2015

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A multifaceted trophic cascade in a detritus-based system: density-, trait-, or processing-chain-mediated effects?

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Citation: Albeny-Simões, D., E. G. Murrell, E. F. Vilela, and S. A. Juliano. 2015. A multifaceted trophic cascade in a detritus-based system: density-, trait-, or processing-chain-mediated effects? Ecosphere 6(3):32. http://dx.doi.org/10.1890/ES14-00365.1

Abstract. We investigated three pathways by which predators on an intermediate trophic level may produce a trophic cascade in detritus-based systems. Predators may increase lower trophic levels (bacteria) by reducing density of bacteriovores, by altering behavior of bacteriovores, and by processing living bacteriovores into carcasses, feces, and dissolved nutrients that are substrates for bacteria. We tested these pathways in laboratory experiments with mosquitoes in water-filled containers. Larval *Toxorhynchites rutilus* prey on larval *Aedes triseriatus*, which feed on bacteria. Using containers stocked with oak leaf infusion as a bacterial substrate, we compared bacterial productivity at 7 and 14 days for: prey alone; prey with a predator; and prey with predation cues but no predator. Controls contained no larvae, either with predation cues or without cues. Predation cues in the control treatment increased bacterial abundance at 7 days, but this effect waned by 14 days. *Aedes triseriatus* larvae reduced bacterial abundance significantly at 14 days. Predator cues and real predation both eliminated the negative effect of *A. triseriatus* on bacterial abundance. Predation cues reduced survivorship of *A. triseriatus* larvae at 14 days, however this effect was smaller than the effect of real predation. We further tested effects of residues from predation as cues or as detritus in a second experiment in which *A. triseriatus* were killed at similar rates by: real predators; mechanical damage without the predator and carcasses left as detritus; or mechanical damage and carcasses removed. No prey larvae were killed in controls. Bacterial productivity was greater with real predation than in all other treatments and greater when prey larvae were killed or killed and removed, than in controls. Thus we find evidence that all three pathways contribute to the trophic cascade from *T. rutilus* to bacteria in tree hole systems.

Key words: *Aedes triseriatus*; density-mediated indirect interaction; predation; processing chain interaction; *Toxorhynchites*; trait-mediated indirect interaction; trophic cascade.

Received 1 October 2014; revised 21 November 2014; accepted 8 December 2014; final version received 14 January 2015; **published** 18 March 2015. Corresponding Editor: D. P. C. Peters.

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Introduction

Trophic cascades occur when direct or indirect effects of predators on prey cause an indirect increase in abundance of the basal trophic level that is the food of the prey (Pace et al. 1999, Shurin et al. 2002). In aquatic detritus-based systems, addition of a predator to the system negatively impacts the abundance of prey, which can then yield cascading positive effects on microorganisms that prey feed on. Although this phenomenon has been documented in a variety of natural and artificial systems under both laboratory and field conditions (Kneitel and Miller 2002, Trzcinski et al. 2005, Cochran-Stafira and von Ende 1998, Yanoviak 2001) the diverse mechanisms by which a third trophic level can have a top-down cascading effect on microbial abundance are less often investigated, and are the subject of this paper.

Predators as a third trophic level can affect the basal trophic level via at least three mechanisms. First, predators may reduce prey abundance, indirectly impacting lower trophic (density mediated indirect interaction, or DMII) (Pace et al. 1999). Second, predator cues may stimulate costly defensive traits (e.g., behavioral changes) in prey that indirectly impact lower trophic levels (trait mediated indirect interaction, or TMII) (reviewed by Werner and Peacor 2003). Third, predator consumption of prey may produce additional detritus in the form of predator feces, excreted nitrogenous waste, and partially eaten prey, releasing nutrients that fuel the growth of the basal trophic level, a phenomenon characterized as "nutrient cycling" in systems dominated by periphyton and phytoplankton (Costa and Vonesh 2013). This effect of predator feeding is likely in detritus-based systems, where bacteria are the basal trophic level, and it is better described as a "processing chain interaction" (PCI) (Heard 1995). A PCI occurs when consumption of a resource by one consumer increases the availability of a modified form of that resource for another consumer. In detritus-based systems, predators may process living tissue of bacteriovores into detritus, making substrates available to decomposers such as bacteria.

Trophic cascades due to DMIIs and TMIIs are well documented in several systems (e.g., Cochran-Stafira and von Ende 1998, Silliman and Bertness 2002, Schwenk et al. 2010, Rosa and DeSouza 2011). TMIIs may be more important than DMIIs in trophic cascades (reviewed by Schmitz et al. 2004) and it is often true that nonconsumptive effects of predators on a prey population may be greater than consumptive effects (e.g., Schmitz et al. 2004, Preisser et al. 2005). Evidence for the contribution of PCIs to trophic cascades is rare, largely because there is a scarcity of studies that investigate DMII, TMII, and PCI effects on trophic cascades simultaneously. The scarcity of such comprehensive studies is surprising, given that detritus-based systems would be ideal for such investigations of trophic cascades and that detritus plays a prominent role in many food webs (Moore et al. 2004). Determining whether density-, trait-, and processing chain-mediated interactions affect trophic cascades requires study systems in which we can investigate all of these effects simultaneously, with and without the presence of the intermediate trophic level (Costa and Vonesh 2013).

Water-filled containers, such as tree-holes, bromeliads, and artificial containers (e.g., automobile tires and plastic buckets) are ideal systems for simultaneously investigating pathways contributing to trophic cascades. These systems support a discrete macro-invertebrate community, including filter feeders/browsers such as mosquito larvae, their microbial food, and their macroinvertebrate predators. Container food webs are usually simple (predators-consumers-microorganisms-detritus) (Walker et al. 1991, Kitching 2000) suggesting that trophic cascades are likely (Strong 1992). Organic detritus, mainly decomposing plant parts and arthropod carcasses, forms the base of the food web and bacteria play a key role in converting that detritus into biomass edible by consumers (Kitching 2000, Moore et al. 2004, Yee et al. 2007*a*, *b*). Although it is clear that detritus-derived productivity affects abundances of decomposers and consumers within natural and artificial containers (e.g., Yee et al. 2007*a*, *b*, Murrell and Juliano 2008), few studies have tested the three pathways by which predators in a bacteria-bacteriovore-predator trophic system may affect bacterial abundance (see Costa and Vonesh 2013). Human-made containers are an ideal system for investigating detritus-based

trophic cascades, because they are small, increasing the potential impact of a single predator on lower trophic levels. Human-made containers are easily replicated and have rapid temporal dynamics (Blaustein and Schwartz 2001, Srivastava et al. 2004), and the discrete physical boundaries facilitate manipulations of species or assembly of an entire experimental community (Blaustein and Schwartz 2001, Srivastava et al. 2004). In addition, bacterial productivity can be quantified (e.g., Yee et al. 2007*a*) and trait-mediated effects of predators on larval mosquito behavior are both well understood and amenable to manipulation (Juliano and Gravel 2002, Kesavaraju et al. 2007*a*, 2010, Costanzo et al. 2011).

Container food webs in North America typically range from three to six living trophic levels, with detritus supporting bacterial and fungal growth, which in turn may support protozoan bacteriovores (Kneitel and Chase 2004, Yee et al. 2007*b*); bacteria, protozoa, and fungi are eaten by invertebrate consumers such as mosquito larvae. The larvae of the mosquito *Aedes triseriatus* (Say) is a common native tree-hole mosquito in the eastern United States (Bradshaw and Holzapfel 1985). Larvae of the mosquito *Toxorhynchites rutilus* (Coquillett) are the dominant predators of container invertebrates in North America (Bradshaw and Holzapfel 1985). Using this simple food web, we manipulated laboratory microcosms to test for the contributions of the different mechanistic pathways (density-, trait-, and processing chain-mediated) by which *T. rutilus* might produce a trophic cascade. We predicted that: (1) predation directly reduces the abundance of consumers and indirectly increases bacterial productivity (DMII), (2) predator cues known to induce behavioral changes in potential prey will indirectly increase bacterial productivity (TMII), and (3) predation and the associated residues (predator feces, uneaten parts of victims, released nutrients) increase the availability of animal detritus in the system, thereby directly increasing bacterial productivity by increasing substrates for bacterial growth (PCI).

Material and Methods

Insect colonies

All *A. triseriatus* and *T. rutilus* larvae used in these experiments came from laboratory colonies maintained at 25° ± 3°C, 80 ± 15% relative humidity with a 14:10 L:D photo-period. *Aedes triseriatus* larvae were kept in 25 × 30 cm plastic trays at a density of approximately 1000 larvae/L of deionized water and fed every other day standard volumes of a liver powder suspension (0.4 g/L deionized (DI) water). *Toxorhynchites rutilus* larvae were individually raised in 20-ml glass vials filled with 10 ml DI water and allowed to feed on *A. aegypti* larvae ad libitum until reaching the fourth instar. Upon eclosion, *A. triseriatus* and *T. rutilus* adults were kept in 60 × 60 × 60 cm and 30 × 30 × 30 cm cages, respectively, and provided continuously with 20% sugar solution. *Aedes triseriatus* females were blood fed on Ketamine: Xylazine-anesthetized guinea pigs (Institutional Animal Assurance number A3762–01, IACUC Protocol 01–2010, Illinois State University). For *T. rutilus*, we used induced-mating technique (Baker et al. 1962) two to four days after emergence to generate fertilized eggs.

General laboratory experimental design

Experimental microcosms were 450-ml plastic cups, filled with 300 ml DI water plus 100 ml white oak (*Quercus alba*) leaf infusion (35 g/L) aged for nine days. The leaves were dried at 50°C for 48 hours. The infusion provided organic matter as a substrate for bacteria that are the food of *A. triseriatus* larvae. Eggs were hatched 24 hours before the start of the experiment in 20-ml glass vials, either individually (*T. rutilus*) or in groups of about 100 (*A. triseriatus*). *Aedes triseriatus* eggs were hatched in an aqueous suspension of 0.4 g/L of lactalbumin.

Experiment I: Effects of predation, predation cues, and processing

This experiment was designed to test for effects of direct predation by *T. rutilus*, predatory cues, and prey larvae on bacterial productivity and survivorship of their prey *A. triseriatus*. Treatments were:

Infusion alone (IA).—This control allowed for bacterial growth in the absence of both *A. triseriatus* grazing pressure and *T. rutilus* indirect predation effects. IA = 300 ml DI water + 100 ml of infusion + 50 ml of aged DI water.

Infusion plus predation cues (IC).—To assess the impact of water-borne predation cues on bacterial growth (processing chain effect), 50 ml of preda-

tion cue-infused water was added to each cup of oak leaf infusion. Predation cues were prepared by holding one *T. rutilus* fourth instar for 5 d in 50 ml of water with 20 *A. triseriatus* fourth instar larvae. This preparation of predation cues has been shown to induce significant reduction in foraging and movement of *A. triseriatus* larvae (Juliano and Gravel 2002, Kesavaraju and Juliano 2004). Prey larvae were counted daily and any missing, dead, or pupated larvae were replaced. Any animal-derived detritus (e.g., predator feces, bits of killed prey) accumulated over the 5-day period remained in the cue-infused water and was added to experimental containers. As a control, 50 ml DI water (aged 5 days) was added to treatments that did not receive predation cues. IC = 300 ml water + 100 ml of infusion + 50 ml of prepared predator cues.

Infusion plus prey alone (IP).—One hundred first instar *A. triseriatus* larvae (=Prey) were added to each cup to assess their DMI effect on bacterial productivity. IP = 300 ml DI water + 100 Prey + 100 ml infusion + 50 ml aged DI water.

Infusion plus prey plus predation cues (IPC).—One hundred first instar *A. triseriatus* larvae plus predator cues prepared as described above were added to assess the combined impact of waterborne predation cues on bacterial productivity via TMII mediated by behavioral effects on *A. triseriatus*, along with the impact of predator-derived detritus (PCI). IPC = 300 ml DI water + 100 Prey + 100 ml infusion + 50 ml prepared cues.

Infusion plus prey plus predator (IPred).—One hundred first instar *A. triseriatus* larvae plus one first instar *T. rutilus* were added to experimental containers to test the combined effects of *T. rutilus* predation on *A. triseriatus* larvae and the resulting DMII on bacterial productivity, along with effects of cues (TMII) and predation-derived detritus (PCI). IPred = 300 ml DI water + 100 Prey + 100 ml infusion + 50 ml aged DI water + 1 predator.

Experimental cups were incubated under insectary conditions (see above) for 14 days. Number of surviving *A. triseriatus* larvae and bacterial productivity (see *Bacterial productivity* below) were recorded on days 7 and 14.

Experiment II: Effects of predation, simulated predation, and prey carcasses

This experiment was designed to control more precisely the density mediated effect of predation and thus to test more clearly the effects of DMII, TMII, and PCI by estimating effects on bacterial abundance and *A. triseriatus* survivorship. Crushing prey with forceps has been shown to produce similar effects on behavior on *A. triseriatus* to real predation (Costanzo et al. 2011). All containers received infusion plus 100 first instar *A. triseriatus* larvae. As in experiment I, the IPred treatment(=actual predation) received these prey larvae plus one first instar *T. rutilus* larva (300 ml of DI water + 100 ml of infusion + one first instar predator + 100 first instar prey = IPred). To test effects of predation cues and prey carcasses, the number of prey larvae consumed by predators was recorded daily and the mean number of consumed prey was removed daily from two simulated predation treatments by crushing the middle of their bodies using forceps, and carcasses either left in the container as detritus (IPCr) or removed (IPCrR). Thus we had treatments of: simulated predation without adding prey carcasses (300 ml of DI water + 100 ml of infusion + 100 first instar prey, with prey larvae crushed and removed from the water = IPCrR); simulated predation with prey carcasses (300 ml of DI water + 100 ml of infusion + 100 first instar prey, with prey crushed and carcasses left in the water = IPCr). We also had a no predation treatment as in experiment I (300 ml of DI water + 100 ml of infusion + 100 first instars prey = IP). Experimental cups were held under insectary conditions (see above) for 10 days. Prey survivorship (including the effects of real and simulated predation) was determined daily; however, only survivorship data from days 5 and 10, when we quantified bacterial productivity, were used in statistical analyses.

Bacterial productivity

Productivity of new bacterial biomass was quantified by estimating protein synthesis using a tritiated L-leucine (4,5-^{3}H, 50 Ci mmol^{-1}; Perkin Elmer, Waltham, MA, USA) incorporation assay that is specific to bacteria in aquatic systems (Riemann and Azam 1992). The assay been used to quantify bacterial productivity in container mosquito experiments (e.g., Yee et al. 2007*a*, Albeny-Simões et al. 2014). We measured water column protein synthesis following Kirchman

(1993) and refined by Kaufman et al. (2001) for container systems. To a 1-ml fluid sample, [^{3}H]-leucine was added, incubated for 30 min, and then protein was precipitated in trichloracetate. [^{3}H]-Leucine incorporation was quantified by liquid scintillation (Beckman LS-6500 scintillation counter; Beckman Coulter, Brea, CA, USA). Decays per minute (DPM) is used as a relative quantification of bacterial protein synthesis.

Statistical analyses

For both experiments the effects of the treatments, time (sampling periods 1 and 2), and interaction on leucine incorporation (DPM) and *A. triseriatus* survival were analyzed using repeated measures analysis of variance (PROC MIXED, SAS Institute Inc. 2011). Significant effects of time within treatments were analyzed via pairwise comparisons of least squares means for the two sample times in each experiment, using a Tukey-Kramer adjustment for all possible pairwise comparisons. For bacterial productivity, four of the five treatments in experiment I form a factorial design with effects of *Aedes* absence/presence (i.e., IA+IC vs. IP+IPC), predator cues absence/presence (i.e., IA+IP vs. IC+IPC), and interaction. Therefore, significant effects in experiment I were further explored using contrasts of least squares means within a sampling period, testing specifically for effects of predator cues, *A. triseriatus* presence, and their interaction. We compared the effect of our fifth treatment (IPred) to other treatments by testing the effects of real predation (IPred) vs. no predation (IP), and real predation (IPred) vs. prey+cues (IPC). Because of the large number (5) of nonorthogonal contrasts done to follow up on experiment I, we adjusted for multiple tests using a sequential Bonferroni approach (Rice 1989). For experiment II follow up tests were pairwise comparisons of least squares means within a sample period, using a Tukey-Kramer adjustment for all possible pairwise comparisons.

For both experiments, data analyzed were taken at times (7, 14 days in experiment I; 5, 10 days in experiment II) prior to pupation of the *A. triseriatus*, so that none of the effects observed on bacterial productivity or on number of surviving larvae derive from larvae of *A. triseriatus* leaving the feeding population (at pupation) or leaving the container (at eclosion of the adult).

Results

Experiment I: Effects of predation, predation-cues, and processing

Bacterial productivity was significantly affected by time ($F_{1,35} = 5.65$, $P = 0.0230$), by treatment ($F_{4,35} = 19.02$, $P = 0.0001$), and by treatment-time interaction ($F_{4,35} = 8.15$, $P = 0.0001$). Thus, trends in bacterial production over the time depended on treatment. Two treatments showed significant increases in DPM from 7 to 14 days: Infusion alone (IA, Fig. 1, compare 7 vs. 14 days) and Infusion + prey + cues (IPC; Fig. 1, compare 7 vs. 14 days); whereas trends for the other three treatments were not significant (Fig. 1). At 7 days there were significant effects of *A. triseriatus* presence and of cues, but the interaction of *A. triseriatus* and cues was marginally nonsignificant after Bonferroni correction (Table 1). *Aedes triseriatus* larvae depressed bacterial productivity relative to no larvae (Fig. 1, 7 days), and addition of cues raised bacterial productivity relative to absence of cues (Fig. 1, 7 days), particularly in the treatments without *A. triseriatus* larvae (Fig. 1, 7 days). The difference between real predation (IPred) and no predation (IP) was also marginally nonsignificant after Bonferroni correction (Table 1) with real predation yielding greater bacterial productivity than no predation (Fig. 1, 7 days). The difference between real predation (IPred) and prey + cues (IPC) was far from significant (Table 1).

Effects on bacterial productivity were much stronger and clear cut by day 14 (Fig. 1). At this time, effects of cues, *A. triseriatus*, and their interaction were all highly significant (Table 1). *Aedes triseriatus* larvae greatly depressed bacterial productivity in the absence of cues (Fig. 1, 14 days, compare IA and IP), but had no effect in the presence of cues (Fig. 1, 14 days, compare IC and IPC). At 14 days, the effect of added cues in the absence of larvae was small and not significant (Fig. 1, 14 days, compare IA and IC). At 14 days both real predation (IPred) and cues (IPC) produced greater bacterial productivity than did prey alone (IP) (Table 1, Fig. 1, 14 days). At day 14, bacterial productivity for real predation (IPred) and prey + cues (IPC) remained statistically indistinguishable (Fig. 1, 14 days).

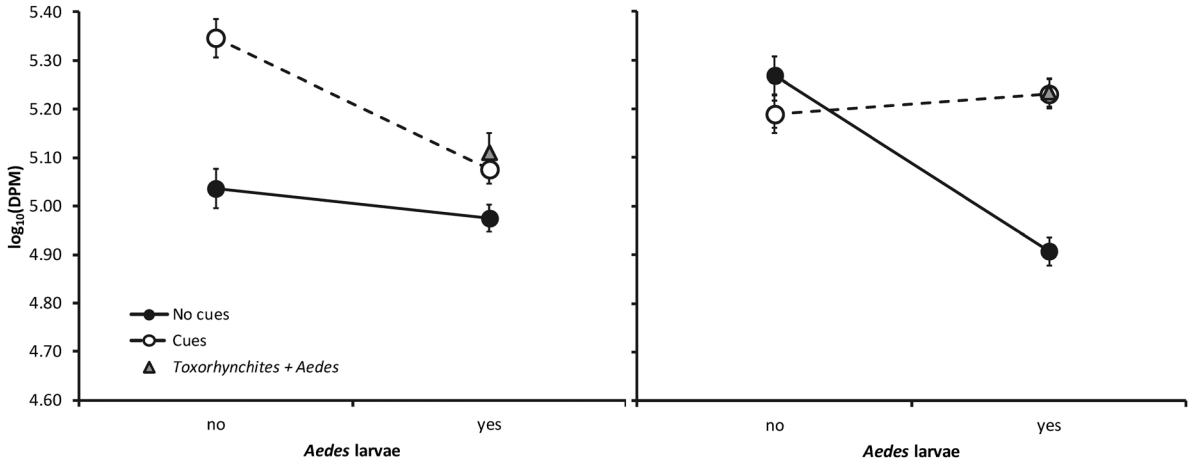

Fig. 1. Experiment I results for days 7 and 14 (least squares means ± SE). Productivity of bacterial biomass based on protein synthesis quantified via incorporation of tritiated H-leucine (4,5-^{3}H). Treatments are: IA: infusion alone; IP: infusion + prey larvae; IC: infusion + cues from predation; IPC: infusion + prey + cues from predation; IPred: infusion + prey + predator. See Table 1 for contrasts testing for effects of *Aedes*, predator cues, interaction of *Aedes* and cues, and comparisons of real predation to other treatments at experimentwise $\alpha = 0.05$.

Survivorship of *A. triseriatus* was significantly affected by treatment ($F_{2,27} = 42.45$, $P < 0.0001$) time ($F_{1,27} = 306.69$, $P < 0.0001$), and treatment-time interaction($F_{2,27} = 39.34$, $P < 0.0001$). Proportion surviving significantly declined from day 7 to day 14 for infusion + prey + cues (IPC) and for infusion + prey + predator (IPred), but not for infusion + prey (IP) (Fig. 2). As expected, real predation (IPred) significantly lowered survivorship relative to prey alone (IP) at both day 7 and day 14 (Fig. 2). Survivorship of larvae in infusion + prey + cues (IPC) did not differ from that in IP at day 7, but was significantly lower than that in IP at day 14 (Fig. 2). Survivorship in IPC was significantly greater than that in IPred at day 14 (Fig. 2).

Experiment II: Effects of predation, simulated predation, and prey carcasses

Bacterial productivity was significantly affected by treatment ($F_{3,24} = 29.10$, $P < 0.0001$), day ($F_{1,24} = 647.54$, $P < 0.0001$), and treatment-day interaction ($F_{3,24} = 20.22$, $P < 0.0001$). DPM declined significantly for all treatments, with greatest decline for infusion + prey (IP) and least for infusion + prey + predator (IPred) (Fig. 3A). At day 5 there were no significant differences among treatments in DPM (Fig. 3A). In contrast, at day 10 DPM was least for infusion + prey (IP), greatest for infusion + prey + predator (IPred), and intermediate for infusion + prey crushed (IPCr) and infusion + prey crushed removed (IPCrR), which did not differ (Fig. 3A). Thus,

Table 1. Contrast analysis for effects on bacterial productivity in experiment I. Because of significant Time × Treatment interaction, contrasts were done within sampling times (7 or 14 days; Fig. 1). Contrasts significant at experiment wise $\alpha = 0.05$ (i.e., less than the associated sequential Bonferroni criterion for each test given for both days) are indicated in boldface type.

	7 days			14 days		
Contrast	$F_{1,35}$	Uncorrected Pr > F	Sequential Bonferroni criterion	$F_{1,34}$	Uncorrected Pr > F	Sequential Bonferroni criterion
Cues vs. no cues	**19.09**	**0.0001**	**0.0100**	**12.02**	**0.0014**	**0.0250**
Prey vs. no prey	**12.49**	**0.0012**	**0.0125**	**21.26**	**<0.0001**	**0.0167**
Cue-prey interaction	4.93	0.0330	0.0250	**32.61**	**<0.0001**	**0.0125**
Real predation vs. cues	0.41	0.5250	0.0500	0.02	0.8929	0.0500
Real predation vs. no predation	6.27	0.0171	0.0167	**66.60**	**<0.0001**	**0.0100**

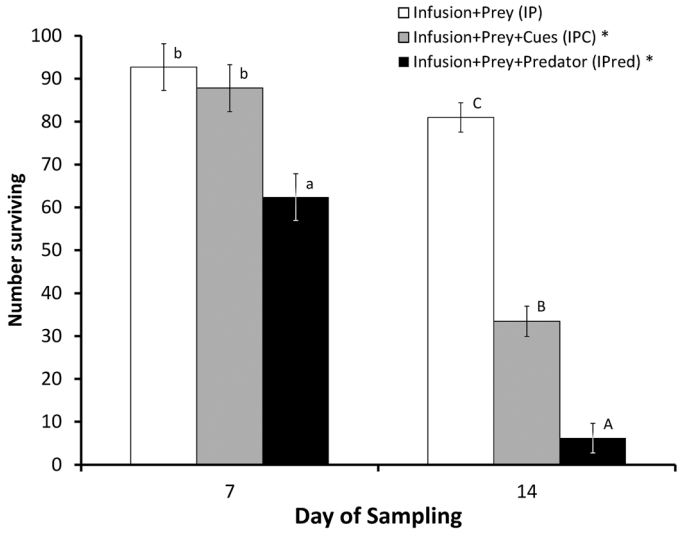

Fig. 2. Experiment I number of surviving *Aedes triseriatus* larvae. Treatments are: IP: infusion + prey larvae; IPC: infusion + prey + cues from predation; IPred: infusion + prey + predator. Within each panel, significant change from 7 to 14 days is indicated by an asterisk (*) adjacent to the treatment in the figure legend, and within weeks, means associated with the same letter are not significantly different, experimentwise $\alpha = 0.05$.

although both real and simulated predation enhanced bacterial productivity at day 10, real predation had a greater effect. Removing victims did not lessen the enhancement of bacterial productivity.

Survivorship of *A. triseriatus* was significantly affected by treatment ($F_{3,24} = 161.09$, $P < 0.0001$), time ($F_{1,24} = 863.95$, $P < 0.0001$), and treatment-time interaction ($F_{2,27} = 43.02$, $P < 0.0001$). Survivorship declined significantly for all treatments, but the decline was least for infusion + prey (IP), and similar for all other treatments (Fig. 3B). At both days 5 and 10, infusion + prey (IP) yielded significantly greater survivorship than all other treatments, and the other three treatments (IPCr, IPCrR, IPred) were statistically indistinguishable (Fig. 3B).

Discussion

We tested hypotheses that density mediated effects of predation, trait mediated effects of cues from predation, and processing chain effects of predation impact trophic cascades in laboratory microcosms that simulate water-filled containers. We found evidence that all three kinds of effects are present in the trophic cascade from *T. rutilus* through *A. triseriatus* to bacteria. Our data suggest the effects act at different time scales and are not additive.

Trophic cascade

Experiment I provides direct evidence for a trophic cascade from *T. rutilus* to the basal level of bacteria. The presence of *A. triseriatus* larvae reduced bacterial productivity at 7 days (significant main effect of *Aedes*, Table 1, Fig. 1). This effect interacted with predator cues at 14 days (Table 1) and was evident only in the absence of predator cues (compare IA vs. IP at 14 days; Fig. 1). When both predator and prey are present (IPred) bacterial productivity at 14 days increased to a level indistinguishable from that of infusion alone (IA vs. IPred; Fig. 1). Our experiments did not consider potential effects on bacteria via protozoa, which are both prey of mosquito larvae and consumers of bacteria (Cochran-Stafira and von Ende 1998, Kitching 2000, Kneitel and Chase 2004). Because protozoa are intraguild predtors on bacteria, their role in this system is likely complex. Grazing mosquitoes affect protozoan diversity and species composition (Cochran-Stafira and von Ende 1998, Kneitel and Chase 2004) but may have positive, negative, or no effect of total protozoan abundance (e.g., Cochran-Stafira and von Ende

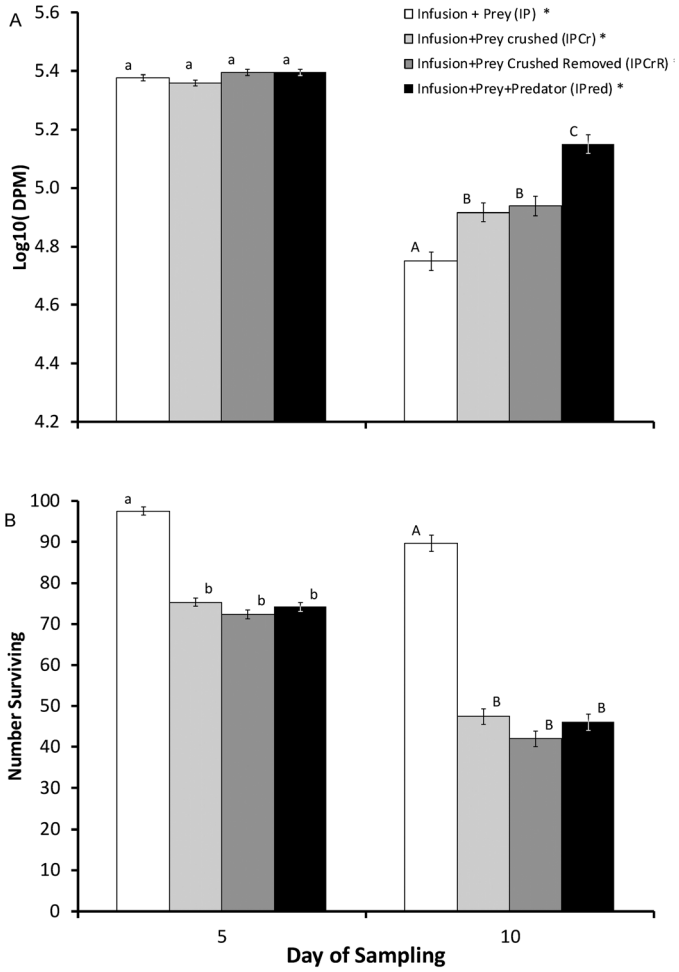

Fig. 3. Experiment II results for days 5 and 10 (least squares means ± SE). (A) Productivity of bacterial biomass based on protein synthesis quantified via incorporation of tritiated H-leucine (4,5-^{3}H). (B) Number of surviving *Aedes triseriatus* larvae. Treatments are: IP: infusion + prey larvae; IPCr: infusion + prey + crushed prey; IPCrR: infusion + prey + prey crushed and removed; IPred: infusion + prey + predator. Within each panel, significant change from day 5 to day 10 is indicated by an asterisk (*) adjacent to the treatment in the figure legend, and within day 5 or day 10, means associated with the same letter are not significantly different, experimentwise $\alpha = 0.05$.

1998, Kneitel and Miller 2002, Kneitel and Chase 2004). Regardless of the unknown role of protozoa, *T. rutilus* has a cascading effect on bacteria, and so we ask: does this trophic cascade involve density-, trait-, or processing-chain-mediated effects via grazing *A. triseriatus*?

Processing chain effects

The most direct evidence for processing chain effects arises when detritus generated by predators produces effects on microbial growth in the absence of intervening bacteriovores (prey) (Costa and Vonesh 2013). Experiment I shows that bacterial abundance increased from day 7 to day 14 in the infusion-alone controls (IA, Fig. 1). In contrast, when we added predation cues (IC), bacterial abundance was significantly enhanced at 7 days by a factor of about 2 relative to IA (Fig. 1) and showed no further significant increase at 14 days (Fig. 1). Thus predator cues in our experiment provided a short-term pulse of nutrients usable by bacteria that was not present

in the leaf infusion we used in these experiments. The effect of that pulse appears to wane by 14 days. In our experiment cues were only added as a single initial pulse consisting of 5 days of predation (see *Material and Methods*), whereas real predation would add cues more slowly over time. Because our preparation of cues, using the standard methods we have used for bioassays of effects of cues on *Aedes* behavior (e.g., Kesavaraju and Juliano 2004), takes place over 5 days, this input is a mix of fresh cues (from the most recent day's predation) and older cues (from earlier days of predation), just as would be the case if predation were occurring within the container. Thus, our estimate of the effect of the processing chain on bacterial productivity (i.e., IC-IA) may be an underestimate of the processing chain contribution because the pulse of cues represented fewer days of predation (5 days) than would real predation (7 or 14 days of predation), and the added cues are older (7 to 12 days old at day 7) and have been available for exploitation longer than would be the case for real predation (0 to 7 days old at day 7). We expect that steady addition of cues occurred in the real predation treatment (IPred), but that treatment necessarily includes the density mediated effects of predation on *A. triseriatus*, because prey were necessary to support the predator over the period of the experiment. Thus, the processing chain effect occurs, but appears to be of short duration, and may be greater than we estimate in this experiment. This is the first demonstration of a predator-induced processing chain effect in this system.

Trait-mediated effects

The presence of *A. triseriatus* larvae along with predator cues (IPC) led to a reduction in bacterial productivity, relative to cues alone (IC) at 7 days (IPC; Fig. 1). The addition of cues with *A. triseriatus* larvae (IPC) also led to significantly increased bacterial productivity relative to larvae alone (IP) at 14 days (Fig. 1) and the level of bacterial productivity in IPC at 14 days was equivalent to that in the treatment with real predation (IPred; Fig. 1). Thus in contrast to the processing chain effect, the trait mediated effects of predator cues seem to be more prominent later in the experiment. We postulate that this effect derives from the well documented impact of predator cues on movement and foraging by *A. triseriatus* (Juliano and Gravel 2002, Kesavaraju and Juliano 2004, 2010, Costanzo et al. 2011). That the combined effects of cues and *A. triseriatus* larvae are different at 7 and 14 days is not surprising because the sizes of larvae change greatly over this time period, with larvae at 14 days mostly in the fourth (last) instar, but at 7 days larvae were mostly in the second or early third instar. The larger larvae are likely to consume bacterial resources at a greater rate, raising their potential impact on bacterial productivity.

These results are consistent with studies showing that mosquito larvae can negatively impact microorganism abundance in detritus-based systems (Walker et al. 1991, Kaufman et al. 1999, 2001, Kneitel and Miller 2002, Trzcinski et al. 2005, Yee et al. 2007*a*), but also show that the magnitude of this effect depends on the time scale considered, and on the presence of predator cues that have been shown to affect behavior of *A. triseriatus* larvae (Juliano and Gravel 2002, Kesavaraju and Juliano 2004, 2010, Costanzo et al. 2011). Similar time-dependent effects of filter feeders on aquatic microorganisms were evident in other investigations of microbial trophic cascades (Costa and Vonesh 2013). These data suggest that our prepared predator cues add both substrates for bacterial growth (predator feces, uneaten prey) and behaviorally active cues from the act of predation that modify the feeding behavior of *A. triseriatus* larvae, as has been demonstrated repeatedly for this species (Juliano and Gravel 2002, Kesavaraju and Juliano 2004, 2010, Kesavaraju et al. 2007).

Density-mediated effects

The most striking effects of the first experiment occurred at 14 days, when the presence of *A. triseriatus* larvae depressed bacterial productivity, yet the presence of predation cues or predators eliminated this effect (compare IP, IPC, and IPred; Fig. 1). The lack of a significant difference between bacterial productivity in infusion alone (IA) and infusion + predator cues (IC) at 14 days (Fig. 1) suggests that any processing chain effects on bacterial productivity had waned by 14 days. If that is so, then the differences in bacterial productivity between these three treatments (IP, IPC, and IPred) at 14 days are evidence for some

combination of trait-mediated behavioral trophic cascades caused by predator cues reducing *A. triseriatus* foraging, and density-mediated trophic cascades caused by reduction in *A. triseriatus* density via predation. If both effects are occurring they appear to be non-additive, as bacterial productivity did not differ between IPC and IPred (Fig. 1). Complicating this interpretation is the significant and large difference in surviving larvae at 14 days, when predator cues (IPC) yielded about 3 times the number of surviving larvae than did real predation (IPred; Fig. 2) and less than half the number of surviving larvae of the prey-only treatment (IP; Fig. 2). This effect of cues on survival of *A. triseriatus* was not expected and experiment I was not designed to test for mechanisms that may produce mortality of larvae in the IPC treatment. We postulate that this mortality effect results from the feeding reduction induced by predator cues (as shown by Juliano and Gravel 2002, Kesavaraju and Juliano 2004, 2010, Kesavaraju et al. 2007, Costanzo et al. 2011) but this remains to be tested. The similarity between IPC and IPred treatments in bacterial productivity at 14 days could be a complex product of a greater density-mediated effect in IPred, with fewer survivors, and greater processing chain effects in IPC resulting from larval mortality without consumption by the predator. In IPC all carcass biomass was available to bacteria, whereas in the IPred treatment, some biomass was assimilated by the predator; this resulted in similar bacterial abundances among the two treatments at the time of sampling. Because the experiment was run for only two weeks, missing larvae all resulted from death rather than pupation and eclosion of adults. Thus we have evidence in experiment I for all three types of effects on this predator-bacterivore-bacteria trophic cascade, but experiment I cannot resolve the relative contributions of density-, trait-, and processing-chain-mediated effects on this trophic cascade.

In experiment II we attempted to keep the density-mediated effects of *A. triseriatus* constant by using simulated predation treatments removing prey at a rate equal to average predator consumption in the experiment. We were successful, in that the real (IPred) and simulated (IPCr, IPCrR) predation treatments did not differ in *A. triseriatus* survival at 5 or 10 days (Fig. 4). Thus, in experiment II we are better able to separate the density-mediated effect from the trait-, and processing-chain-mediated effects of predation on the trophic cascade. Treatments differed in microbial productivity only at day 10, not at day 5 (Fig. 3). Crushed prey (IPCr), or crushed and removed prey (IPCrR) produced increased microbial productivity relative to prey alone (IP), and yielded virtually identical levels of productivity (Fig. 3). This suggests that the presence of prey carcasses (IPCr) had no detectable effect on microbial productivity, suggesting that insect carcasses had little impact on the trophic cascade. In contrast, real predation (IPred) also increased microbial productivity, not only relative to IP, but also relative to IPCr and IPCrR. Because mortality, and therefore density-mediated effects, were similar for all three treatments (Fig. 4), this result suggests that the differences between IPred and IPCr and IPCrR arise either because predation by *T. rutilus* produces more effective predation cues yielding greater shift in *A. triseriatus* behavior, or that processing of prey into predator feces or excretion by the predator of ammonium (Walker et al. 1991) has a greater effect on bacterial productivity than does simple mechanical killing of prey even leaving carcasses behind. Costanzo et al. (2011) showed that crushed prey left in the container (equivalent to our IPCr treatment) produced the same behavioral effect on *A. triseriatus* larvae as predator killing of twice that number of prey larvae, suggesting that if anything, real predation yields less behaviorally active cue per victim than does mechanical killing. Costanzo et al. (2011) observed behavior of *A. triseriatus* in the presence of water-borne cues, but in absence of the predator itself. Thus, another interpretation of our results is that the physical presence of a feeding *T. rutilus* larva had a greater trait-mediated effect on *A. triseriatus* feeding and bacterial suppression than did water-borne cues from killed prey, and so resulted in greater microbial productivity in the IPred treatment. Other hypotheses for the greater effect of real predation on bacterial productivity include the predator converting prey to feces that are qualitatively better (but quantitatively less, because of assimilation by the predator) microbial substrates than simply killing prey larvae, that predator excretion of ammonium enhanced

the availability of a critical nutrient such as nitrogen, in much the same way that *Aedes* may enhance nitrogen (Walker et al. 1991), or that the predator added bacteria to the water, perhaps from its gut microbiome. We cannot exclude the possibility that predator itself was perceived by prey as more threatening than the forceps used to kill prey larvae, which would enhance TMII effects in IPred. Though we cannot resolve the relative importance of density-, trait-, and processing-chain-mediated trophic cascades in this system, our experiments suggest that all three are present, and acting in complex, interactive, and time-dependent ways.

Top-down theory, in which predators regulate prey species abundance, predicts negative predator effects one trophic level below the predator and positive predator effects two trophic levels below the predator (Pace et al. 1999, Shurin et al. 2002). Trophic cacades have proved to be widespread in terrestrial and freshwater systems, (reviewed by Pace et al. 1999, Shurin et al. 2002, Schmitz et al. 2004) including containers primarily based on bacterial processing of detritus (Cochran-Stafira and von Ende 1998, Yanoviak 2001, Kneitel and Miller 2002). Density-mediated effects (DMII) are typically assumed to be the basis for trophic cascades, but there is growing evidence for the importance of trait-mediated behavioral effects (Albeny-Simões; Schmitz et al. 2004). Our results, and those of others (Schmitz et al. 2010, Costa and Vonesh 2013) suggest that processing chain effects of predation on bacterial (our results) or periphyton/phytoplankton abundance (Costa and Vonesh 2013) can also contribute to trophic cascades, at least in freshwater systems. Nutrient poor (Costa and Vonesh 2013) and relatively small systems, like the tree hole systems we investigated, seem to be those most likely to exhibit multifaceted trophic cascades influenced by density, traits, and resource processing.

Cues by which aquatic prey perceive the risk of predation can originate with predator presence, or can be created by the act of the predation (Chivers and Smith 1998). Kesavaraju and Juliano (2010) demonstrated that *A. triseriatus* larvae increased low-risk-behavior in water containing filtered solids from predation (uneaten body parts and predator feces) compared to their behavior in the absence of such solids. This result suggests that contact of *A. triseriatus* larvae with uneaten conspecific body parts (e.g., head capsules) and predator feces provided the cues to the presence of the predation threat. Our treatment with mechanically killed and removed prey (IPCrR) had no solid residues from predation, yet produced similar productivity of bacteria to mechanically killed prey left in the container (IPCr), which has been shown to induce anti-predator behavior among *A. triseriatus* larvae (Costanzo et al. 2011). We expected that these two treatments would differ primarily in the amount of dead animal matter added to the containers as carcasses; hence we expected that IPCrR would yield reduced bacterial productivity relative to IPCr, but this was not the case. This may be interpreted as indicating that most of the increase in bacterial productivity in IPCrR and IPCr arises because of TMII and DMII due to cue-induced behavioral changes in *A. triseriatus* and removal of *A. triseriatus*, respectively. Alternatively, the effect of carcasses may be reduced because *Aedes* larvae (including *A. triseriatus*) feed directly on insect carcasses and prefer feeding on insect carcasses to plant material or filter feeding when a choice is present (Merritt et al. 1992, Kesavaraju et al. 2007*b*). This behavioral trait may have resulted in preemption of insect carcasses in IPCr, yielding no detectable increase in bacterial productivity due to addition of dead larvae. This behavioral trait also suggests that the greater productivity of bacteria with real predation (IPred) derives in part from predation specifically processing *A. triseriatus* larvae into *T. rutilus* feces, which may be less attractive as a food source for surviving *A. triseriatus* larvae, and thus more likely to provide a bacterial substrate.

Effects of predators on developing mosquitoes can be counterintuitive including greater individual biomass and reduced development time for survivors (e.g., Grill and Juliano 1996, Alto and Griswold 2005) or survivorship that equals or exceeds that in the absence of predation, due to reductions in density dependent mortality (compensatory or overcompensatory effects; Juliano 2007). Usually, these kinds of effects are postulated to result primarily from density-mediated trophic cascades, with reductions in larval density via predation making greater per capita bacterial food available to survivors

(Juliano 2007). The results presented here indicate that these counterintuitive effects of predation may have multiple causes, including trait-mediated trophic cascades and processing chain effects that also contribute to greater per capita bacteria availability for survivors. Models describing mechanisms capable of producing overcompensatory mortality effects of predation (Abrams 2009) show that trait-mediated effects leading to predator-induced reduced foraging could be sufficient to yield overcompensation by prey in response to predators. The tree hole mosquito-bacteria system we investigated would be ideal for tests of these models, and for developing more complete models of the multifaceted ways in which predation can produce trophic cascades in detritus-based systems.

Acknowledgments

We thank G. F. Martins, N. Honório, R. I. Campos, V. A. Borowicz, and J. A. Breaux for useful discussion or review of the manuscript, two anonymous referees for constructive comments that helped improve this paper, and the School of Biological Sciences, Illinois State University, USA, for providing facilities. A fellowship from CNPq Brazil supported D. Albeny-Simões' visit to S. A. Juliano's laboratory. This research was supported by NIAID grant R15 AI075306-01 to S. A. Juliano.

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