filling the bromeliad with a measured amount of water until the bromeliad capacity was reached. Number of tanks refers to the number of green, live leaves that form the tanks of the bromeliad. Detritus was quantified by retaining all the detritus content from each bromeliad in 53  $\mu\text{m}$  soil sieves, drying (60°C for 72h) and weighing.

	Damselfly absent	Damselfly presence only	Damselfly presence and consumption cues
Plant volume (ml)	890 $\pm$ 16.06 (800–980)	1056 $\pm$ 61.54 (700–1500)	950 $\pm$ 83.66 (500–1600)
Number of tanks	18.8 $\pm$ 0.51 (16–23)	21 $\pm$ 0.83 (17–26)	19.2 $\pm$ 0.96 (13–25)
Detritus (mg dry mass)	14.23 $\pm$ 1.74 (11.98–16.03)	14.4 $\pm$ 1.18 (11.38–24.99)	14.78 $\pm$ 0.80 (11.27–19.76)

were qualitatively similar for both abundance (Bray–Curtis dissimilarity) and occurrence (Jaccard dissimilarity) data. Therefore, we will only present results for analyses of community composition based on abundance data. Principal components analysis and community composition data were analysed using functions from package *vegan* (Oksanen et al. 2015).

We found a large tabanid larva in one of the damselfly presence and consumption cues replicates, which, given its biomass, must have escaped the washing and drying procedure. Diagnostic plots showed that this replicate was an outlier in our analysis whenever we considered macroinvertebrate biomass as a response variable, and we therefore excluded this replicate from further analysis considering this response variable. In addition, we carried out our initial analysis by separating macroinvertebrates into trophic levels (detritivores, mosquitoes and intermediate predators), but responses were qualitatively similar to the ones with the entire macroinvertebrate community. Thus, we report analyses considering the entire macroinvertebrate community.

## Data deposition

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.3m2c3>> (Marino et al. 2015).

## Results

### Effects of predation risk cues on aquatic macroinvertebrates

Community structure responded to our three treatments (Fig. 1, Table 2). When damselflies were present, the risk of damselfly predation reduced macroinvertebrate richness by 21.6% (Fig. 1A; contrast 1, Table 2), abundance by 36.6% (Fig. 1B; contrast 1, Table 2) and biomass by 72.3% (Fig. 1C; contrast 1, Table 2). We found no differences in community composition due to predation risk (Bray–Curtis dissimilarity: PERMANOVA,  $F_{2,12} = 1.2913$ ,  $p = 0.198$ ; PERMDISP,  $F_{2,12} = 0.0706$ ,  $p = 0.953$ ). Adding cues from consumed prey to any kairomone effects of the damselfly did not alter any of the observed results (contrast 2, Table 2). Bromeliad size affected community structure independently of predation risk (Table 2). Bromeliad size had a positive effect on macroinvertebrate richness (Table 2) and abundance (Table 2), but no effect on biomass.

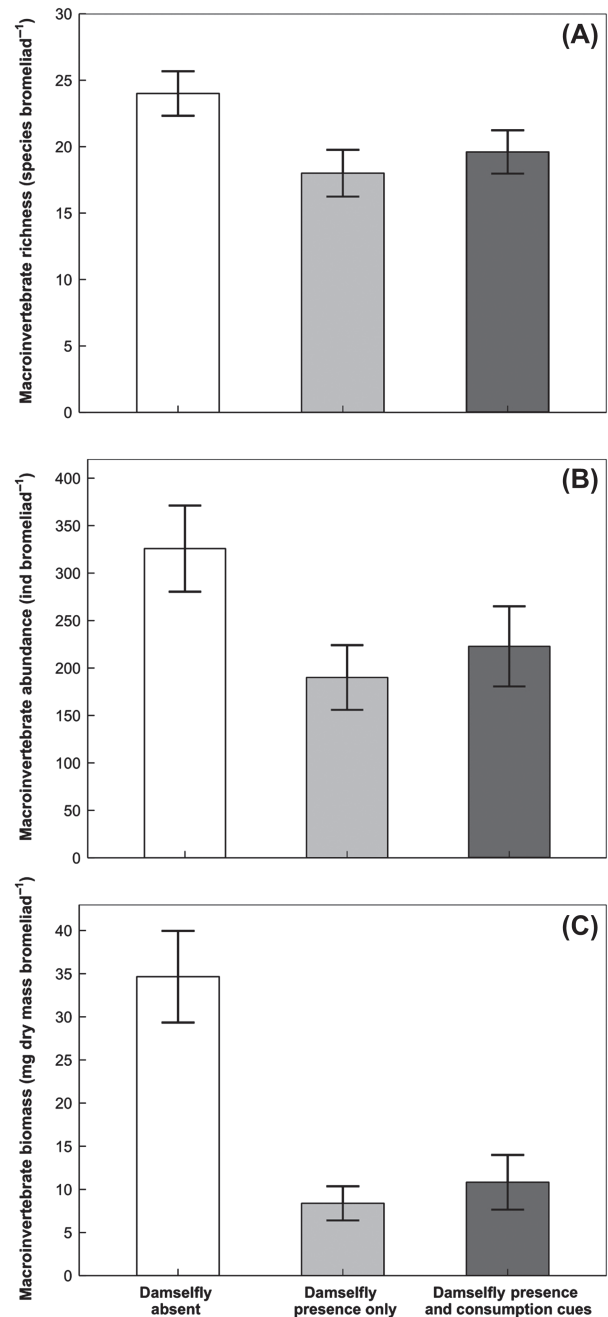


Figure 1. Effects of predation risk cues on aquatic macroinvertebrate community structure, in terms of (A) species richness, (B) abundance and (C) biomass. The damselfly is either absent (white bars), caged and starved (damselfly presence only treatment; light grey bars) or caged and fed (damselfly presence and consumption cues treatment; dark grey bars). The bars represent the mean  $\pm$  SE.

Table 2. Effects of bromeliad size and predation risk cues in macroinvertebrates, bacteria and ecosystem function as determined by generalized linear models and a priori contrasts. Bromeliad size was measured as the scores of the first axis of a PCA ordination: high values of the PCA score describe bromeliads with a greater number of leaves and a larger capacity to accumulate water and detritus. We used the gamma distribution to calculate parameter estimates for macroinvertebrate response variables (log link), bacterial biomass (log link), and nitrogen (log link) and CO<sub>2</sub> fluxes (identity link, log transformed data). We used the Gaussian distribution for bacterial production data (identity link, log transformed). All analysis were made with F<sub>2,11</sub> degrees of freedom, except for macroinvertebrate biomass (F<sub>2,10</sub>). Statistically significant results are shown in bold.

Response						Contrasts			
	Bromeliad size			Predation risk cues		1) Damsselfy Absence vs Presence		2) Damsselfy presence and consumption cues vs Damsselfy presence only	
	Slope	F	p	F	P	t	p	t	p
Macroinvertebrate									
Species richness	0.195	18.966	<b>0.001</b>	12.576	<b>0.001</b>	4.701	<b>0.001</b>	1.876	0.087
Abundance	0.323	4.943	<b>0.048</b>	4.289	<b>0.042</b>	2.718	<b>0.020</b>	0.742	0.473
Biomass	0.192	0.905	0.364	12.882	<b>0.002</b>	4.702	<b>0.001</b>	0.567	0.583
Bacteria and ecosystem									
Bacterial biomass	0.070	0.448	0.517	7.081	<b>0.011</b>	-3.881	<b>0.003</b>	0.969	0.354
Bacterial production	-0.056	0.358	0.562	5.458	<b>0.023</b>	3.187	<b>0.009</b>	-0.804	0.439
Nitrogen flux	-0.262	1.313	0.276	4.930	<b>0.030</b>	-2.624	<b>0.024</b>	1.844	0.092
CO <sub>2</sub> flux	0.075	0.394	0.543	0.114	0.893	-0.439	0.669	-0.212	0.836

### Cascading effects of predation risk cues in bacteria and ecosystem functioning

Damsselfy presence affected bacterial biomass, bacterial production, and nitrogen flux, but had no effect on CO<sub>2</sub> flux (Table 2; Fig. 2, 3). The presence of the damsselfy increased bacterial biomass by 44.5% (Fig. 2A; contrast 1, Table 2), but decreased bacterial production by 50% (Fig. 2B; contrast 1,

Table 2). Nitrogen flux increased by 60% in the presence of the damsselfy (Fig. 2B; contrast 1, Table 2). There was no additional effect of adding cues from consumed prey to any kairomone effects of the predator, in terms of bacterial biomass, bacterial production and nitrogen cycling (contrast 2, Table 2). These ecosystem functions were also unaffected by bromeliad size (Table 2).

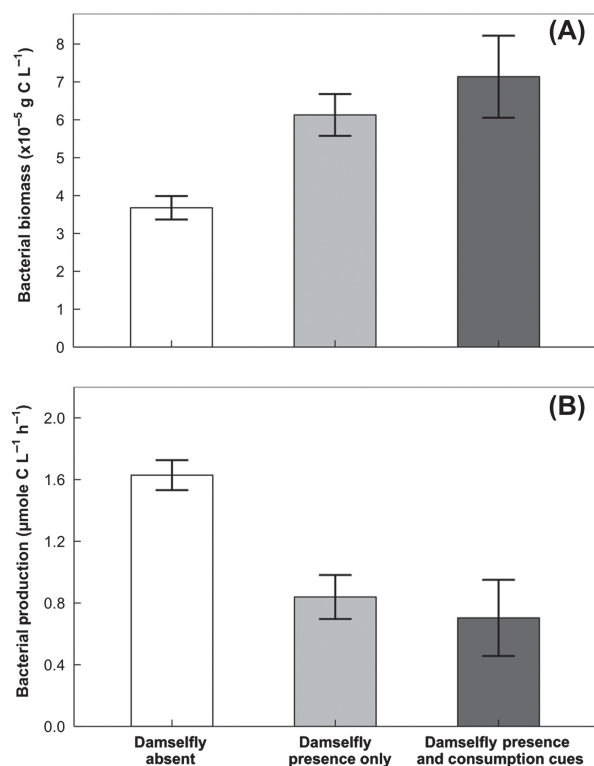


Figure 2. Effects of predation risk cues on (A) bacterial biomass and (B) bacterial production. The damsselfy is either absent (white bars), caged and starved (damsselfy presence only treatment; light grey bars) or caged and fed (damsselfy presence and consumption cues treatment; dark grey bars). The bars represent the mean  $\pm$  SE.

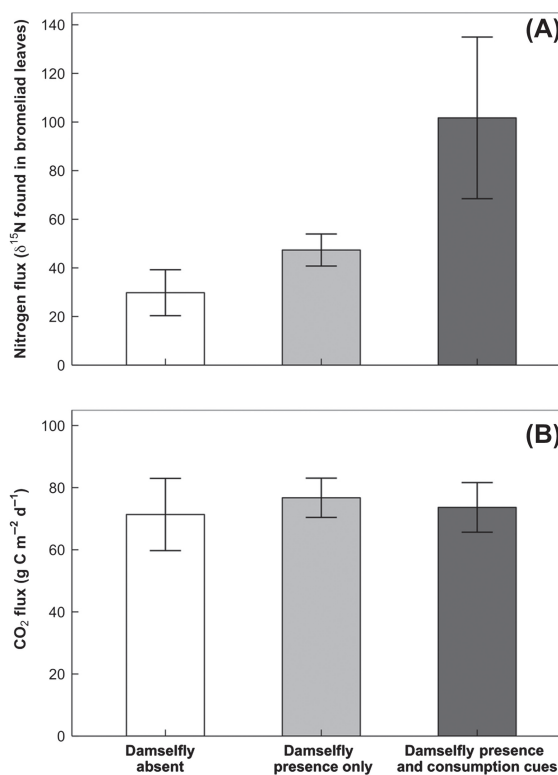


Figure 3. Effects of predation risk cues on (A) nitrogen flux ( $\delta^{15}\text{N}$  from  $^{15}\text{N}$  enriched detritus of bromeliad leaves) and (B) CO<sub>2</sub> flux from the bromeliad tanks. The damsselfy is either absent (white bars), caged and starved (damsselfy presence only treatment; light grey bars) or caged and fed (damsselfy presence and consumption cues treatment; dark grey bars). The bars represent the mean  $\pm$  SE.

## Discussion

Our main aim was to test whether the addition of chemical cues from consumed prey to those from predators are necessary to produce food web and ecosystem responses to predation risk. Given that predation risk should be important for the bromeliad food web (Hammill et al. 2015b) and other systems (Schoeppner and Relyea 2005, 2009, Schoeppner et al. 2009, Vonesh et al. 2009, Wesner et al. 2012), we predicted that the availability of more predation risk cues would lead to stronger responses. In agreement with a previous study, we found that predation risk affects ecological processes in tank bromeliads (Hammill et al. 2015b) and may explain the strength of previous observed effects of damselflies in this system (Ngai and Srivastava 2006, Srivastava 2006, Petermann et al. 2015). However, contrary to our expectation, we found no evidence that adding cues from consumed prey to predator kairomones increased the responsiveness to predation risk (contrast 2, Table 1); rather, the effect of predator kairomones alone were indistinguishable from that of the addition of cues from consumed prey to affect the biomass or abundance of multiple trophic levels (Fig. 1, 2) and ecosystem function (Fig. 3). Since the damselfly was not allowed to consume macroinvertebrates outside the cage, we suggest that predation risk had a negative effect on macroinvertebrate colonization and foraging behaviour (Fig. 1). This non-consumptive effect of the damselfly induced a trophic cascade that released bacteria from top-down effect of macroinvertebrates (Fig. 2) and increased the uptake of nitrogen by the bromeliad (Fig. 3A), but did not affect ecosystem respiration (Fig. 3B).

Predation risk can have effects that extend beyond the colonization process to affect community assembly (Vonesh et al. 2009). Our results show that predation risk did not affect the identity of the colonizing species (community composition and dispersion did not vary among treatments), but affected the abundance and biomass of macroinvertebrate larvae. As our experimental treatments did not allow macroinvertebrates to be consumed outside the damselfly cages, the decrease in macroinvertebrate abundance and biomass cannot be explained by consumptive effects of the damselfly. Therefore, non-consumptive effects of the damselfly are the likely explanation for these responses, through its effects on oviposition decisions by females (as suggested by Hammill et al. 2015a) and changes on macroinvertebrate foraging behaviour. When exposed to predation risk, some insects and amphibians can partition the number of eggs laid in each habitat along a predation risk gradient (Silberbush and Blaustein 2011). This results in habitats with high predation risk receiving fewer colonizers than habitats with no or low predation risk (Silberbush and Blaustein 2011). On the other hand, predation risk may also affect the larval stages by reducing macroinvertebrate foraging activity (Hammill et al. 2015a), which may lead to slower development rates and increased mortality (McCauley et al. 2011, Davenport et al. 2014). Both mechanisms could account for the observed responses of macroinvertebrates to predation risk. Nevertheless, we acknowledge that our experiment does not allow partitioning the effects of changes on adult oviposition behaviour from changes on larval behaviour and

development, and further experiments would be needed to disentangle this effect.

Macroinvertebrates could potentially benefit bacteria by releasing waste products and processing detritus, but they can also passively feed on bacteria when consuming detritus (Srivastava and Bell 2009). Our results show that predation risk had an indirect, positive effect on bacterial biomass and an indirect, negative effect on bacterial production. Damselfly larvae exert strong predation pressure on macroinvertebrates (Starzomski et al. 2010, Petermann et al. 2015), initiating a trophic cascade in tank bromeliads (Ngai and Srivastava 2006, Srivastava 2006, Atwood et al. 2013, Hammill et al. 2015b). Similarly, predation risk may also induce trophic cascades through effects on the macroinvertebrate trophic level (Hammill et al. 2015b). Our results show that predation risk reduced the rate at which bacteria were replaced (i.e. the ratio between bacterial production and bacterial biomass), suggesting that predation risk reduced the loss of bacteria and increased the stock of bacteria in tank bromeliads. Likewise, our results suggests that bacterial turnover rates were primarily regulated by the top-down effect of macroinvertebrate prey in the absence of predation risk. Furthermore, while carcasses from dead prey may act as a nutrient subsidy (Costa and Vonesh 2013) to increase bacterial production (Albeny-Simões et al. 2014), we found no positive effect of predation risk in bacterial production. Alternatively, the final pathway by which predation risk could affect bacteria is via direct effects (either toxic or nutritive) of the kairomones on bacteria. We are not aware of any example whereby invertebrate kairomones have antibiotic effects; indeed it is not clear what the adaptive benefit to the damselfly would be, and if kairomones had antibiotic effects on bacteria we would expect a decrease in bacteria biomass, not an increase. In addition, although there are reports that bacteria can degrade invertebrate kairomones (Tollrian 1993), this would presumably increase bacterial production, not reduce it. Thus, the effect of the damselfly in releasing bacteria from top-down control is more likely to explain this trophic cascade than the possibility of kairomones acting as a resource subsidy.

Bacteria are critical in conditioning detritus for consumption by detritivores, and may also contribute to methane production in the bromeliad ecosystem (Martinson et al. 2010). Our results show that predation risk had a positive effect on the bacteria trophic level (increased biomass and reduced bacteria loss), suggesting that the contribution of bacteria to energy flux and nutrient recycling likely increased. This result highlights that bacteria may be a very important link in the bromeliad food web. However, the responses of the bacteria trophic level to food web manipulations has been largely underappreciated in previous studies, when compared to the responses of the macroinvertebrate trophic level (Ngai and Srivastava 2006, Srivastava 2006, Starzomski et al. 2010, Petermann et al. 2015, Hammill et al. 2015b). Therefore, further consideration on the role of bacteria may provide new insights into the functioning of the bromeliad ecosystem.

Predation risk increased the rates of nutrient remineralization, as observed by the increase in nitrogen flux from detritus to bromeliad leaves. The insects found in tank bromeliads may act as a nutrient sink after they complete their

larval stage and emerge as adults (Ngai and Srivastava 2006). Predators may have a positive effect on nutrient cycling by consuming prey larvae before they emerge (Ngai and Srivastava 2006). Alternatively, the positive effect of predators on nutrient cycling has also been explained by an input of high quality resources from prey carcasses (as opposed to low quality leaf litter) entering the detritus pool (Romero and Srivastava 2010). In our experiment, however, the damselfly was not allowed to prey on macroinvertebrates consuming the labelled detritus outside the cages. As such, the damselfly could not decrease the number of emerging insects and the amount of prey carcasses entering the detritus pool through direct consumption. In this case, insect emergence and the amount of nutrients in insect biomass should have increased, with a negative effect of predation risk on nutrient cycling as observed in other studies (Hawlena and Schmitz 2010, Hawlena et al. 2012). Instead, our results show a positive effect of predation risk on nutrient cycling, which is not consistent with those predictions. Two mechanisms may explain this effect: the damselfly must have increased the uptake of nitrogen by the bromeliad either through 1) a reduction in the amount of nutrients stocked in macroinvertebrate biomass (a physiological response, Hawlena and Schmitz 2010), leaving more nutrients to be absorbed by the bromeliad, or by 2) the positive effect on the bacteria trophic level, promoting an increase in bacterial decomposition. We did not measure the amount of nitrogen in macroinvertebrate biomass nor bacterial decomposition, so that we cannot rule out either explanations. Nevertheless, in both cases, the trophic cascade occurs through an effect of predation risk on the macroinvertebrate trophic level, providing further support that the trophic cascade was mediated by impacts on this trophic level. These results are novel in demonstrating that the positive, indirect effect of a behaviour-mediated trophic cascade on nutrient cycling are not restricted to controlled experiments with simplified food webs, but may also occur in a natural, species-rich aquatic system.

The presence of predators in natural systems, including tank bromeliads, is predicted to affect the CO<sub>2</sub> flux to the atmosphere (Atwood et al. 2013), with an increase or decrease in CO<sub>2</sub> flux for food webs with, respectively, even (Hammill et al. 2015b) and odd (Atwood et al. 2013) numbers of trophic levels. Although the food web observed in our experiment was functionally similar to that in Hammill et al. (2015a), and we observed an increase in bacterial biomass in response to predation risk, we found no evidence that predation risk either increased or decreased CO<sub>2</sub> flux. This points to strong context-dependence of the effects of predation risk on CO<sub>2</sub> flux. CO<sub>2</sub> flux is an aggregate measure from the metabolism of an ecosystem, integrating the results of several processes, such as bacterial respiration, primary production and anaerobic processes that consume and produce CO<sub>2</sub> (e.g. methanogenesis and methanotrophy, respectively). The magnitude of each of these processes can vary according to organic matter content and microsite characteristics (Martinson et al. 2010, Marino et al. 2011) and measuring each process can be logistically difficult, if not infeasible. Therefore, although measuring CO<sub>2</sub> flux is still the best way to integrate across processes, changes in CO<sub>2</sub> flux mediated by predators are more likely to be observed when predation has a disproportionate effect on one of the processes

described above. Moreover, previous studies at our field site have found a high abundance of algae (Marino et al. 2011) and substantial methane concentration in these bromeliads (N. Marino unpubl.). It is therefore possible that predators can also have an impact on primary production and methane production, counteracting any effect of increased bacterial respiration, but any attempt to link our results to these processes will be very speculative.

There are a few caveats to our study. First, the duration of our experiment covered the length of the life cycle of many of the organisms of the bromeliad ecosystem. We acknowledge that during this period some early insect colonists such as mosquitoes and chironomids may have emerged and left the bromeliad, but given the slow rate of colonization by the major detritivores (Tipulidae and Scirtidae), this is unlikely to have caused the observed detrital decomposition and nitrogen uptake responses to predation risk. It should also be remembered that both colonization and emergence are continuous processes, so emerged insects are replaced by new colonists, maintaining abundance and richness. Second, our experiment was designed to test whether adding cues from consumed prey to predator kairomones could produce ecological patterns different from that of predator kairomones alone. Therefore, we did not test the relative importance of prey cues versus predator kairomones. A factorial experiment (prey cues and predator kairomones in isolation and combined) could have determined the relative importance of consumed prey versus predator derived chemical cues (Schoeppner and Relyea 2005, 2009), but we chose not to focus on this question as consumed prey by definition do not occur in the absence of a predator. Finally, we acknowledge that kairomones from starved predators may elicit stronger responses on prey than kairomones from satiated predators (Bell et al. 2006). As such, while we cannot rule out this effect, we also emphasize that our main aim was not to test the relative importance of these two sources of cues. Although these caveats may limit our ability to generalize our conclusions, we believe our results provide evidence that predator kairomones alone may influence a species-rich food web with cascading consequences for ecosystem functioning, which effect is indistinguishable from that of the combination of predator kairomones and cues from consumed prey. These results are in agreement with evidence that even low levels of predation risk may induce responses on prey (Schoeppner and Relyea 2008). Therefore, we suggest that the role of predators in transmitting predation risk should not be overlooked in multispecies communities.

Here, we show that predation risk can induce a community-wide, behaviour-mediated trophic cascade. These results are in line with recent evidence that predation risk can play a large role in the net effect of predation in bromeliads (Hammill et al. 2015b), and expands this conclusion by showing that these responses are not amplified by the provision of additional cues (Schoeppner and Relyea 2008). Moreover, we show that the effects of predators in nutrient remineralisation can occur even when there is no consumptive effect of predators on prey, in contrast to consumptive mechanisms that have been usually evoked to explain such pattern (Ngai and Srivastava 2006). These results also show that the effects of predation risk on nutrient cycling are not restricted to experiments using simplified food webs (Stief



et al. 2006, Hawlena and Schmitz 2010, Hawlena et al. 2012, Sitvarin and Rypstra 2014), and may also occur in a species-rich food web. The fact that the mere presence of predators affected the entire food web highlights the pervasive effect that the fear of predation may play in natural systems. We suggest that the consequences of predation risk in complex systems can be better understood by moving beyond experiments with pairwise species interactions in simplified food webs.

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Supplementary material (available online as Appendix oik-02664 at <[www.oikosjournal.org/appendix/oik-02664](http://www.oikosjournal.org/appendix/oik-02664)>). Appendix 1.