

ISOLATING TOP-DOWN EFFECTS OF AQUATIC MACROCONSUMERS ON BENTHIC
STRUCTURE AND FUNCTION IN A NEOTROPICAL STREAM

by

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(Under the Direction of Catherine M. Pringle)

Macroconsumer taxa, such as fishes and crustaceans, can exert strong top-down effects on ecosystem properties and processes in streams and rivers. Previous work in montane streams in Trinidad showed that different assemblages of consumers exert different effects on benthic ecosystems. This study employed novel methodology to separate out effects of individual consumer taxa, using diurnally- and nocturnally- active electric fields. We found that one species in particular, the guppy (*Poecilia reticulata*) exerted both direct and indirect top-down effects, directly slowing rates of algal accrual via direct consumption of benthic biofilm material, while indirectly speeding up the process of leaf decomposition, likely due to an interruptive effect on a trophic cascade that occurs between an insectivorous fish, *Rivulus hartii*, a shredding invertebrate, *Phylloicus hansonii*, and the decay rate of leaf litter. This study helped to further our understanding of ecological-evolutionary interactions *in situ*, using guppies as a model organism.

INDEX WORDS: top-down effects, macroconsumer, guppy, *Poecilia reticulata*, trophic cascade, biofilms, leaf decomposition, Trinidad, Neotropical streams

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The top-down control that consumer taxa exert on their basal resources is of fundamental importance in understanding the functioning of ecosystems (e.g. Hairston et al. 1960, Oksanen et al. 1981); a single species of consumer has the potential to strongly affect both structure and function in lotic ecosystems (e.g. Flecker 1996, Taylor et al. 2006). Hairston et al. (1960) introduced the idea of the Green World Hypothesis, which proposed that primary producers dominate many ecosystems because top predatory consumers limit the consumptive effects of herbivores. In lotic systems, macroconsumer fauna can also exert direct top-down control on primary producers. Shurin et al. (2002) showed that the top-down effects of top consumers on primary producers were generally stronger in aquatic ecosystems than in terrestrial ecosystems. A meta-analysis of herbivory effects on stream primary production suggested that large-bodied macroconsumer taxa, such as fishes and crustaceans, ultimately exerted the stronger effects on the biomass of algal communities in streams than invertebrate grazers (Feminella and Hawkins 1995). Accordingly, freshwater shrimp assemblages in the Neotropics can significantly control algal communities. Pringle and Blake (1994) found that Atyid shrimp communities significantly impacted primary producers, reducing standing crops of algae and algal biovolumes along with organic and inorganic sediments, while a subsequent study showed the strong effects of freshwater shrimps in controlling the distribution and patchiness of algal communities within

the stream (Pringle 1996). Similar effects of shrimp communities in directly controlling and regulating algal communities as well as organic and inorganic sediment stocks were also observed in other studies (Pringle and Hamazaki 1998, March et al. 2002). Shrimps controlled the algal community in a Brazilian stream by ingesting and removing certain diatom taxa, which when combined with the associated strong effects on sediments, determined the species composition of the dominant algal taxa in the community (De Souza and Moulton 2005).

Freshwater fishes can also play a significant role in the top-down control of primary producers. In Panamanian streams, armored catfishes significantly affected the distribution of algal communities throughout the entire stream channel, with areas accessible to catfish grazing almost entirely denuded of algal growth (Power 1987). Using cage exclusion experiments in Costa Rica, Wootton and Oemke showed the prevalence of herbivory in diets of a diverse freshwater fish assemblage, and the significant top-down effects of fish herbivory on aquatic macrophytes and periphyton (Wootton and Oemke 1992). Flecker (1996) showed that a detritivorous fish species was functionally dominant in tropical streams in the Orinoco basin, regulating the species composition of algal communities by exerting strong top-down effects on sediments and stream benthic environments, resulting in significant reductions in diatoms as components of the algal community. The top-down effects of consumer taxa were found to be stronger in influencing algal biomass and community composition than bottom-up effects such as nutrient limitation in the same Orinoco system (Flecker et al. 2002). Pringle and Hamazaki (1997) showed that tropical freshwater fish assemblages in Costa Rica controlled the biomass of periphyton, algal succession and the structure of the algal community, while also increasing the stability of the algal community in response to high discharge storm events.

Headwater or high-gradient tropical streams generally have high inputs of allochthonous leaf litter, a very important basal resource when canopy cover is dense and light inputs (which function to stimulate primary productivity in many tropical systems) are low (e.g. Vannote et al. 1980). Allochthonous litter inputs are fundamentally important in maintaining the stability and diversity of food webs (Wallace et al. 1997), and the top-down effects of macroconsumer taxa on the breakdown and processing of leaf litter can be very significant on both local and reach scales.

Freshwater crustaceans have been shown to exert strong top-down effects on leaf litter in tropical streams and rivers. Atyid and xiphocarid shrimps significantly increased the decay rates of leaf litter in high-gradient tropical streams in Puerto Rico (Crowl et al. 2001, March et al. 2001). In tropical streams in Kenya, freshwater crabs rapidly shredded leaf litter, reducing stocks significantly in a matter of days (Moss 2007). Moulton et al. (2010) showed that in some Brazilian streams, macroconsumer assemblage contributed significantly more to the decay of leaf material than specialist shredding invertebrates.

Freshwater fishes in tropical streams and rivers are much more likely to feed directly on allochthonous leaf litter inputs than fishes in temperate systems (Bowen 1983, Wootton and Oemke 1992). A combined freshwater tropical fish assemblage consumed the majority of introduced allochthonous leaf litter within 48 hours in a manipulative experiment in Costa Rica (Wootton and Oemke 1992). The combined effects of omnivorous fishes and shrimps caused significant mass loss of leaf packs in lowland streams in Costa Rica (Rosemond et al. 1998).

Top macroconsumers can directly affect basal resources through feeding or foraging, but can also indirectly affect basal resources via 'cascading' effects exerted on intermediate consumers or herbivores, a phenomenon commonly known as the trophic cascade (e.g. Polis et al. 2000).

For example, Boyero et al. (2008) found that the direct (predatory) and indirect (behavior-modifying) effects of Australian rainbowfish in tropical streams in Queensland worked synergistically in significantly impacting shredding invertebrate communities, and caused declines in the processing rates of allochthonous leaf litter. Generally speaking, trophic cascades are strengthened in systems with low species diversity and simple, well-defined food webs; the presence of omnivorous species may result in weak trophic ‘trickles’ rather than strong cascades (Strong 1992). In lotic systems, omnivorous macroconsumers may ‘disrupt’ potential trophic cascades by foraging at multiple trophic levels, masking any effect of intermediate consumers on basal resources. Pringle and Hamazaki (1998) found that the omnivorous foraging of nocturnal shrimps on both basal resources and intermediate consumers precluded the existence of a trophic cascade, while Usio (2000) showed how a potential trophic cascade was decoupled due to concomitant reductions in leaf litter and leaf-dwelling invertebrate abundance as a result of the foraging of omnivorous crayfish in headwater streams in New Zealand. Accordingly, the presence of omnivores in a macroconsumer assemblage may complicate the study of top-down effects if the assemblage is assessed as a whole; the necessity of isolating species-specific top-down effects when macroconsumer omnivory is prevalent was one of the driving factors behind this study.

Stream and river systems in Trinidad are defined by diverse assemblages of fishes (Kenny 1995) and decapod crustaceans (Rostant 2005). One very well-known fish species is the common guppy (*Poecilia reticulata*), a small live-bearer that is distributed throughout lotic systems across the entire island (Kenny 1995). Guppies are omnivorous (Dussault and Kramer 1981, Palkovacs et al. 2009, Bassar et al. 2010), feeding on detritus, algae, and invertebrates,

though guppy diets differ significantly as a result of both habitat and fish assemblage structure. Guppies have been used as a model organism for the study of evolution for decades. The vast majority of this work has focused on the role of predation on guppies as a driver of evolutionary divergence, as the presence or absence of predators can significantly affect guppy phenotype (Reznick and Endler 1982, Reznick 1989) and genotype (Reznick 1982). In higher-order streams and rivers, piscivores like the pike cichlid (*Crenicichla alta*) and wolf fish (*Hoplias malabaricus*) exert strong top-down pressure on guppy populations (Reznick and Endler 1982), causing significant mortality and resulting in the development of a high-predation, or 'HP' phenotype.

Meanwhile, in low-order, high-gradient streams, piscivores drop out of the fish assemblage, and guppies share habitat primarily with the killifish *Rivulus hartii* (Seghers 1978). *Rivulus* are often found in low-order streams and pools that guppies cannot naturally access due to *Rivulus*' ability to traverse waterfalls and cross large expanses of dry land to colonize new habitat (Seghers 1978, Walsh and Reznick 2008), while the upstream migration of guppies is often limited by naturally-occurring barriers such as waterfalls (Reznick 1982, Reznick and Endler 1982). While *Rivulus* are generally insectivorous (Gilliam et al. 1993, Fraser et al. 1999), opportunistic predation on guppies may occur, resulting in a low-predation or 'LP' phenotype (Reznick and Endler 1982, Reznick 1989) when guppies and *Rivulus* occupy the same habitat. The life-history consequences for predator-driven evolution in guppy populations is profound: LP guppies have much lower rates of mortality, and consequently, higher population densities (Reznick et al. 1996, Rodd and Reznick 1997, Reznick and Ghalambor 2001), reach sexual maturity later and at larger sizes (Reznick 1982, Reznick and Endler 1982), exert lower reproductive effort as a function of reproductive allotment as well as produce fewer but larger

offspring (Reznick 1982, Reznick and Endler 1982), and demonstrate far lower occurrences of defensive behaviors such as schooling (Magurran and Seghers 1994) than HP guppies.

Meanwhile, both guppies and *Rivulus* share habitat with the ubiquitous Manicou crab, *Eudaniela garmani*, a pseudothelphusid that is widely distributed in montane streams in Trinidad, and is the only remaining decapod crustacean due to the local extirpation of amphidromous shrimps (Rostant 2005).

An examination of the literature yields many examples of evolution in experimental populations facilitated by ecological manipulation (Strauss et al. 2008). However, the magnitude and significance of interactions between evolutionary and ecological processes in natural systems and how they “feed back” to affect each other are virtually unknown (e.g. Post and Palkovacs 2009). That is the overarching goal of the NSF-FIBR (Frontiers in Integrative Biological Research) project in Trinidad, of which this study is a component. Guppies are particularly useful as a model organism in assessing these ‘eco-evo’ interactions, as previous studies have shown that guppies evolve on ecologically relevant time scales (Reznick et al. 1997, Fussmann et al. 2007), while previous manipulative experiments have shown the highly significant effects of guppy phenotypic divergence (i.e. LP versus HP) on ecosystem properties and processes in artificial stream mesocosms (Palkovacs et al. 2009, Bassar et al. 2010). Ongoing work involves introductions of HP guppies into sites containing only *Rivulus*, with careful monitoring of fish evolutionary responses and associated ecosystem-level changes as guppy populations evolve over time.

In this introductory chapter, we have reviewed the top-down effects of macroconsumers on basal resources in tropical streams. We touched on the disruptive effects of omnivory on

evaluating trophic dynamics, and the importance of separating out species-specific effects in assemblages where omnivory is prevalent in order to fully understand top-down control on basal resources. We reviewed the life history of the major focal consumer of this study, the omnivorous guppy, as well as the conceptual basis for ecological-evolutionary interactions related to guppy introductions in tropical streams in Trinidad as part of the NSF-FIBR Trinidad project. This information all leads to the major questions proposed in this study: (1) *Can we isolate species-specific, top-down effects on basal resources (i.e. primary producers, allochthonous leaf litter) in a high-gradient Neotropical stream in Trinidad?* (2) *Do guppies have strong top-down effects on basal resources, and does the presence of guppies have the potential to alter the effects of other macroconsumer taxa relative to their effects when guppies are absent?* In order to answer these questions, we first developed a method to isolate taxa-specific macroconsumer effects based on observations of their diel activity patterns. Using two connected reaches, one containing *Rivulus* and *Eudaniela*, and the other containing the aforementioned taxa and a population of guppies, we ran randomized block experiments *in situ*, manipulating the presence and absence of macroconsumer taxa based on our diel isolation method to determine the top-down effects of individual taxa on stream benthic ecosystem structure and function. Our results showed both the magnitude and direction of individual macroconsumer taxa in both reaches, and allowed us to examine the ecosystem-level effects of guppy introductions post-evolution, and compare them to ecosystem structure and function in a guppy-free site. The *in situ* nature of our results can serve as a benchmark for future work to evaluate the ‘endpoint’ of the effects of guppy evolution on ecosystem structure and function, allowing for comparative study of our results not only to *ex situ* experiments examining guppy eco-evo interactions, but also in the context of ongoing work evaluating interactions between

evolutionary and ecosystem processes in recently introduced guppy populations in experimental streams in the same watershed.

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CHAPTER 2

EXPERIMENTAL QUANTIFICATION OF GUPPY (*POECILIA RETICULATA*) EFFECTS
ON ALGAL BASAL RESOURCES, *IN SITU*, REVEALS CONTEXT-DEPENDENT
EFFECTS OF OTHER MACROCONSUMERS¹

¹Binderup, A.J., Pringle, C.M., Marshall, M.C., Thomas, S.A., Flecker, A.S., El-Sabaawi, R.W., López-Sepulcre, A., and D.N. Reznick. To be submitted to *Ecosphere*.

Introduction

A major challenge in understanding food web dynamics in natural systems is distinguishing between effects of specific consumer species within a given assemblage, and quantifying species-specific effects, *in situ*. The top-down effects of one particular species may be functionally important (and/or dominant) in determining trophic dynamics and associated ecosystem properties and processes. While several studies have effectively isolated species-specific effects within an assemblage (Terrestrial references to be added) (Flecker 1996, Taylor et al. 2006), it remains a challenge to isolate and quantify species-specific effects *in situ* given the confounding effects of other species and potential artifacts of experimental manipulation.

This study is part of a collaborative project funded through the National Science Foundation's FIBR (Frontiers in Integrative Biological Research) Program. The larger project focuses on guppies (*Poecilia reticulata*) as a model for evaluating the strengths of interactions between evolutionary and ecological processes and their concomitant feedbacks. Guppy evolution occurs at ecologically relevant time-scales, making guppies well suited for the study of *eco-evo* interactions (Reznick et al. 1997, Fussmann et al. 2007). In Trinidad, guppies are an abundant macroconsumer species, widely distributed in lotic systems, from high-gradient, low-order streams to the main channels of large rivers (Kenny 1995).

We focused on *in situ* experimental isolation of the top-down effects of the common guppy from the rest of the macroconsumer assemblage, and quantification of the top-down effects of this species on algal standing crop and algal accrual rates. Our *in situ* experiments build on previous *ex situ* studies (Palkovacs et al. 2009, Bassar et al. 2010) that were conducted in artificial stream mesocosms. While mesocosm studies showed that guppies reduce algal standing crop, no studies have yet isolated and quantified guppy-specific effects in the natural

stream system. Do guppies reduce rates of algal standing crop accrual in nature and, if so, does guppy presence or absence determine context-specific effects of the rest of the macroconsumer assemblage?

Here we build on this previous research by attempting to experimentally isolate the direct, top-down effects of guppies from the rest of the macroconsumer assemblage in a natural system. We ran manipulative experiments in two connected stream reaches characterized by different yet overlapping macroconsumer assemblages: an upstream reach dominated by a killifish (*Rivulus hartii*) and pseudothelphusid crab (*Eudaniela garmani*) naïve to guppy presence, and a downstream reach containing guppies plus the aforementioned killifish and crab, *coevolved* with guppies.

We addressed the following two questions: (1) Can we isolate the effects of diurnally-active guppies from the rest of the consumer assemblage by manipulating their diel access to the stream bottom, thereby separating and quantifying species-level effects on algal accrual rate and standing crop?; and (2) are the combined top-down effects of *Rivulus* and *Eudaniela* on algal primary producers context-dependent (i.e. are they affected by guppy presence/absence and/or natural abiotic variation between stream reaches such that their combined effects are different between reaches)? Specific objectives of our study were to: (1) Isolate and quantify the effects of guppies (from the rest of the macroconsumer community) on algal accrual, algal standing crop, AFDM, and associated invertebrate communities by manipulating the presence and absence of all 3 macroconsumer species to replicated areas of the stream bottom based on their diel activity patterns; and (2) To quantify the combined top-down effects of coevolved (with guppies) versus naïve (no guppies) *Rivulus* and *Eudaniela* on the same parameters. We predicted that guppies would exert strong top-down effects on algal communities, resulting in direct

reductions in algal accrual rate and algal standing crop, and that these effects would be stronger than the effects of both coevolved and naïve *Rivulus* and *Eudaniela*.

Quantifying the effects of guppies on algal communities as well as the combined effects of both naïve and coevolved *Rivulus* and *Eudaniela* will, for the first time, allow us to determine if guppies exert direct control on algal standing crop and accrual in *natural systems*, and if guppy introduction or invasion can potentially alter the top-down effects of killifish and crabs on these parameters. This information will allow us to more accurately predict both the magnitude and direction of ecosystem-level effects of guppy introductions, and will inform future research linking ecosystem processes to guppy evolution in Trinidadian streams.

Methods

Site description

Our experiments were conducted in the mountainous Northern Range of Trinidad in the Caigual, a second-order headwater stream and tributary of the Guanapo River. We used two connected reaches of the Caigual, with similar, simple macroconsumer assemblages separated by barrier waterfalls. For ease of description, the reaches were abbreviated based on the dominant macroconsumer taxa present: the upstream reach was the **RE** (*Rivulus* + *Eudaniela*) reach, while the downstream was the **REP** (*Rivulus* + *Eudaniela* + *Poecilia*) reach. Both study reaches were heavily forested and had abundant canopy cover, while stream benthic substrate was dominated by a combination of gravel and cobble, with some boulders found in riffles. The measured physicochemical characteristics were very similar between our chosen replicate pools (Table 2.1), with the exception of canopy cover, which was approximately twice as open on average in the **REP** reach.

Experimental Design

We used an electric exclosure technique based on Pringle and Blake (1994) and modified from Connelly et al. (2008) to exclude macroconsumer fauna from standardized areas of the stream benthic environment. Underwater electric fields have been used to successfully exclude macrofauna *in situ* in both tropical (Pringle and Hamazaki 1997, Moulton et al. 2004, Ranvestel et al. 2004) and temperate (Schofield et al. 2004) systems, with a major advantage of the technique being the lack of ‘cage effects’ associated with physical exclusion methods (Pringle and Blake 1994). Exclosure quadrats were constructed of uninsulated 8-gauge copper wire shaped into two nested rectangles (outer rectangle: 25cm x 50cm, inner rectangle: 8cm x 30cm) and connected using plastic cable “zip” ties. Quadrats were connected to Speedrite 1000 electric fence chargers (TruTest Group, Amarillo TX) and were powered by rechargeable 12V batteries (which were replaced as they depleted in the field), resulting in an electric field output pulse strength of approximately 1 joule every two seconds covering the entire quadrat area. Four quadrats were placed in pools with similar physicochemical characteristics and anchored with steel tent stakes. Five replicate pools with similar physicochemical characteristics were chosen across approximately 250m of the stream reach, and 5 complete sets of exclosures were deployed. Three of the quadrats were randomly attached to the fence chargers, with two of the three quadrats additionally wired into Flexcharge 12V real time programmable timers (Flexcharge Corporation, Charlevoix MI). This design resulted in a total of four treatment types, designed to capture the variability in diel cycles displayed by focal macroconsumers: Control (C), with no electricity; Diurnal (D), with diurnal (06:00 – 18:00) electrification; Nocturnal (N),

with nocturnal (18:00 – 06:00) electrification; and Electric (E), with complete electrification (24 hrs./day).

Trinidad has a distinct dry season, with a tropical maritime climate dominating from January to May, while the wet season, characterized by a modified moist equatorial climate, dominates from June to December. In order to avoid unpredictable weather and potential confounding effects of high discharge on the stream ecosystem during the wet season, we conducted our experiments during the dry season in the **RE** reach from February 22nd – March 23rd, 2009, and in the **REP** reach from April 16th – May 13th, 2009 (Table 2.1). Temporally distinct experiments were required due to logistic limitations, and we assumed stable populations of macroconsumers in both reaches due to the stable weather and lack of stochastic events that could potentially ‘reset’ the benthic environment during our experimental periods.

Macroconsumer visitation and observations

We estimated the visitation response of each species of macroconsumer via timed observation periods. We used visual point-counts over a total of twenty hrs., in four observational periods per reach: two diurnal and two nocturnal. We made timed observations in two treatments within each of five pools per reach during both night and day (the control in both nocturnal and diurnal observations paired with the nighttime electricity treatment for diurnal observations and the daytime electricity treatment for nocturnal observations). Nocturnal observations all occurred on relatively clear nights with the moon in either first or last quarter phase. We observed each accessible (non-electrified) quadrat for fifteen min., with point-counts occurring every min. We quantified the species composition of the community within the treatment area during point-counts, noting the number of each species present. The number

found in each treatment area was averaged over the fifteen-minute observational period and converted to number of visits $\text{m}^{-2} \text{hr}^{-1}$.

Biofilm analysis

We determined biofilm responses to the effects of macroconsumers by measuring algal standing crop accrual through time on 5cm x 5cm unglazed, pre-ashed ceramic tiles as a proxy for hard benthic substrate. Ten tiles were placed inside of each experimental quadrat and were secured to quadrat frames using 19mm binder clips and plastic cables 'zip' ties. Tiles were retrieved two times during the first week, and weekly for the remainder of the experiment for a total of four weeks. We scraped tiles with a steel wire brush and rinsed them with deionized water to make a slurry of known volume, then subsampled this slurry for both chlorophyll *a* and ash-free dry mass (AFDM) (e.g. Steinman et al. 2006). Chlorophyll *a* subsamples were pipetted onto a 25-mm diameter Pall AE glass fiber filter and AFDM subsamples onto a pre-ashed, pre-weighed 47 mm diameter Whatman glass fiber filter. We used a 24-hour incubation in 90% ethanol at room temperature to extract chlorophyll *a* from filters, and then measured fluorescence on replicated aliquots of the extract subsample using a Turner Aquafluor handheld fluorometer fitted with a chlorophyll-specific wavelength channel. Algal accrual rate was determined by examining the exponential phase of accrual over the first 21 days (e.g. Biggs 1996), until an asymptote was reached. Chlorophyll *a* values for the first 21 days were log-transformed and regressed against incubation day with the slope of this regression estimating the rate of accrual. We used chlorophyll *a* standing crop values at Day 21 in our analyses to directly correspond with the asymptotic, or 'peak' algal accrual and to avoid underestimating algal standing crop due to algal sloughing that could potentially occur after a peak has been reached.

Benthic invertebrates

We sampled benthic invertebrates within each quadrat area on the final day of the experiment in both reaches using a stovepipe sampler and a dip cup (e.g. Merritt et al. 2008). Samples were dyed with Rose Bengal stain, picked, and sorted, and all invertebrates greater than 250 μm in length were counted and identified using taxonomic keys by Merritt et al. (2008) and Perez (1988). We used length-mass regressions (Benke et al. 1999, Becker et al. 2009) to generate biomass values for insects and volume-mass regressions (Feller and Warwick 1988) for non-insect invertebrates. Both Diptera and Chironomidae were analyzed separately in addition to the entire assemblage due to their high abundance in guppy guts (Bassar et al. 2010).

Statistical analysis

We used *a priori* contrasts in a mixed-effects linear model to examine the effects of macroconsumers in both reaches. Pool was assigned as a random effect to account for inter-pool variability, while treatment was a fixed effect. Our four diel exclusion manipulation treatments, Control (C), Diurnal (D), Exclusion (E), and Nocturnal (N), were combined to yield three contrasts per reach. The effect of all macroconsumers in each reach was obtained by contrasting C treatment versus all other treatments (D,E,N). In the **RE** reach, we contrasted CN versus ED as a generalized effect of diurnal *Rivulus* activity, while CD versus EN yielded the nocturnal effects of *Rivulus* and *Eudaniela*. In the **REP** reach, we contrasted CN versus ED as an effect of strongly diurnal guppies, while CD versus EN yielded nocturnal effects of *Rivulus* and *Eudaniela*. Natural inter-reach variation was examined with one-way analysis of variance (ANOVA) of response variables in full exclusion (E) treatments (which exclude all macrofauna) using reach as a fixed factor, followed by *post hoc* Tukey-Kramer HSD tests. Finally, the

differential effect sizes of macroconsumer taxa on ecosystem response variables were examined both between and within reaches by calculation and comparison of Cohen's d index values based on F-statistics (e.g. Cohen 1988, 1992). All statistical analyses were performed using using JMP 8.0.2 (SAS Institute 2009) and figures were made using SigmaPlot 10 (Systat Software 2008).

Results

Diel patterns in macroconsumer activity

Guppies displayed significant (One-tailed t-test, $p < 0.01$) diurnal activity patterns, and guppy activity was observed much more frequently than the activity of any other taxa, over four times more frequently on average than the next most active macroconsumer (Figure 2.1). Coevolved *Rivulus* in the **REP** reach were significantly (1-tailed t-test, $p < 0.05$) more nocturnal than diurnal (Figure 2.1), while *Eudaniela* in both reaches were significantly ($p < 0.05$) more nocturnal than diurnal; observations of *Eudaniela* during daytime hours in both reaches were consistently less than 1 observed visitation $m^{-2} hr^{-1}$ (Figure 2.1). In the **RE** reach, naïve *Rivulus* showed no significantly distinct preference for either diurnal or nocturnal activity, with a nonsignificant trend toward increased nighttime activity (Figure 2.1). Naïve *Eudaniela* activity was observed at a 6-fold greater rate in the **RE** reach versus the **REP** reach (Figure 2.1), suggesting that *Eudaniela* populations reach higher densities and/or behave differently in the absence of guppies. The catfish *Rhamdia quelen*, which was present in very low densities in the **REP** reach, was never observed entering or interacting with any of treatment quadrats during observational periods (see Figure 2.1), but was rarely sighted at night in some of the reach's deepest pools, away from the shallower areas where we deployed our experimental quadrats.

Isolating macroconsumer effects

Guppies dominated the daytime assemblage in the **REP** reach (close to 30 visitations $\text{m}^{-2} \text{hr}^{-1}$) and were virtually inactive at night (Figure 2.1), while crabs were almost entirely nocturnal, and coevolved *Rivulus* were 2.5-fold more active at night versus day (Figure 2.1). We were able to successfully isolate the diurnal effects of guppies from the effects of coevolved *Rivulus* and *Eudaniela* using our diel exclusion method. *Rivulus* and *Eudaniela* were both nocturnally active in both reaches (see Figure 2.1), and consequently, we could not separate their species-specific effects based on diel activity in the same manner we used for guppies. Instead, we quantified their combined effects in both reaches. Daytime effects of **RE** *Rivulus* were examined by contrasting diurnal access treatments to diurnal exclusion treatments, while the nocturnal effects of both *Rivulus* and *Eudaniela* were examined by contrasting nocturnal access to nocturnal exclusion treatments (see Table 2.2). For the purposes of evaluating the total combined effects of naïve *Rivulus* and *Eudaniela* in the **RE** reach (and for subsequent comparison to the top-down effects of coevolved *Rivulus* and *Eudaniela* in the **REP** reach), we contrasted the whole assemblage (i.e. control treatment, both diurnal and nocturnal effects of naïve *Rivulus* and *Eudaniela*) to all exclusion treatments.

Specific effects of guppies

Guppies directly affected algal communities in the **REP** reach. Guppies significantly ($F_{1,12} = 21.99$, $p = 0.001$, Table 2.2) reduced algal accrual rate from $1.63 \text{ mg chl } a \text{ m}^{-2} \text{ day}^{-1}$ to $1.07 \text{ mg chl } a \text{ m}^{-2} \text{ day}^{-1}$ (Figure 2.2a), and also significantly ($F_{1,12} = 21.36$, $p = 0.0001$, Table 2.2) decreased algal standing crop from $39.98 \text{ mg chl } a \text{ m}^{-2}$ to $23.22 \text{ mg chl } a \text{ m}^{-2}$ (Figure 2.2b). Ash-free dry mass (AFDM) was not significantly affected by guppies ($F_{1,12} = 2.42$, $p=0.14$) but

decreased from 234.2 g m⁻² to 167.9 g m⁻² (Figure 2.2c). Guppies had no significant top-down effects on benthic invertebrate abundance, benthic invertebrate biomass, or chironomid abundance and biomass (Table 2.2) were found, and no guppy effect trends were observed (Figure 2.2d – 2.2f).

Effects of Rivulus and Eudaniela

In the presence of guppies, total combined effects of coevolved *Rivulus* and *Eudaniela* did not significantly affect any of the measured ecosystem response variables (Table 2.2). In the **RE** reach (where guppies were not present) the total combined effects of naïve *Rivulus* and *Eudaniela* significantly ($F_{1,12} = 4.77$, $p = 0.049$) reduced algal accrual rate from 1.02 (± 0.24) mg chl *a* m⁻² day⁻¹ to 0.58 (± 0.13) mg chl *a* m⁻² day⁻¹ (Table 2.2, Figure 2.3b), but did not affect algal standing crop (Table 2.2, Figure 2.4b). *Rivulus* and *Eudaniela* also exerted a marginally significant ($F_{1,12} = 4.53$, $p = 0.055$) effect on tile AFDM, reducing tile AFDM from 47.62 (± 9.17) g m⁻² to a mean of 20.15 (± 7.58) g m⁻².

Comparing effects of macroconsumers

We examined effect size (expressed as Cohen's *d*) to compare three top-down effects: (1) guppies, (2) coevolved *Rivulus* and *Eudaniela*, and (3) naïve *Rivulus* and *Eudaniela*. Guppies had the largest effect size with regards to algal accrual rate, nearly triple the effect size of coevolved *Rivulus* and *Eudaniela*, and nearly double the effect size of naïve *Rivulus* and *Eudaniela* (Figure 2.5). Guppies also had the largest effect size with regards to algal standing crop, over twice as large as the effect size of coevolved *Rivulus* and *Eudaniela*, and over ten-fold larger than the effect size of coevolved *Rivulus* and *Eudaniela* (Figure 2.5). Naïve *Rivulus* and

Eudaniela exerted the largest effect on tile AFDM, which decreased two-fold in the presence of guppies (Figure 2.5).

Assessment of natural inter-reach variation

We examined differences between the total exclusion (E) treatment in **RE** and **REP** to quantify natural variation in stream benthic structure and function between reaches independent of our experimental manipulation. Algal accrual rate ($1.76 \text{ mg chl } a \text{ m}^{-2} \text{ day}^{-1}$ vs. $1.02 \text{ mg chl } a \text{ m}^{-2} \text{ day}^{-1}$), mean algal standing crop ($39.98 \text{ mg chl } a \text{ m}^{-2}$ vs. $15.08 \text{ mg chl } a \text{ m}^{-2}$), and mean tile biofilm AFDM (277.3 g m^{-2} vs. 38.31 g m^{-2}) were all significantly ($p < 0.05$) higher in **REP** relative to the **RE** reach (Table 2.3). No inter-reach differences were observed for any of the invertebrate response variables.

Discussion

In this study, we successfully separated out the top-down effects of guppies *in situ* by manipulating their presence and absence to the stream bottom based on guppy-specific diurnal behavior. While we could not separate out species-level effects of *Rivulus* and *Eudaniela*, we did successfully quantify their combined effects both in the presence and absence of guppies. Here we showed for the first time that guppies exert strong, direct top-down control on algal accrual and standing crop in a natural system, and that the combined top-down effects of *Rivulus* and *Eudaniela*, (which significantly decreased algal accrual rate in the reach when *Rivulus* and *Eudaniela* are naïve to guppy presence) have no significant top-down effects on algal accrual when these two organisms are coevolved with guppies.

Effects of guppies

The top-down effects of guppies on algal accrual rate, standing crop, and AFDM can be attributed to direct foraging on benthic and epilithic biofilms, a behavior we observed directly in the field (Binderup, *unpublished data*) that has also been observed in other studies (e.g. Dussault and Kramer 1981). Another line of evidence of direct guppy foraging/ingestion of algae is the high-proportion of algae and detritus relative to other food items in the guts of guppies adapted to high-gradient, piscivore-free environments such as our study reaches (Palkovacs et al. 2009, Bassar et al. 2010). Moreover, guppies that are not subjected to high levels of predation by piscivores typically reach high population densities and experience very low mortality (Rodd and Reznick 1997, Reznick and Ghalambor 2001); foraging pressure on algal and detrital basal resources likely increases with population density, contributing to the strength of top-down guppy effects observed in this study.

Our *in situ* study showed guppy effects that were in the same direction as guppy effects measured in *ex situ* studies. Bassar et al. (2010) found that top-down effects of guppies in artificial stream mesocosms significantly reduced algal standing crop. In a similar *ex situ* mesocosm study, Palkovacs et al. (2009) also found that the top-down effects of guppies from piscivore-free environments significantly reduced both algal accrual rates and algal standing crop. However, the effect size of guppies on algal standing crop described by Bassar (2010) was over ten-fold greater than the guppy effect size observed in our study (see Figure 2.5). *Ex situ* isolation of guppy-specific effects may overestimate their magnitude relative to what occurs in a natural system. We attribute the strength of the observed effects in *ex situ* studies to the relative homogeneity of artificial mesocosms; guppy density, light, and flow rate were all directly regulated, and all mesocosm channels were equal in area. *In situ* estimation of top-down guppy

effects places them in the context of natural variation and pool-specific heterogeneity, important criteria when considering how top-down effects in natural systems “scale up” to a reach or whole ecosystem. Greathouse et al. (2006) found that small-scale local manipulations excluding shrimps from small areas of tropical stream bottoms accurately predicted both the direction and magnitude of shrimp extirpations in Puerto Rico when compared with sites above dams where shrimps had actually been fully extirpated. Connelly et al. (2008) found that small-scale local manipulations excluding tadpoles accurately predicted the direction of the effects of tadpole extirpation in Panamanian streams, but underestimated the effect magnitude relative to streams where tadpoles were extirpated by disease. We recognize the difficulties in scaling up the effects of localized experimental manipulation to reach or ecosystem scales, and suggest that caution be used when evaluating magnitudes of effects both *ex situ* and *in situ*; complementary examination of both types of studies may enable a greater understanding of the underlying mechanisms behind quantified macroconsumer effects.

While guppies clearly exerted direct top-down effects on algal accrual rate and standing crop, they may have also exerted indirect, bottom-up effects on algal communities. Algal accrual rates as well as baseline standing crops of algae (measured in exclusion treatments) were significantly higher in the **REP** relative to the **RE** reach (Table 2.3). Palkovacs et al. (2009) showed that in experimental mesocosms, guppy excretion increased nitrogen concentrations seven-fold and phosphorus concentrations nine-fold compared to the increases in nutrient concentrations due to *Rivulus* excretion. It is possible that guppies, which were present only in the **REP** reach, excreted enough nitrogen and phosphorus relative to the concentrations found in the **RE** reach to cause baseline increases in primary productivity and hence, algal standing crop, on a reach scale, though water chemistry assays in the Caigual in the previous year’s dry season

did not show differences in stream nutrient levels between **RE** and **REP** reaches (Marshall et al. *In manuscript*).

Combined effects of Rivulus and Eudaniela

Our study strongly suggests that the significant negative combined effects of naïve *Rivulus* and *Eudaniela* on algae in the **RE** reach is due to bioturbation. The total combined effects of naïve *Rivulus* and *Eudaniela* significantly ($p < 0.05$) decreased algal accrual rates and marginally affected tile AFDM (Table 2.2, Figure 2.3b). Tropical fishes can potentially exert very strong top-down control on algae and benthic organic matter in tropical systems (e.g Power 1987, Wootton and Oemke 1992, Flecker 1997). However, *Rivulus* do not typically consume algae or detritus, with an almost entirely insectivorous diet comprised mainly of terrestrial invertebrate ‘fall-in’ and large aquatic insect larvae (Gilliam et al. 1993, Fraser et al. 1999). It is most likely that any *Rivulus* effects on algal accrual rate and tile biofilm AFDM were due to bioturbation and not feeding or foraging behavior. *Rivulus* adults tend to rest directly on the benthic substrate, and their quick, explosive displays of swimming speed when engaging in territorial displays or striking at prey items coupled with their large body size (relative to guppies) can noticeably stir up detritus in the benthos and potentially dislodge or ‘slough’ layers of the algal community.

Naïve populations of *Eudaniela* showed significantly higher activity rates than *Eudaniela* coevolved with guppies (See Figure 2.1). These higher activity levels suggest that *Eudaniela* effects on algal accrual rate and tile AFDM would potentially be greater in the **RE** reach. In previous studies, decapod crustaceans have been shown to strongly affect stream basal resources in tropical streams. Shrimps can significantly reduce algal biovolumes and standing crops

(Pringle and Blake 1994). March et al. (2002) showed that tropical shrimp fauna in high-gradient island streams exerted strong top-down effects on chlorophyll *a*, algal biovolume, and AFDM. In our study system, heavy pollution in the Caroni estuary downstream of the watershed has resulted in the complete extirpation of all amphidromous shrimp fauna (Rostant 2005). However, *Eudaniela* are widespread and ubiquitous in the Guanapo drainage and surrounding watersheds, and it is possible that they have expanded both their habitat and foraging behavior to fill the functional ecological niche of extirpated freshwater shrimp taxa such as *Atya*, *Macrobrachium*, and *Xiphocaris*.

Our results strongly suggest that the combined top-down effects of *Rivulus* and *Eudaniela* are strongly context-dependent, both on the presence of guppies as well as natural variation in abiotic factors between the reaches. In the presence of guppies, the combined effects of coevolved *Eudaniela* and *Rivulus* had no significant effects on any of the measured ecosystem response variables (Table 2.2), contrary to the strong observed top-down effects of naïve *Rivulus* and *Eudaniela* on algal accrual rate in the absence of guppies. The effect size of coevolved *Rivulus* and *Eudaniela* on algal accrual rate dropped by over 80% relative to the effect of naïve *Rivulus* and *Eudaniela* (Figure 2.5). Moreover, the top-down effects of guppies on both algal accrual rate and algal standing crop did not differ significantly from the effects of the whole assemblage (Figures 2.3a, 2.3b), while the effects of coevolved *Rivulus* and *Eudaniela* did not differ significantly from full consumer exclusion treatments, illustrating the direct top-down effects of guppies and loss of *Rivulus* and *Eudaniela* top-down effects in the reach containing guppies (Figures 2.3a, 2.3b).

We propose that this context-dependency is driven by two mechanisms: (1) interreach differences in abiotic factors due to natural variation, and (2) interactions between guppies and

Rivulus negatively impacting *Rivulus* populations. Natural variation between stream reaches may confound direct statistical comparisons of treatments between reaches, but may also help explain the differential effects of naïve versus coevolved *Rivulus* and *Eudaniela* communities between the reaches. Here we discuss the natural variation between reaches and the potential effects on stream environments. In general, longitudinal effects in lotic systems may be responsible for increases in sediment bedload as the river or stream flows downstream from its source. However, the very short distance between our study reaches and relative similarities in physicochemical characteristics (Table 2.1) suggests that a river continuum explanation (e.g. Vannote et al. 1980) for the observed differences in mean benthic biofilm ecosystem responses in the absence of predators (see Table 2.4) observed between the reaches is not likely. Baseline algal accrual rate and algal standing crop were both significantly higher in the **REP** reach (Table 2.3). Algal communities in the **REP** reach received more light as the canopy openness was on average twice as high as in the **RE** reach (Table 2.1), and the accrual rate in the **REP** reach was marginally ($p = 0.059$, $r^2 = 0.18$) correlated with canopy openness (Figure 2.6b), while other algal responses were not (Figs. 2.6a, 2.6c, 2.6d). The lack of correlation between canopy and algal standing crop in the **REP** reach (Figure 2.6d) supports our earlier findings that guppies strongly controlled the standing crops of algae, while the marginally significant correlation between accrual rate and canopy suggests that light intensity may have played a role in the quick establishment of algal communities on experimental tiles in the **REP** reach. Algal standing crop and tile AFDM were positively correlated in the **REP** reach (linear regression, $r^2 = 0.76$, $p = 0.052$) suggesting that the increased organic material load may have driven increases in algal standing crop as a result of the increased surface area for algal biofilm accrual and the concentrated nutrients found in the organic material. Furthermore, the fact that algal accrual,

algal standing crop, and AFDM were significantly lower at a baseline measurement (i.e. Table 2.3) in **RE** versus **REP** may have increased the strength of naïve *Rivulus* and *Eudaniela* top-down effects that we quantified; if less deposition of organic material (i.e. AFDM) and less algal growth occurred in the **RE** reach as the experiment progressed over time, the effects of macroconsumer taxa would be markedly higher relative to effects in the **REP** reach, where algae grew more quickly and baseline AFDM levels suggested higher rates of organic material deposition in stream channels.

Despite the differences in abiotic factors between reaches, it is important to note that the demography of *Rivulus* populations changes very significantly in the presence of guppies. When occurring with guppies, coevolved *Rivulus* produce smaller, more numerous eggs as a response to guppy egg predation as well as the increased competition for food resources between guppies and *Rivulus* juveniles (Walsh and Reznick 2010). These factors influence the observed densities of *Rivulus*, which have been shown to be significantly lower in the presence of guppies in streams across Trinidad (Gilliam et al. 1993, Walsh and Reznick 2008), by as much as 300-400% (B. Lamphere, unpublished data). The high densities of naïve *Rivulus* in the **RE** reach (in the absence of guppies) and the associated increase in *Rivulus*' bioturbative effects were likely the driving forces in decreasing the rate of algal accrual and disturbing sediments and associated AFDM on the stream bottom.

A final consumer, *Rhamdia quelen*, occurs in very low densities in the **REP** reach, and was only observed at night (though not interacting with our experimental quadrats, see Figure 2.1). Though it is possible that due to their large body size, *Rhamdia* may potentially exert strong effects on the benthic environment due to foraging and bioturbation, the effects of the nocturnal assemblage in the **REP** reach was not significant with regards to all of the measured

ecosystem response variables (see Table 2.2). In streams and rivers with higher densities of *Rhamdia*, their top-down effects on invertebrate abundance and biomass would likely be more apparent as *Rhamdia* commonly consume insect larvae from stream benthic environments as a major component of their diets (Gilliam et al. 1993, Ferreira 2007).

Temporal differences between experimental manipulations have the potential to affect stream ecosystems independent of the manipulation. Our experiments were conducted in the **RE** reach from 22 February 2009 – 23 March 2009, and in the **REP** reach from 16 April 2009 – 13 May 2009 (Table 2.1). Trinidad's dry season typically begins in January, and intensifies to a peak level by late April or early May. The reduced precipitation and reduced cloud cover with concomitant increases in sunlight intensity associated with the peak of the dry season may have contributed to the higher baseline levels of chlorophyll *a* observed in the **REP** reach (Table 2.3), though, interestingly, light was not a significant covariate in any of our statistical models. While the lack of rainfall and associated decreases in stream velocity and storm flow in the stream channel may have resulted in greater deposition of organic material in the benthic environment in the **REP** reach relative to the **RE** reach, accounting for the higher stocks of AFDM (see Table 2.3) mean pool depth and size as well as measured water velocity were still very similar between reaches (Table 2.1) despite temporal differences in our experiments.

Conclusions

In this study, we successfully isolated the effects of guppies (*Poecilia reticulata*) from the combined effects of killifish (*Rivulus hartii*) and crabs (*Eudaniela garmani*) based on the diurnal activity of guppies. We compared the top-down effects of guppies on algal standing crop and accrual (and associated properties and processes) with the effects of naïve *Rivulus* and *Eudaniela*

in the **RE** reach (not accustomed to guppy presence), and coevolved *Rivulus* and *Eudaniela* cohabitating with guppies in the **REP** reach. In our *in situ* study, the combined effects of naïve *Rivulus* and *Eudaniela* significantly reduced algal accrual rates (but not algal standing crop) in the reach without guppies. However, in the reach with guppies, this significant effect was lost, and combined effects of coevolved *Rivulus* and *Eudaniela* were insignificant with regards to all ecosystem parameters. Overall, guppies exerted very strong top-down control on algal standing crop and algal accrual rates, with effects in the same direction (but lesser in magnitude) to those effects shown in previous studies conducted *ex situ* in artificial stream mesocosms (e.g. Palkovacs et al. 2009, Bassar et al. 2010), indicating that localized *in situ* experimental manipulations can effectively mechanistically assess species-specific effects in natural systems subject to abiotic heterogeneity. Measured effect sizes of guppies on algal accrual rate and algal standing crop were over 2-fold greater than effect sizes exerted by naïve killifish and crabs in the absence of guppies and over 3-fold greater than the effect sizes exerted by coevolved *Rivulus* and *Eudaniela*, further suggesting that introductions or invasions of guppies have the potential to significantly reduce algal accrual rates and standing crop in reaches natively dominated by *Rivulus* and *Eudaniela*.

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Table 2.1. Physical characteristics of both study reaches, **RE** (*Rivulus* + *Eudaniela*) and **REP** (*Rivulus* + *Eudaniela* + *Poecilia*). Canopy Openness, Water Velocity, and Water Depth are means (\pm SE) across replicate pools (n=5 per site).

Parameter	RE	REP
Experimental Period	22 Feb - 23 March 2009	16 April – 13 May 2009
Stream Order	2	2
Reach length (m)	~200	~200
Canopy Openness (%)	11.30 (1.37)	25.00 (1.78)
Water Velocity (m s^{-1})	0.10 (0.01)	0.07 (0.01)
Water Depth (cm)	25.50 (2.49)	22.90 (1.07)
Macroconsumers	<i>Rivulus</i> , <i>Eudaniela</i>	<i>Rivulus</i> , <i>Eudaniela</i> , <i>Poecilia</i> , <i>Rhamdia</i>

Table 2.2. Results of planned contrasts for macroconsumer effects in **RE** and **REP** reaches based on a linear mixed-effects model. C,D,E,N are diel treatments defined in Methods section. Numbers are p-values, while NS = not significant ($p > 0.10$). (+) or (-) indicates effect direction.

(RE) ecosystem response	<i>Total Naïve Rivulus + Eudaniela effect (C v DEN)</i>	<i>Naïve Rivulus daytime effect (CN v DE)</i>	<i>Naïve Rivulus + Eudaniela nighttime effect (CD v EN)</i>
Algal accrual rate (mg chl <i>a</i> m ⁻² day ⁻¹)	*0.049 (-)	0.09 (-)	NS
Algal standing crop (mg chl <i>a</i> m ⁻²)	NS	NS	NS
AFDM (g m ⁻²)	0.055 (-)	0.079 (-)	NS
Invertebrate abundance (m ⁻²)	NS	NS	NS
Invertebrate biomass (mg m ⁻²)	NS	NS	NS
Diptera abundance (m ⁻²)	NS	NS	NS
Diptera biomass (mg m ⁻²)	NS	NS	NS
Chironomid abundance (m ⁻²)	NS	NS	NS
Chironomid biomass (mg m ⁻²)	NS	NS	NS
(REP) ecosystem response	<i>Guppy + Rivulus + Eudaniela effect (C v DEN)</i>	<i>Guppy effect (CN v DE)</i>	<i>Coevolved Rivulus + Eudaniela effect (CD v EN)</i>
Algal accrual rate (mg chl <i>a</i> m ⁻² day ⁻¹)	*0.002 (-)	*0.001 (-)	NS
Algal standing crop (mg chl <i>a</i> m ⁻²)	*0.008 (-)	*0.0001 (-)	NS
AFDM (g m ⁻²)	NS	NS	NS
Invertebrate abundance (m ⁻²)	NS	NS	NS
Invertebrate biomass (mg m ⁻²)	NS	NS	NS
Diptera abundance (m ⁻²)	NS	NS	NS
Diptera biomass (mg m ⁻²)	NS	NS	NS
Chironomid abundance (m ⁻²)	NS	NS	NS
Chironomid biomass (mg m ⁻²)	NS	NS	NS

Table 2.3. Summary of ecosystem response variables in macroconsumer exclusion (E) treatment in both reaches. Values are means (\pm SE), n = 5 in both reaches. Different letters indicate significant mean pairwise differences between reaches (Tukey's HSD).

<i>Ecosystem response variable</i>	RE	REP
Algal Accrual Rate (mg chl <i>a</i> m ⁻² day ⁻¹)	1.02 (0.24) A	1.76 (0.20) B
Algal Standing Crop (mg chl <i>a</i> m ⁻²)	15.08 (4.75) A	39.98 (3.91) B
Ash-free Dry Mass (g m ⁻²)	38.31 (8.37) A	277.3 (47.8) B
Invertebrate Abundance (number m ⁻²)	14,126.6 (4013.9) A	13,537.1 (2729.9) A
Invertebrate Biomass (DM mg m ⁻²)	268.9 (50.33) A	740.4 (528.2) A
Chironomid Abundance (number m ⁻²)	5458.5 (3968.7) A	10,720.5 (1004.7) A
Chironomid Biomass (DM mg m ⁻²)	115.6 (38.8) A	32.5 (10.5) A

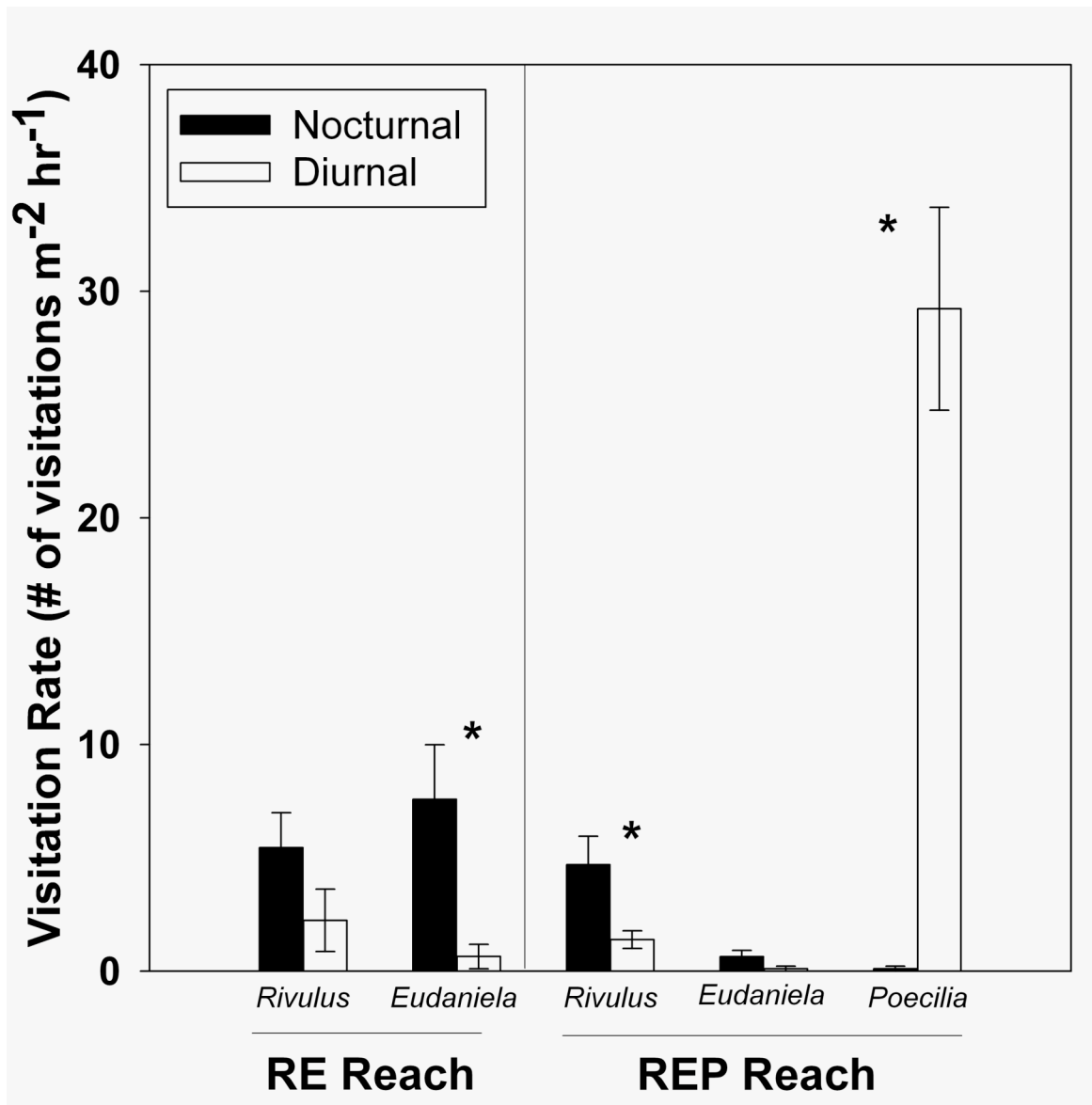


Figure 2.1: Diurnal and nocturnal visitation estimates of macroconsumers to macroconsumer access treatments in study pools in both **REP** (*Rivulus* + *Eudaniela* + *Poecilia*) and **RE** (*Rivulus* + *Eudaniela*) reaches. Diurnal estimates based on pooled visitation from Control (C) and Nocturnal (N) treatments, while Nocturnal estimates are based on Control (C) and Diurnal (D) treatments. Values are means ($n=5$) \pm SE (* = significant ($p < 0.05$) differences between nocturnal and diurnal visitation rates).

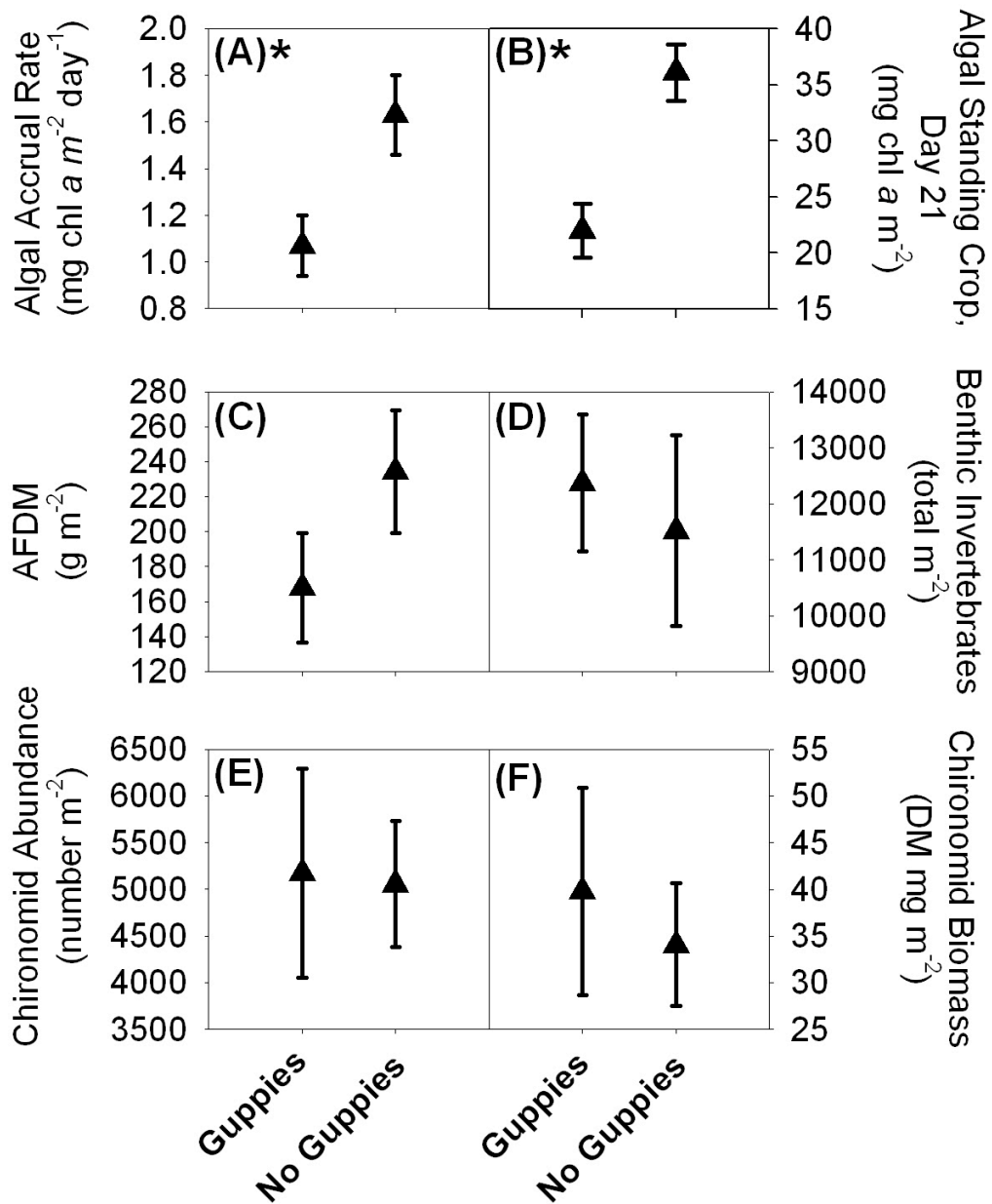


Figure 2.2. Response variable results (\pm SE) for treatments in the **REP** reach with guppies (C,N) relative to treatments without guppies (D,E). Values are means (\pm SE), $n=10$ for each mean. (*) indicates significant difference between means at $p = 0.05$ level.

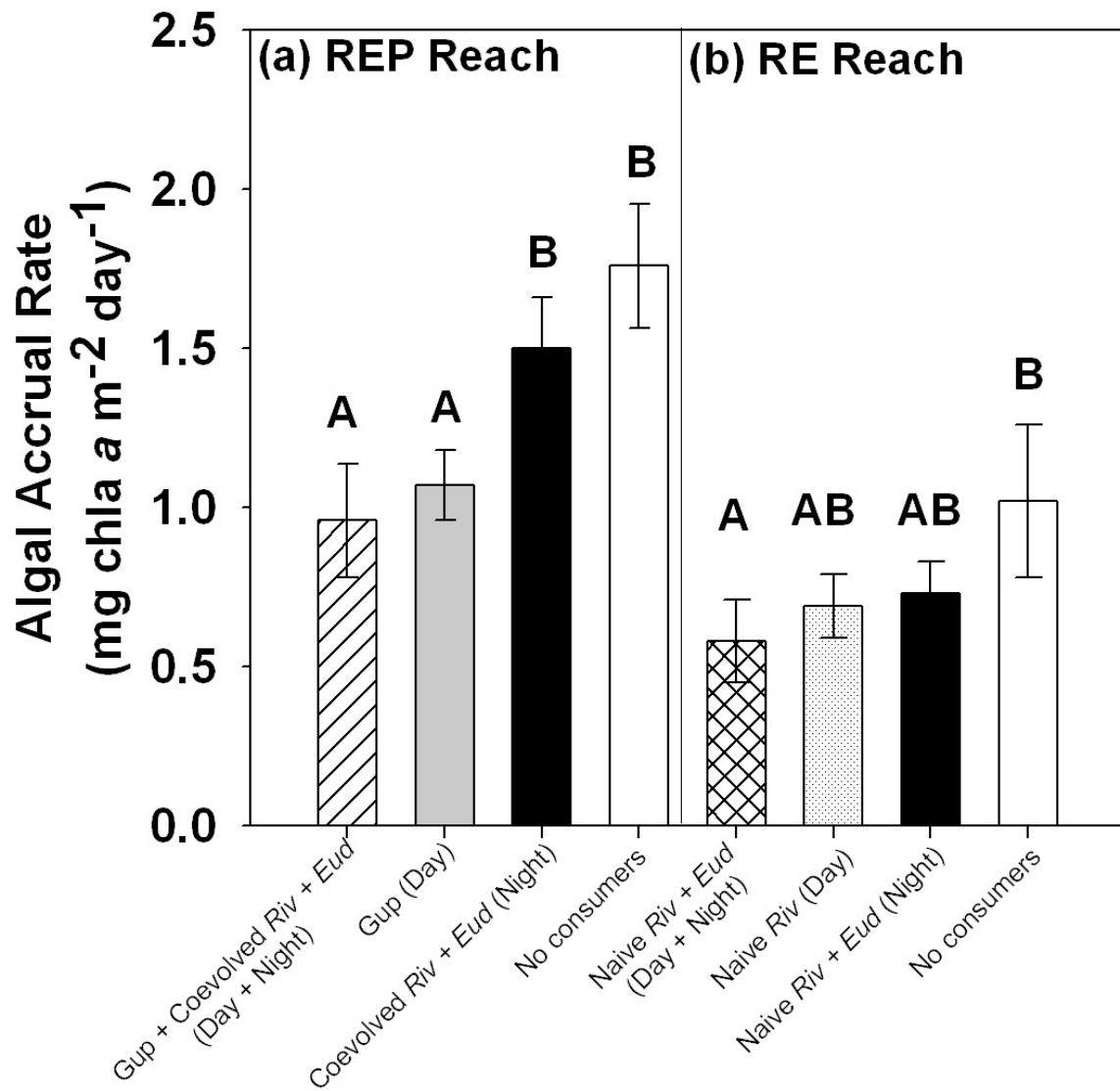


Figure 2.3. Effects of macroconsumers on algal accrual rate (\pm SE) in (a) **REP** and (b) **RE** reaches. X-axis labels indicate the specific macroconsumer effect on algal accrual rate being displayed as well as the diel type for that effect. Within reaches, bars with different letters are significantly different (Tukey's HSD).

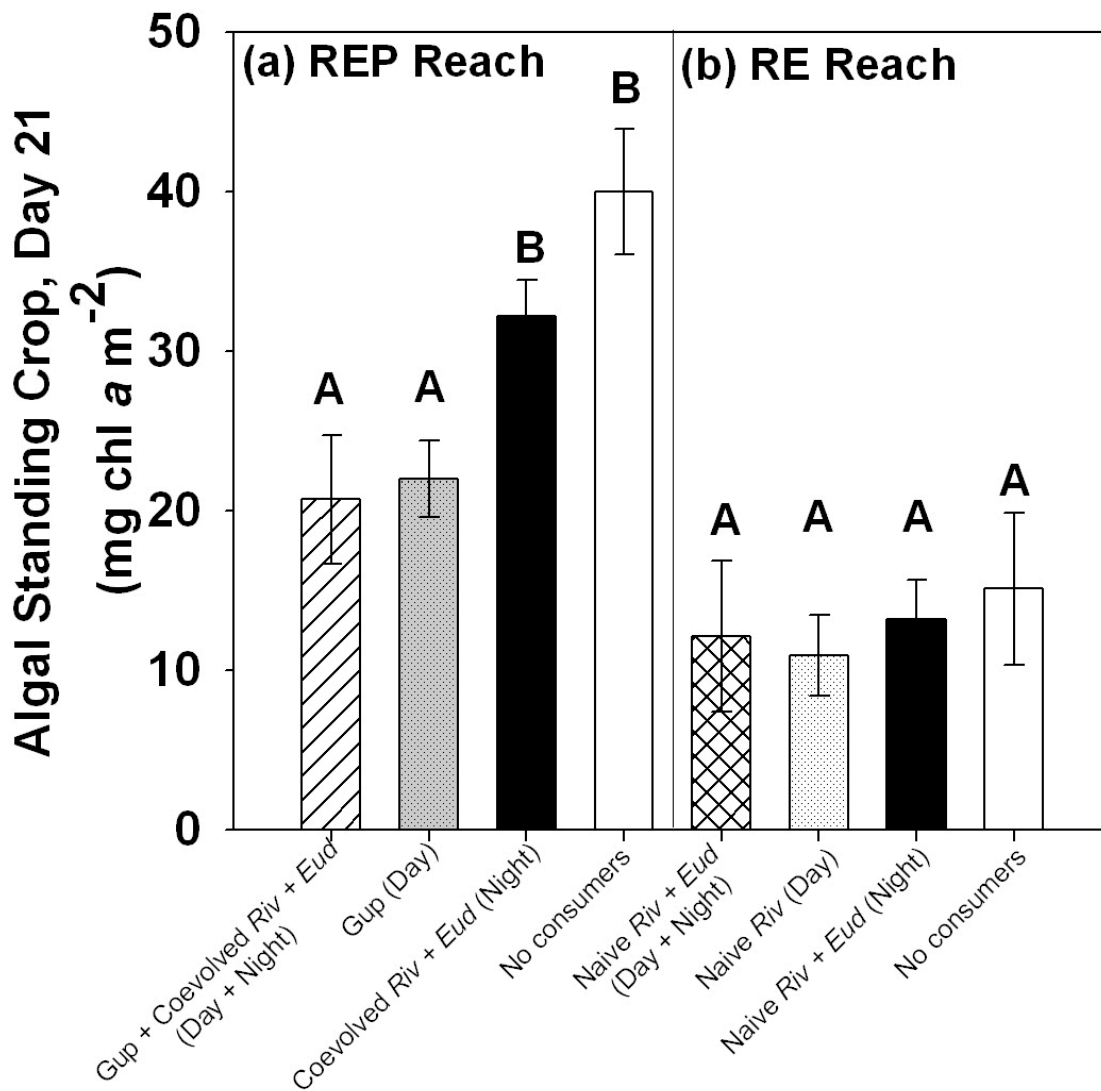


Figure 2.4. Effects of macroconsumers on algal standing crop (\pm SE) in (a) **REP** and (b) **RE** reaches. X-axis labels indicate the specific macroconsumer effect on algal standing crop being displayed as well as the diel type for that effect. Within reaches, bars with different letters are significantly different (Tukey's HSD).

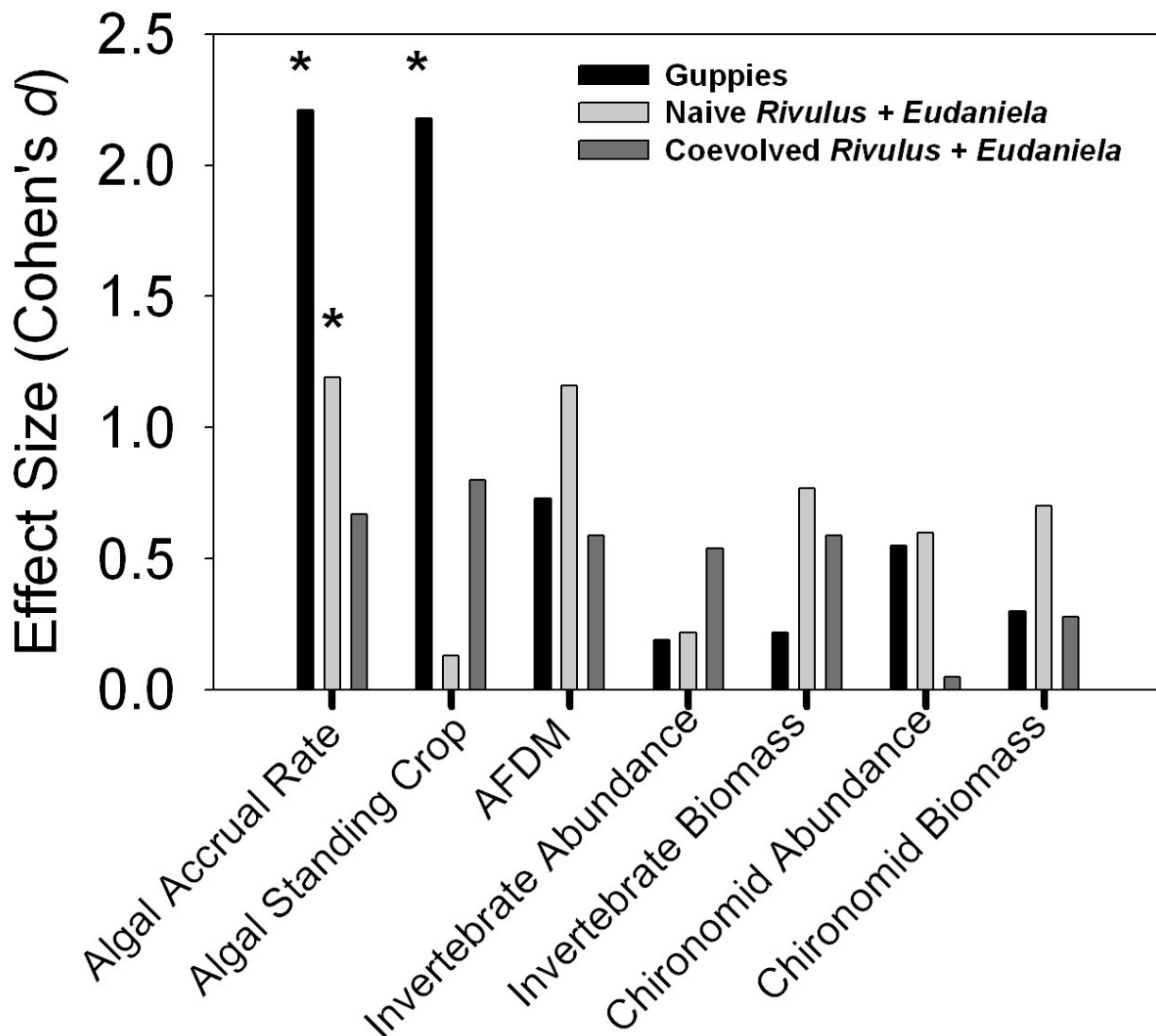


Figure 2.5. Effect size (expressed as Cohen's *d*) estimates for macroconsumers in both reaches on benthic ecosystem response variables. Black bars indicate effect sizes of guppies, light gray bars indicate effect sizes of naïve *Rivulus* and *Eudaniela* (i.e. in the **RE** reach), and dark gray bars indicate effect sizes of coevolved *Rivulus* and *Eudaniela* (i.e. in the **REP** reach). Higher values indicate a greater effect size (larger reductions in ecosystem response variable), while (*) indicates effects that were significant at the $p < 0.05$ level.

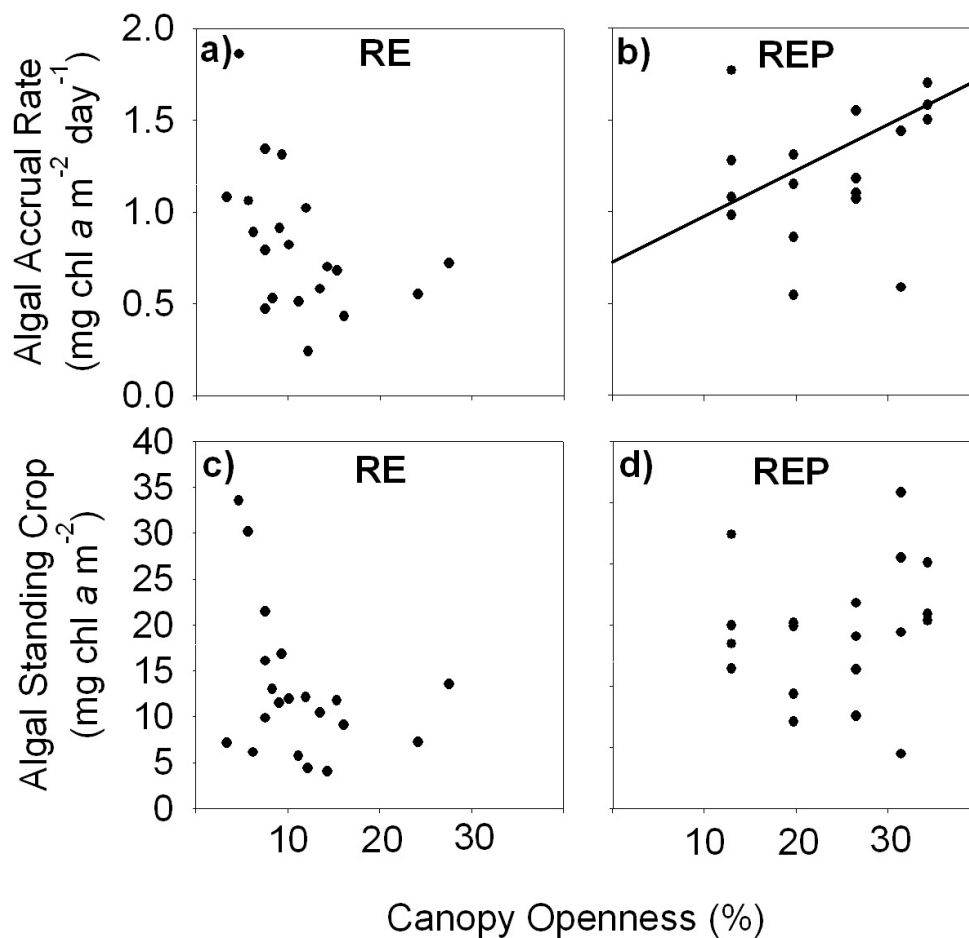


Figure 2.6. Bivariate linear regressions of algal accretion rate ($\text{mg chl } a \text{ m}^{-2} \text{ day}^{-1}$) versus overstory canopy openness, in a) **RE** reach ($p > 0.10$, $r^2 = 0.21$) and b) **REP** reach ($p = 0.059$, $r^2 = 0.18$); and algal standing crop ($\text{g chl } a \text{ m}^{-2}$) in c) **RE** ($p > 0.10$, $r^2 = 0.12$) and d) **REP** ($p > 0.10$, $r^2 = 0.02$) reaches ($n = 20$ in both reaches).

CHAPTER 3

GUPPY PRESENCE DECOUPLES A DETRITUS-BASED TROPHIC CASCADE¹

¹Binderup, A.J., Pringle, C.M., Marshall, M.C., Thomas, S.A., Flecker, A.S., El-Sabaawi, R.W., López-Sepulcre, A., Bassar, R.D., and D.N. Reznick. To be submitted to *Freshwater Biology*.

Introduction

Top-down control of basal resources by top consumers, both direct and indirect, is of fundamental importance in understanding food webs and trophic dynamics in natural systems. Predators or top consumers can regulate ecosystem structure and function (e.g. basal resources and associated processes) via effects on lower trophic levels (e.g. Hairston *et al.*, 1960, Paine, 1980), and these indirect effects often ‘cascade’ through food webs (Carpenter *et al.*, 1985, Paine, 1980). This phenomenon has been observed across terrestrial, aquatic, and marine systems, in both temperate and tropical latitudes (Pace *et al.*, 1999, Shurin *et al.*, 2002).

Here we examine both direct and indirect top-down macroconsumer effects on leaf litter (coarse detritus), an important allochthonous basal resource in lower-order, forested streams. In stream systems, much of the work regarding the indirect effects of trophic cascades has been framed in the context of top-down control of the standing crop of algal basal resources (Flecker and Townsend, 1994, Power, 1992, Power, 1990, Huryn, 1998), where the food web is based on algal primary production. In contrast, few studies have demonstrated trophic cascades in detritus-based systems (e.g. Konishi *et al.*, 2001, Oberndorfer *et al.*, 1984), and there are virtually no studies in tropical detritus-based systems (but see Boyero *et al.*, 2008). In forested headwaters, heavy canopy cover often limits primary productivity, and terrestrial leaf litter constitutes the primary basal resource of the detritus-based food web (Vannote *et al.*, 1980, Wallace *et al.*, 1997). In temperate streams, shredding invertebrates are responsible for much of the detrital processing (Wallace and Webster, 1996, Cummins, 1974, Cummins and Klug, 1979). However, in many tropical systems, leaf detritus may be directly broken down by macroconsumers such as fishes (Wootton and Oemke, 1992) and decapod crustaceans (e.g. Robertson and Daniel, 1989, March *et al.*, 2001, Nordhaus *et al.*, 2006). Several studies have

shown that in some forested tropical streams, the macroconsumer assemblage contributed more to the breakdown of leaf material than specialist macroinvertebrate shredders (Moulton *et al.*, 2010, Rosemond *et al.*, 1998). Trophic cascades may be buffered when top consumers are omnivorous, feeding on both basal resources and primary consumers (Pringle and Hamazaki, 1998); indirect effects of top consumers on basal resources (such as leaf litter or algae) mediated by consumption of primary consumers can be masked by strong, direct top-down effects.

In this study, we examined the top-down effects of two different macroconsumer assemblages, one with guppies and one without, on leaf decomposition in a stream draining Trinidad's Northern mountain range. We asked: What are the relative strengths of direct and indirect effects of macroconsumers on leaf decomposition in Trinidadian streams? In accordance with much of the published literature, we initially predicted that: (1) leaf litter decay would primarily be driven by the direct, top-down effects of macroconsumers and would be significantly elevated in areas fully accessed by macroconsumers; and (2) A trophic cascade would not be observed indirectly linking macroconsumers to the rate of leaf litter decay, due to both the effects of macroconsumer omnivory and the paucity of invertebrate shredding specialists common in Neotropical streams and rivers.

Methods

Description of the Study Site

Our experiments were conducted in the Guanapo River watershed, located in the Guanapo Valley in the Northern Range of Trinidad. We used two distinct reaches of the Caigual, a second-order headwater stream and tributary of the Guanapo River. The two reaches were approximately 200 m apart and were separated by barrier waterfalls. Reaches differed in

their macroconsumer assemblage, with the upstream reach containing the killifish, *Rivulus hartii*, and the freshwater pseudophlepusid crab, *Eudaniela garmani*, and the downstream reach with these two species plus *Poecilia reticulata*, commonly known as guppies and a very low density of the catfish *Rhamdia quelen*. For ease of description, the reaches are abbreviated based on the dominant macroconsumer taxa present; the upstream reach is designated the **RE** (*Rivulus* + *Eudaniela*) reach, while the downstream is designated as the **REP** (*Rivulus* + *Eudaniela* + *Poecilia*) reach. Both study reaches are representative of heavily forested, detritus-based headwater streams, with common riparian tree species such as *Cecropia peltata*, and *Theobroma cacao* along with the riparian shrub *Pachystachys coccinea* providing year-round shading. Stream substrate was dominated by a combination of gravel and cobble, with boulders also common in riffles, and physicochemical characteristics were very similar between replicate pools (Table 3.1), with the exception of canopy cover, which was greater in the **RE** reach.

Focal Taxa

Poecilia reticulata, the Trinidadian guppy, is an ovo-viviparous fish attaining a maximum total length of approximately 55-60 mm. They are distributed throughout Trinidad and have a long history of study on the island (Reznick, 1982, Reznick and Endler, 1982, Reznick, 1989) due to rapid evolution of their life histories under changes in predation pressure and resource availability (Reznick *et al.*, 1997). Although guppies are only native to Trinidad and Venezuela, they have been introduced throughout the world where, due to their high fecundity and ability to survive in highly variable conditions they have become invasive. Guppies are omnivorous and feed primarily on periphyton, fine detritus and small invertebrates, including chironomids and mayflies (Fraser *et al.*, 2004, Bassar *et al.*, 2010).

Rivulus hartii, commonly known as Hart's killifish is an oviparous Cyprinodontid fish with a maximum length of 85-90 mm. This species is distributed widely throughout Trinidad due to its ability to traverse waterfalls and areas of fast-moving water, as well as across expanses of dry land. *Rivulus* are also able to tolerate long periods of drought, and survive in very small bodies of water, such as rain puddles (Seghers, 1978). *Rivulus* diets are typically dominated by terrestrial and aquatic insects (Gilliam *et al.*, 1993, Fraser *et al.*, 1999). Typically *Rivulus* are found at higher densities in headwater streams when guppies are absent (Gilliam *et al.*, 1993, Walsh and Reznick, 2008).

Eudaniela garmani (Family: Pseudothelphusidae) is the only freshwater crustacean found with regularity on the South Slope of Trinidad's Northern Range and the only crustacean in the Guanapo drainage (Rostant, 2005). Sclerotized gill edges enable this organism to spend large periods of time out of the water (Diaz and Rodriguez, 1977). *Eudaniela* is an omnivorous nocturnal scavenger, often found actively foraging at night in pools (Binderup & Zandoná, unpublished data).

Experimental Design

The use of electric fields to experimentally manipulate macrobiotic assemblage *in situ* has proven effective in both tropical (e.g. Pringle and Hamazaki, 1997, Rosemond *et al.*, 1998, Pringle and Hamazaki, 1998) and temperate (e.g. Schofield *et al.*, 2004, 2008) streams. Macroconsumers were excluded using an electric exclosure technique modified from an earlier method (Pringle and Blake, 1994) and described in detail by Connelly *et al.* (2008). Exclosure quadrats were constructed using two nested rectangles made of uninsulated 8-gauge copper wire (outer rectangle: 25cm x 50cm, inner rectangle: 8cm x 30cm) connected using standard plastic cable

“zip” ties. Quadrats were connected to Speedrite 1000 electric fence chargers (TruTest Group, Amarillo TX) and powered by rechargeable 12V batteries, resulting in an electric field output pulse strength of 1 joule with a pulse rate of 30 pulses per second covering the entire quadrat area. Four quadrats were placed in pools with similar physicochemical characteristics and anchored with metal tent stakes. Three of the quadrats were randomly selected and attached to separate fence chargers, with two of the three quadrats additionally wired into 12V DC programmable voltage timers (Flexcharge Corporation). This design resulted in a total of four treatment types: Control (C), with no electricity; Diurnal (D), with diurnal (06:00 – 18:00) electrification; Nocturnal (N), with nocturnal (18:00 – 06:00) electrification; and Electric (E), with complete electrification (24 hrs./day). Five replicate pools with similar physicochemical characteristics were chosen across approximately 250m of the stream reach, and 5 complete sets of exclosures were deployed. In order to avoid unpredictable weather and potential confounding effects of the increased pressure of extremely high discharge on the stream ecosystem during the wet season, we conducted our experiments during the dry season in the **RE** reach from February 22nd – March 23rd, 2009, and in the **REP** reach from April 16th – May 13th, 2009. Temporally distinct experiments were required due to logistic limitations (manpower and equipment availability) hence our interpretation of the results assumes that populations of macroconsumers remain stable in both reaches throughout the dry season due to the absence of disturbance.

In order to test the effectiveness of our exclosures, macroconsumers (*R. hartii*, *E. garmani*, and *Poecilia reticulata*) were placed inside electrified quadrats and observed. Fishes exited quadrats immediately (2-4 seconds) after exposure to electric fields, while crabs generally exited within 30 seconds (Binderup, unpublished data). Macroinvertebrate taxa (e.g. odonates,

trichopterans >5mm) placed inside the electrified area were unaffected by the electric field and did not migrate out.

Macroconsumer Visitation Estimates

We estimated the visitation response of each species of macroconsumer to our four treatments via timed observation periods. Visitation data were collected using visual point-count assessments over a total of twenty hrs., including a total of four observational periods per reach: two diurnal and two nocturnal. Nocturnal observations were made on clear nights, with two of the observations taking place during first quarter phase, one around full moon, and one in 1st quarter phase. We made timed observations in two of the four treatments (the two that were not electrified at a given time) within each of five pools per reach during both night and day. Furthermore, electrified treatments were observed one time in each pool to check whether macroconsumer taxa were being excluded effectively. Each treatment was observed for fifteen minutes, with point-counts occurring every minute. During point-counts, we noted the number of individuals from each species present within the treatment area. These were averaged over the fifteen-minute observational period and converted to number of visits $\text{m}^{-2} \text{hr}^{-1}$.

Leaf Decomposition Rates and Invertebrates

Cecropia is a common Neotropical tree genus found across Central and South America and throughout many of the islands of the Caribbean Basin (Rickson, 1977), with leaves that tend to be high in nutrients and low in secondary compounds (Zou *et al.*, 1995). We collected freshly-abscised leaves from the locally abundant *Cecropia peltata* in the Guanapo Valley and dried them at approximately 40°C for 72 hours. Leaf packs were constructed from dried *C.*

peltata leaves collected into 5g packs that simulated natural accumulation of leaf pack material (Petersen and Cummins, 1974). Packs were held together at the base using binderclips with tags of acetate sheeting inserted into them to identify individual leaf packs. Mesh bags were not used to contain leaf pack material since we wanted to allow macroconsumer and invertebrate access (e.g. March *et al.*, 2001, Bassar *et al.*, 2010). Mesh bags can isolate leaf material from both macroconsumers and shredding invertebrates, thus leading to underestimation of their respective roles in driving leaf decomposition in tropical streams (Rosemond *et al.*, 1998). Leaf packs were attached to the copper wire quadrats using plastic zip ties, and were collected on days 4,8,15,22, and 29 using a 63 μ m mesh net placed downstream of the pack to avoid loss of invertebrates or fractionated leaf material. Packs were rinsed over a 250 μ m sieve, with all leaf particles being placed into pre-weighed paper bags, and the >250 μ m size fraction saved for quantification of invertebrate taxa. Paper bags containing leaf packs were dried at 40°C for 72 hours and weighed. To correct for leaf pack mass loss due to transport and handling, a sample of packs were constructed in an identical manner as experimental leaf packs, placed into the stream and transported back to the lab, dried, and weighed. The average DM loss in handling packs was subtracted from the initial DM of experimental leaf packs. Final leaf dry mass percent remaining was natural log-transformed and regressed with day of collection to generate *k*-values (Benfield, 2006).

All invertebrates exceeding 1mm in length were picked from each sample, counted and identified (Perez, 1988). Invertebrate abundances were normalized per gram of leaf dry mass collected with the invertebrate sample, Length-mass regressions (Benke *et al.*, 1999, Becker *et al.*, 2009) were used to generate invertebrate biomass in dry mass, which was normalized per

gram of leaf mass for each specific sample. Finally, invertebrate taxa were assigned to functional feeding groups as described by Merritt et al. (2008) and de Carvalho and Uieda (2009).

Statistical Analysis

Visitation data for diurnal and nocturnal observations were pooled separately (there were no significant differences between visitation rates to treatments in the same diel period, e.g. C and N observed during the diurnal period) and the means of these data were compared using t-tests to examine intra- and inter-reach and species-level variation in visitation rate. Leaf decay rate was first examined using analysis of covariance (ANCOVA) on natural log percentage DM remaining, using day as the covariate. Significant interaction effects led to examination of pairwise differences in decay rates (calculated as k) using Tukey-Kramer HSD *post-hoc* tests. Differences in both *Phylloicus* abundance and resource-specific biomass between treatments were determined using repeated measures ANOVA, while pairwise total abundance and biomass over the 29-day experiment were compared among treatments using Tukey-Kramer HSDs. Leaf decay rates and *Phylloicus* abundance were correlated using bivariate fit plots. All statistical analyses were performed using JMP 8.0.2 (SAS Institute 2009) on Mac OS10.5 (Apple Inc. 2008), and figures were made using SigmaPlot (Systat Software 2008).

Results

Macroconsumer Visitation

Each species displayed different levels of diurnal and nocturnal activity (Table 3.2). *Rivulus hartii* visited unelectrified treatments at a significantly higher ($p < 0.05$, one-way

ANOVA) rate during the night than day in the **REP** reach, but not in the **RE** reach. *Rivulus* were three times more likely to visit study quadrats in the **REP** reach at night than during the day (4.69 visitations $\text{m}^{-2} \text{hr}^{-1}$ vs. 1.49 visitations $\text{m}^{-2} \text{hr}^{-1}$) (Table 3.2). In the **RE** reach, *Rivulus* visitation to unelectrified treatments was uniformly spread between day and night (4.05 visitations $\text{m}^{-2} \text{hr}^{-1}$ in daytime versus 6.18 visitations $\text{m}^{-2} \text{hr}^{-1}$ in at nighttime) and diel visitation did not differ significantly ($p > 0.10$, one-way ANOVA). These results suggest that in **REP** reaches (where guppies are present) *Rivulus* are significantly more nocturnal than in **RE** reaches, where they exhibit no diel activity preference. *Eudaniela garmani* visited unelectrified treatments at a significantly higher ($p < 0.05$, one-way ANOVA) rate at night versus day in both reaches (Table 3.2), indicating primarily nocturnal activity patterns. In the **REP** reach, guppies visited accessible treatment areas nearly thirty times more often in the day versus night ($p < 0.05$, one-way ANOVA) (Table 3.2), indicating strongly diurnal activity patterns.

Leaf Decay

We found no significant differences in leaf decay rate (k_{day}) between the three exclusion treatment types (D,E,N) in either reach (Figure 3.1). Leaves decayed at a significantly faster rate (k) in all macroconsumer exclusion treatments (D,E,N) than macroconsumer access treatments (C) in the **RE** reach (Figure 3.1a, Tukey-Kramer HSD), while no significant difference in decay rate was detected between treatments in the **REP** reach (Figure 3.1b). Mean instantaneous decay rate in the **RE** reach was 1.98% day^{-1} (± 0.2 SE) in the control (C) treatment, and was 3.42% day^{-1} (± 0.68 SE) in the electric (E) treatment. Mean decay rate in the **REP** reach was 3.55% day^{-1} (± 1.2 SE) in the control (C) treatment, and 2.83% day^{-1} (± 0.35 SE) in the electric (E) treatment. No significant differences in decay rate were observed between reaches, though there was a trend

toward faster decay in the control (C) treatment in the **REP** reach relative to the **RE** reach (1-tailed t-test, $p=0.1193$) (Figure 3.1).

Invertebrate Community Composition

The invertebrate community in both reaches was similar in both total invertebrate biomass and diversity, but differed in abundance (Table 3.3). In the **RE** reach, 3406 invertebrates were collected and identified with a total resource-specific biomass (RSB) of 262.75 mg invertebrate dry mass g^{-1} leaf material. In the **REP** reach, 1502 invertebrates were collected and identified, with a total RSB of 252.27 mg invertebrate dry mass g^{-1} leaf material. In total, 15 distinct taxa were identified in the **RE** reach and 17 were identified in the **REP** reach (Table 3.3). In both reaches, the only shredder identified was the Calamoceratid caddisfly *Phylloicus hansonii*. *Phylloicus* was the single greatest contributor of all taxa to total invertebrate RSB in both reaches (Table 3.3). In both **RE** and **REP** reaches, chironomids (both Tanypodinae and non-Tanypodinae) were dominant in terms of total abundance (Table 3.3). *Phylloicus* were the third most abundant invertebrate taxa in both reaches, comprising 8.69% and 16.78% of the total invertebrate abundance in **RE** and **REP** reaches respectively (Table 3.3).

Phylloicus Biomass, Abundance, and Population Size Structure

Phylloicus resource-specific biomass (mg dry mass g^{-1} *Cecropia* dry mass) varied with treatment in the **RE** reach ($F_{3,16} = 0.604$, $p=0.0507$, repeated measures ANOVA), but treatment had no significant effect in the **REP** reach ($F_{3,16} = 0.044$, $p=0.869$, repeated measures ANOVA). *Phylloicus* normalized abundance (No. individuals g^{-1} *Cecropia* dry mass) in the **RE** reach showed a non-significant trend with regards to treatment ($F_{3,16} = 0.344$, $p = 0.18$, repeated

measures ANOVA), while *Phylloicus* abundance in the **REP** reach did not correlate with treatment ($F_{3,16} = 0.093$, $p = 0.69$, repeated measures ANOVA).

The electric (E) treatment had significantly higher *Phylloicus* biomass ($p = 0.0028$) and *Phylloicus* abundance ($p = 0.0441$) in the **RE** reach relative to the control (C) treatment but no treatment differences were observed in the **REP** reach. Both biomass and abundance of *Phylloicus* were similar between treatments in the **RE** reach for the first three sampling dates, but diverged significantly by day 15 (abundance) and day 22 (biomass). Conversely, no significant differences in resource-specific *Phylloicus* biomass or abundance were found between diurnal exclusion (D) and nocturnal exclusion (N) treatments in either reach, and furthermore, the three exclusion treatments (D, E, N) did not differ significantly in either reach.

Both total (sum over the 4-week experimental period) normalized *Phylloicus* biomass and normalized *Phylloicus* abundance were significantly higher in all three macroconsumer exclusion treatments (D,E,N) than in the control (C) treatment in the **RE** reach, but did not differ between treatments in the **REP** reach (Figures 3.2, 3.3). Total *Phylloicus* biomass was significantly higher in all treatments in the **REP** (Fig. 3.2b) reach versus the **RE** reach (Fig. 3.2a), but total *Phylloicus* abundance was not (Figure 3.3a, 3.3b), suggesting that larger *Phylloicus* individuals rather than a greater number of total number of individuals resulted in the higher biomass observed in the **REP** reach. In both **RE** and **REP** reaches, treatment-pooled leaf decay rates (k_{day}) were significantly correlated with normalized *Phylloicus* abundance (one-way linear regression, **RE** $p=0.0090$, $r^2 = 0.32$, Fig. 3.4a; **REP**: $p < 0.0001$, $r^2 = 0.75$, Fig. 3.4b)

The size structure of *Phylloicus* populations differed significantly ($p < 0.0001$) between reaches; mean *Phylloicus* length was 44.6% higher in **REP** versus **RE** reach (Figure 3.5a). Furthermore, in the **RE** reach, *Phylloicus* individuals in the control (C) treatment were

significantly ($p < 0.05$) smaller than individuals from the three exclusion treatment types (D,N,E) (Figure 3.5b), which mirrored the trends for reductions in *Phylloicus* resource-specific abundance, *Phylloicus* resource-specific biomass, and leaf decay rate measured in the control treatment in the **RE** reach.

Discussion

Examining a Novel Detritus-Based Trophic Cascade

Our results suggest that indirect macroconsumer effects in the **RE** reach facilitated a trophic cascade: specifically, that the insectivorous killifish, *Rivulus hartii*, indirectly decreased rates of leaf decay by consuming *Phylloicus hansonii*, the only shredder in the system. We predicted that macroconsumers would directly stimulate increased rates of leaf litter decomposition. While both fishes (Wootton and Oemke, 1992) and crustaceans (Robertson and Daniel, 1989, March *et al.*, 2001) can significantly enhance the rate of detrital processing, our results indicate that macroconsumer breakdown of leaf material via consumption or bioturbation (if existent) did not represent a significant contribution to leaf decay, since we did not find elevated rates of leaf decay in control relative to exclusion treatments. In the **REP** reach, no significant differences in decay rate were found between treatments, while in the **RE** reach, controls allowing for 24-hour macroconsumer access actually had the slowest rates of leaf decay measured in the experiment (Figure 3.1).

The shredding invertebrate *Phylloicus hansonii*, a Calamoceratid caddisfly (described by Prather, 2003, Denning *et al.*, 1983), constituted a substantial component of the macroinvertebrate community in our leaf packs, with respect to both abundance and biomass (Table 3.3). These results contrast with previous tropical studies, which indicate a relative lack

of shredders and their associated effects on the processing of leaf litter (Wantzen *et al.*, 2008, Pringle and Ramirez, 1998). Rosemond (1998) found overall increases in invertebrate community abundance when macroconsumers were excluded in a lowland Costa Rican stream, but no shredders were observed and there was no relationship between leaf decay rate and invertebrate abundance. More recent work suggests that shredders are not necessarily rare in tropical systems, but may simply be understudied or undersampled due to the paucity of tropical studies relative to temperate studies (Camacho *et al.*, 2009). High shredder diversity has been observed in detritus-based tropical streams in Australia (Cheshire *et al.*, 2005), and Landeiro and colleagues (2008) found two taxa of actively-shredding caddisfly larvae in upland Amazonian streams (including *Phylloicus*). Overall, while invertebrate resource-specific biomass in our system was dominated by *Phylloicus*, chironomids were found at high abundances in both reaches (Table 3.3). However, a previous study in the neotropics found no link between chironomid density and leaf decay rate (Rosemond *et al.*, 1998) despite the possible leaf-mining behavior of some chironomid taxa, and no statistically significant correlation was established between either chironomid abundance or RSB and leaf decay rate in either of our reaches (Bivariate fit, **RE** and **REP** $p > 0.10$),

Our results indicate that *Phylloicus* abundance directly affected the rate of *Cecropia* leaf decay. Recent studies across tropical systems have shown that shredding invertebrates can be key players in the process of allochthonous leaf litter breakdown. Li and Dudgeon (2009) found a positive relationship between increasing shredder abundance and increasing decay rate of leaves in tropical streams in Hong Kong, while Landeiro *et al.* (2008) found a similar relationship in Amazonian streams. Concurrently, we found that increasing *Phylloicus* abundance was correlated with increasing leaf decay rates in both **RE** and **REP** reaches (Figs.

3.4a, 3.4b). Generally, caddisflies in the genus *Phylloicus* are specialist shredders in the Neotropics, with a diet consisting mostly of coarse detritus (de Carvalho and Uieda, 2009). *Phylloicus* often prefer to consume leaves that are relatively high in nutrient content and low in lignins and other secondary compounds (Rincon and Martinez, 2006), properties common to *Cecropia peltata*, the leaf species used in this study (Zou *et al.*, 1995). Additionally, *Phylloicus* in the Caigual often constructed leaf cases from *C. peltata*, and many of the collected leaf packs showed evidence of this, such as adjacent circular holes cut away from the interior of the leaf (Binderup, personal observation).

Our results suggest that *Phylloicus* biomass and abundance was significantly depressed by *Rivulus* in the **RE** reach where guppies were not present (Figs. 3.2a, 3.3a). In Trinidadian stream reaches without guppies, *Rivulus* are commonly found at high densities (Gilliam *et al.*, 1993, Walsh and Reznick, 2008) and accordingly, their top-down predatory effects on aquatic invertebrates are likely magnified. The lack of significant differences in *Phylloicus* biomass and abundance among all exclusion (D,E,N) treatment types (in both **RE** and **REP** reaches, Figs. 3.2 and 3.3) suggests that neither nocturnal crabs (in both reaches) or diurnal guppies (in **REP** reach) have significant top-down effects on shredder communities. The small adult size of guppies would likely inhibit their ability to consume relatively large invertebrates such as *Phylloicus*, due to gape limitation. *Rivulus* attain significantly larger adult sizes than guppies, and *Rivulus* adults have been observed to consume the largest *Phylloicus* individuals without being limited by gape size. A previous study (Fraser *et al.*, 1999) showed that *Rivulus* consume an approximately even proportion of aquatic versus terrestrial invertebrates; in **RE** reaches, where *Rivulus* densities are high, intraspecific competition for food resources is high, and top-down pressure on large-bodied macroinvertebrates such as *Phylloicus* likely increases. Furthermore, the significant reduction in

mean *Phylloicus* size in control relative to exclusion (D,E,N) treatments (Figure 3.5b) in the **RE** reach suggests a predatory effect of *Rivulus* on larger-bodied *Phylloicus* individuals.

Decoupling the Trophic Cascade: The Guppy Effect

Predatory taxa have been shown to generate trophic cascades in detritus-based streams and rivers. For example, by preying on leaf-consuming detritivores, stonefly predators indirectly reduced leaf decay rates (Oberndorfer *et al.*, 1984, Malmqvist, 1993). The predatory effects of fishes on detritivores (such as caddisflies and amphipods) can also slow decay rates of leaves in temperate streams (Konishi *et al.*, 2001, Ruetz *et al.*, 2002, Greig and McIntosh, 2006, Woodward *et al.*, 2008) (see Table 3.4). In tropical detritus-based streams in Australia, the presence of predatory rainbowfish significantly reduced the breakdown rate of leaf material by inhibiting activity across all taxa of shredding macroinvertebrates (Boyero *et al.*, 2008), the only study demonstrating a detritus-based trophic cascade in a tropical system. Accordingly, we found many lines of evidence suggesting that the insectivorous predator *Rivulus* facilitated a detritus-based trophic cascade. However, this cascade did not occur and was decoupled in the presence of guppies in the downstream **REP** reach (see Figure 3.6). *Rivulus* densities have been shown to decrease significantly in the presence of guppies (as observed in similar Trinidadian streams by Gilliam *et al.*, 1993, Walsh and Reznick, 2008). Our results suggest that this reduction in *Rivulus* decreased top-down pressure on *Phylloicus* shredders (which have been observed to be preyed upon by *Rivulus*) in the **REP** reach. We found that increased guppy visitation to control treatments in the **REP** reach correlated strongly (one-way linear regression, $p = 0.0318$, $r^2 = 0.82$) with an increase in *Phylloicus* biomass, while mean *Phylloicus* length was over two-fold greater in the **REP** reach versus **RE** (Figure 3.5a), suggesting that guppies play a

role in releasing *Phylloicus* from *Rivulus* predation pressure. In addition to documented reductions in *Rivulus* densities associated with guppy presence in other Trinidadian streams, a recent study by de Villemereuil and López-Sepulcre (2010) examined the functional responses of guppies and *Rivulus* under different types of interference competition. *Rivulus* foraging efficiency was shown to decline in the presence of guppies, which likely contributes to the increased biomass and mean length of *Phylloicus* observed in the **REP** reach, and consequently, the decoupling of the trophic cascade observed in the **RE** reach where guppies are absent (see Figure 6).

The increase in *Phylloicus* abundance and biomass in the **REP** reach also resulted in a marginally significant trend ($p=0.11$) trend toward increases in leaf decay rate in the **REP** control relative to the **RE** control treatment, suggesting that the presence of guppies in a stream can indirectly stimulate increases in leaf decay rate. Trophic cascades are more pronounced and have a greater magnitude effect size in systems with low species diversity and simple food webs (Strong, 1992), and it appears that the loss of the trophic cascade in the three-species assemblage versus the two-species assemblage upstream supports this general paradigm.

Ecological-Evolutionary Implications

Our study is part of an NSF-funded FIBR (Frontiers in Integrative Biological Research) project that examines reciprocal feedbacks between guppy evolution and ecosystem properties and processes. An understanding of the ecosystem-level effects of guppy populations allows us to make predictions regarding potential ecological – evolutionary interactions found occurring between guppies and their environment. It is possible that changes in food web structure due to guppy invasions or introductions (in this case, the decoupling of a trophic cascade) could have

feedbacks which affect microevolution of guppy populations. Since guppy presence depresses *Rivulus* density (Gilliam *et al.*, 1993, Walsh and Reznick, 2008) and indirectly increases the biomass of *Phylloicus* shredders (this study), an increase in fine particulate organic matter (FPOM) export as a consequence of coarse leaf material being broken down and egested by shredders (e.g. Wallace *et al.*, 1982) may occur. FPOM is an important food resource for many invertebrate filter feeders in streams (Wallace and Merritt, 1980, Benke *et al.*, 1984), and an increase in the food source of these taxa may stimulate increased secondary production. Similarly, increased deposition of fine benthic organic material (FBOM) may provide an increased basal resource for deposit feeders (collector – gatherer invertebrates) with potential stimulation of the secondary production of common larval deposit feeders such as chironomids, which compose a large proportion of guppy diets (Dussault and Kramer, 1981, Bassar *et al.*, 2010). By potentially altering the stream food web (increasing fine organic material stocks and increasing the secondary production of invertebrate prey taxa), guppies may receive an ecological ‘feedback’ whereby their microevolution is affected by an increase in high-quality food (invertebrates) in their diets.

Previous studies have used small-scale manipulations to document both the direction and magnitude of large-scale responses to events such as localized species extirpations (e.g. Greathouse *et al.*, 2006, Connelly *et al.*, 2008). Mesocosm studies have determined the effects of fish co-evolution (Palkovacs *et al.*, 2009), guppy density and guppy phenotype (Bassar *et al.*, 2010) on ecosystem properties and processes in Trinidad, and we believe that our study has similar predictive value for the reach-scale effects of guppy introductions and/or invasions on coarse detrital basal resources.

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Table 3.1: Physical characteristics of both study reaches, **RE** (*Rivulus* + *Eudaniela*) and **REP** (*Rivulus* + *Eudaniela* + *Poecilia*). Canopy Openness, Water Velocity, and Water Depth are means (\pm SE) across replicate pools (n=5 per site).

Parameter	RE	REP
Experimental Period	22 Feb - 23 March 2009	16 April – 13 May 2009
Stream Order	2	2
Reach length (m)	~200	~200
Canopy Openness (%)	11.30 (1.37)	25.00 (1.78)
Water Velocity (m s^{-1})	0.10 (0.01)	0.07 (0.01)
Water Depth (cm)	25.50 (2.49)	22.90 (1.07)
Macroconsumers	<i>Rivulus</i> , <i>Eudaniela</i>	<i>Rivulus</i> , <i>Eudaniela</i> , <i>Poecilia</i> , <i>Rhamdia</i>

Table 3.2. Summary of diel macroconsumer visitation observations ($\# \text{ m}^{-2} \text{ hr}^{-1}$). Data comprised of means (\pm SE) over 10 hours of observation per reach, n=20 observation periods for each reach.

Macroconsumer	RE Reach	REP Reach
<u>Daytime</u>		
Guppy	N/A	29.2 (4.48)
<i>Rivulus</i>	2.24 (1.38)	1.39 (0.39)
<i>Eudaniela</i>	0.64 (0.54)	0.11 (0.10)
<u>Nighttime</u>		
Guppy	N/A	0.11 (0.11)
<i>Rivulus</i>	5.44 (1.54)	4.69 (1.26)
<i>Eudaniela</i>	7.57 (2.41)	0.64 (0.27)

Table 3.3: Insect community composition in (A) **RE** reach and (B) **REP** reach. FFG refers to functional feeding group guilds, while RSB is a measure of biomass (mg) per unit of leaf mass (g) remaining. %RSB and %Abundance indicate contributions of each taxon to the overall RSB and abundance of the entire community. In the second column, standard font indicates Family while italics indicate genera.

(A)				Total		
Order	Lowest Taxonomic Grouping	FFG	RSB	Abundance	% RSB	% Abundance
Coleoptera	Elmidae	CG	21.86	186	8.32%	5.46%
Diptera	Ceratopogonidae	CG	0.84	15	0.32%	0.44%
Diptera	Chironomidae	CG	35.83	2006	13.64%	58.90%
Ephemeroptera	<i>Baetis</i>	CG	3.59	39	1.37%	1.15%
Ephemeroptera	<i>Baetodes</i>	CG	3.74	32	1.42%	0.94%
Ephemeroptera	<i>Leptohyphes</i>	CG	14.16	81	5.39%	2.38%
Ephemeroptera	<i>Tricorythodes</i>	CG	0.95	20	0.36%	0.59%
Diptera	Tanypodinae	PR	6.46	382	2.46%	11.22%
Ephemeroptera	<i>Euthyplocia</i>	PR	4.22	1	1.61%	0.03%
Odonata	<i>Argia</i>	PR	45.57	83	17.34%	2.44%
Odonata	<i>Hetaerina</i>	PR	15.18	77	5.78%	2.26%
Plecoptera	<i>Anacroneuria</i>	PR	11.20	7	4.26%	0.21%
Coleoptera	<i>Ectopria</i>	SC	5.55	33	2.11%	0.97%
Ephemeroptera	<i>Thraulodes</i>	SC	9.65	148	3.67%	4.35%
Trichoptera	<i>Phylloicus</i>	SH	83.94	296	31.95%	8.69%
		TOTAL:	262.74	3406		
(B)						
Coleoptera	Elmidae	CG	5.18	32	2.05%	2.13%
Diptera	Ceratopogonidae	CG	4.50	68	1.79%	4.53%
Diptera	Chironomidae	CG	17.82	631	7.06%	42.01%
Ephemeroptera	<i>Baetis</i>	CG	0.76	4	0.30%	0.27%
Ephemeroptera	<i>Baetodes</i>	CG	0.20	1	0.08%	0.07%
Ephemeroptera	<i>Leptohyphes</i>	CG	1.82	6	0.72%	0.40%
Ephemeroptera	<i>Tricorythodes</i>	CG	2.02	27	0.80%	1.80%
Trichoptera	Philopotamidae	CG	35.45	21	14.05%	1.40%
Trichoptera	Hydropsychidae	F	0.96	13	0.38%	0.87%
Diptera	Tanypodinae	PR	24.71	349	9.80%	23.24%
Odonata	<i>Argia</i>	PR	15.08	22	5.98%	1.46%
Odonata	<i>Hetaerina</i>	PR	12.78	37	5.07%	2.46%
Plecoptera	<i>Anacroneuria</i>	PR	1.02	1	0.40%	0.07%
Coleoptera	<i>Ectopria</i>	SC	1.17	4	0.46%	0.27%
Ephemeroptera	<i>Thraulodes</i>	SC	1.52	24	0.60%	1.60%
Trichoptera	Glossosomatidae	SC	0.92	10	0.36%	0.67%
Trichoptera	<i>Phylloicus</i>	SH	126.35	252	50.09%	16.78%
		TOTAL:	252.27	1502		

Table 3.4: Comparison of studies which observed trophic cascades in detritus-based stream ecosystems whereby leaf processing rates were affected by fish predators. Leaf processing was enhanced in the absence of predators in all studies due to release of invertebrate detritivores from predation. ‡ = increase in DM (dry mass) day⁻¹, * = estimate based on CPOM remaining, ** = estimate based on k_{day} values, *** = estimate based on leaf mass loss (mg)

Study	Site	Latitude	1° Detritivore	Predatory fish	(%) increase in decay rate (predators excluded)
Konishi 2001	Hokkaido, Japan (<i>in situ</i>)	Temperate	Amphipod: <i>Jesogammarus jezoensis</i>	Rainbow trout, freshwater sculpin	75%‡
Ruetz et al. 2002	Minnesota, USA (<i>ex situ, in situ</i>)	Temperate	Amphipod: <i>Gammarus pseudolimnaeus</i> ; Trichoptera: <i>Lepidostoma</i> sp., <i>Hesperophylax</i> sp.	Rainbow trout, brook trout, brown trout, slimy sculpin	27.2%‡
Greig & McIntosh 2006	S. Island, New Zealand (<i>in situ</i>)	Temperate	Trichoptera: <i>Zelandopsycha ingens</i>	Brown trout	>25%*
Woodward et al. 2008	England, UK (<i>in situ</i>)	Temperate	Amphipod: <i>Gammarus pulex</i>	Bullhead	53.8%**
Boyero et al. 2008	QLD, Australia (<i>ex situ</i>)	Tropical	Trichoptera: <i>Anisocentropus kirramus</i> ; Ephemeroptera	Eastern rainbowfish	>25%***
This Study.	Northern Range, Trinidad (<i>in situ</i>)	Tropical	Trichoptera: <i>Phylloicus hansonii</i>	Hart's killifish	41.2%‡

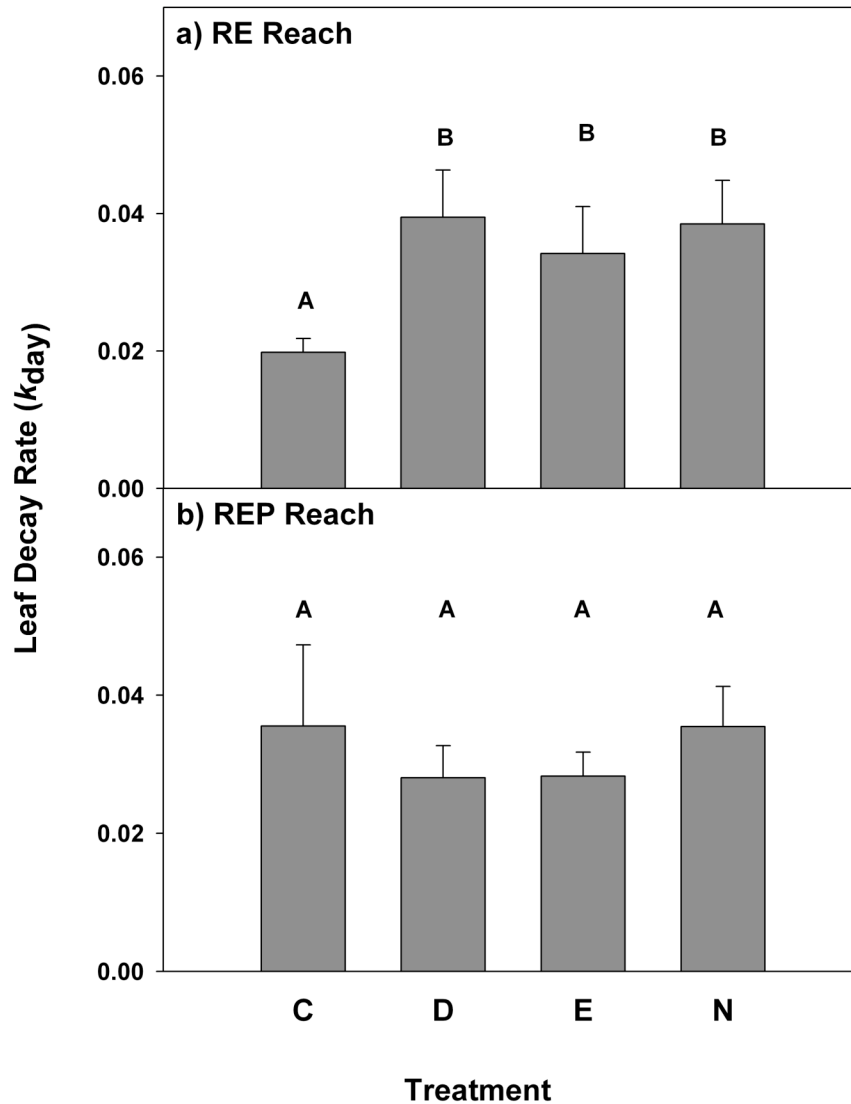


Figure 3.1: Leaf decay rates (k_{day}) \pm SE for the four treatment types (C = macroconsumer access, D = diurnal exclusion, E = total macroconsumer exclusion, N = nocturnal exclusion) in a) **RE** (*Rivulus* + *Eudaniela*) and b) **REP** (*Rivulus* + *Eudaniela* + *Poecilia*) reaches. Letters between bars indicate results of post-hoc Tukey-Kramer HSD tests, with different letters indicating significant intra-reach differences in pairs of means between treatments.

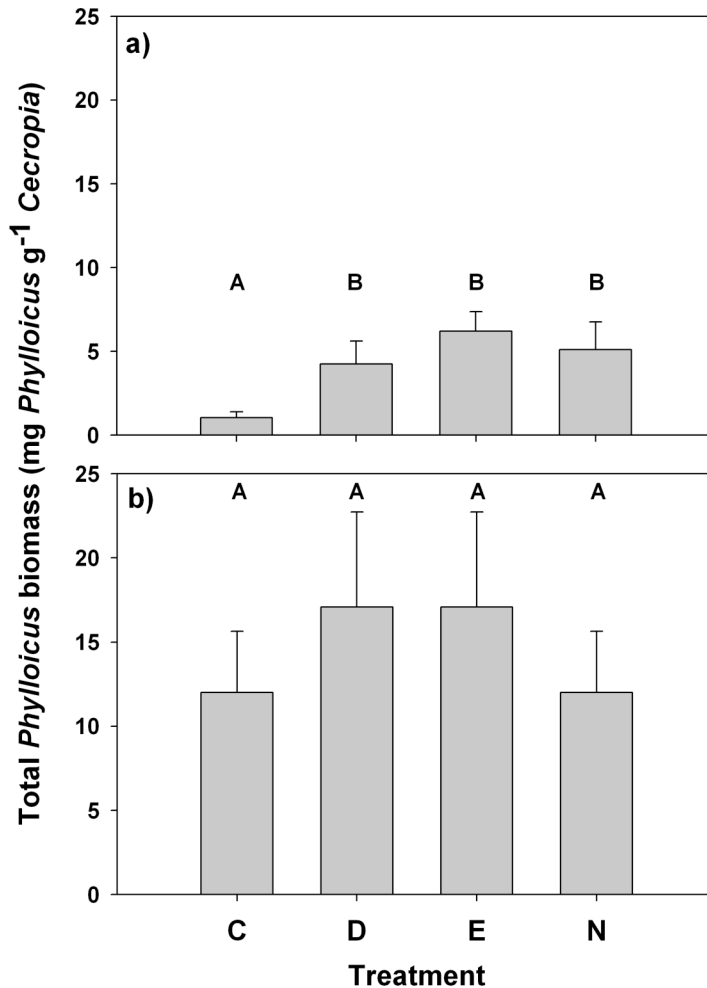


Figure 3.2: Comparison of total normalized *Phylloicus* resource-specific biomass (mg dry mass g⁻¹ *Cecropia* dry mass) measured over the duration of the 4-week experimental manipulation in both **a) RE** (*Rivulus* + *Eudaniela*) and **b) REP** (*Rivulus* + *Eudaniela* + *Poecilia*) reaches for all 4 treatment types (C = macroconsumer access, D = diurnal exclusion, E= total macroconsumer exclusion, N = nocturnal exclusion). All values ($n=5$) represent means \pm SE. Letters between bars indicate results of post-hoc Tukey-Kramer HSD tests, with different letters indicating significant intra-reach differences in pairs of means between treatments.

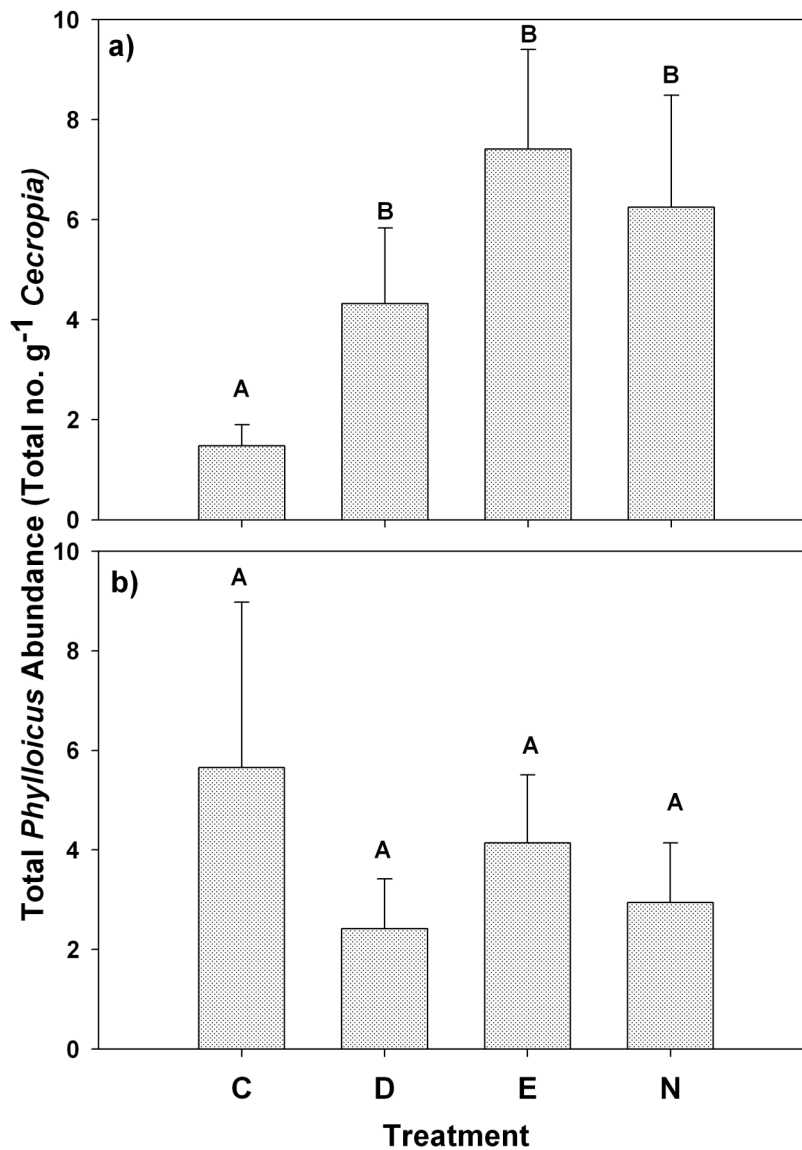


Figure 3.3: Comparison of total normalized *Phylloicus* abundance (No. of individuals g⁻¹ *Cecropia* dry mass) measured over the duration of the 4-week experimental manipulation in both **a) RE** (*Rivulus* + *Eudaniela*) and **b) REP** (*Rivulus* + *Eudaniela* + *Poecilia*) reaches for all 4 treatment types (C = macroconsumer access, D = diurnal exclusion, E= total macroconsumer exclusion, N = nocturnal exclusion). All values ($n=5$) represent means \pm SE. Letters between bars indicate results of post-hoc Tukey-Kramer HSD tests, with different letters indicating significant intra-reach differences in pairs of means between treatments.

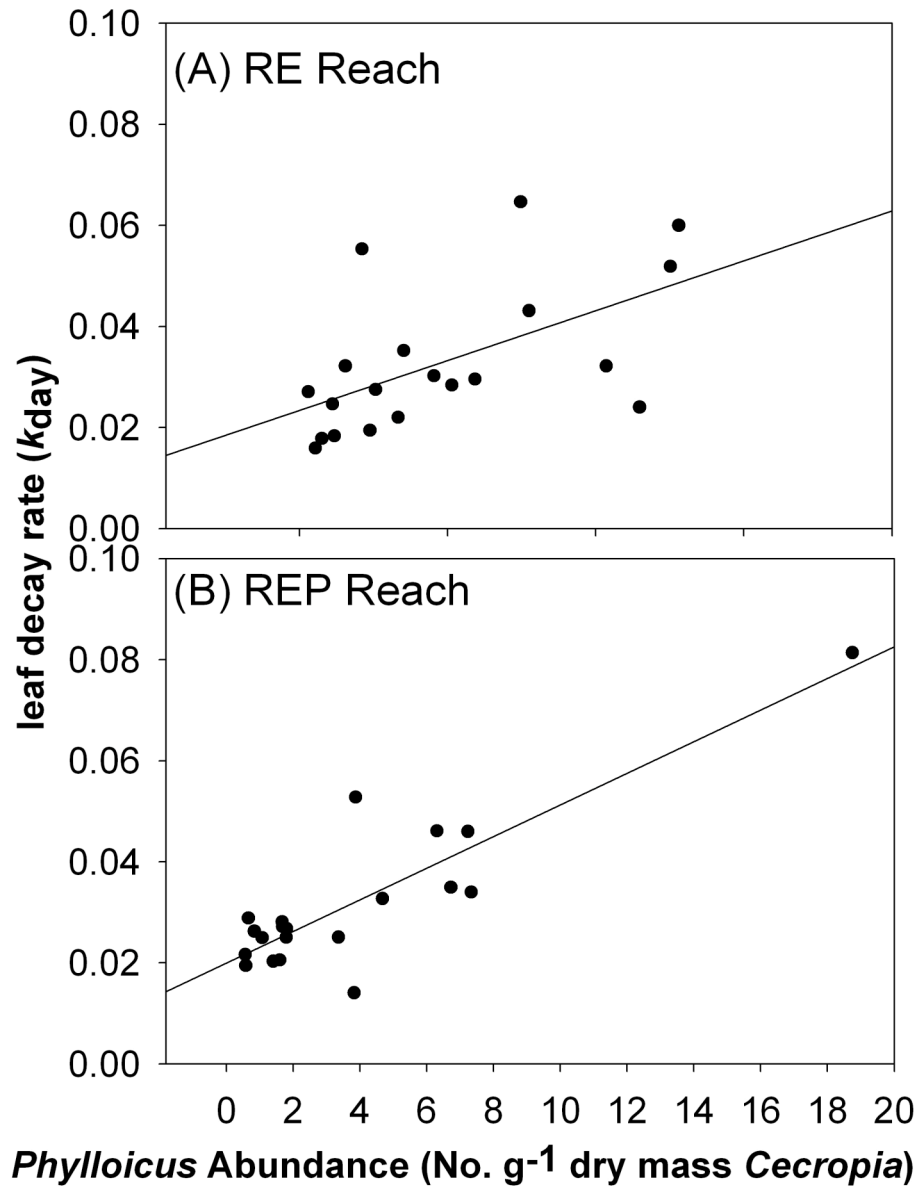


Figure 3.4: Bivariate fits of leaf decay rate (k_{day}) versus total normalized *Phylloicus* abundance (No. of individuals g^{-1} *Cecropia* dry mass) in (A) RE (*Rivulus* + *Eudaniela*, $p = 0.0090$, $r^2 = 0.32$) and (B) REP (*Rivulus* + *Eudaniela* + *Poecilia*, $p < 0.0001$, $r^2 = 0.75$) reaches ($n=20$ in both reaches)

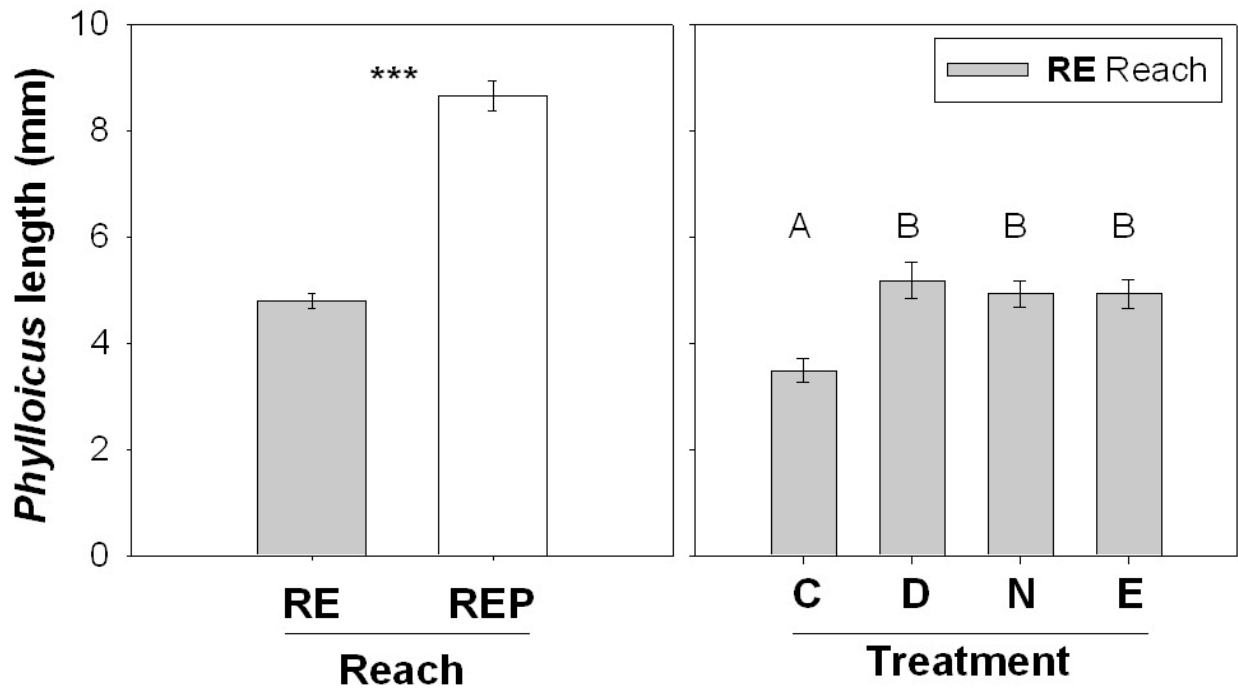


Figure 3.5: Mean *Phylloicus* lengths in mm (\pm SE) in a) **RE** versus **REP** reaches (**RE**: n=296; **REP**: n=218; *** = $p < 0.0001$); and b) treatments within **RE** reach, n = 38 (C), 63 (D), 84 (N), 111 (E); letters above bars signify results of posthoc Tukey-Kramer HSD, with different letters indicating significant differences in pairs of means between treatments.

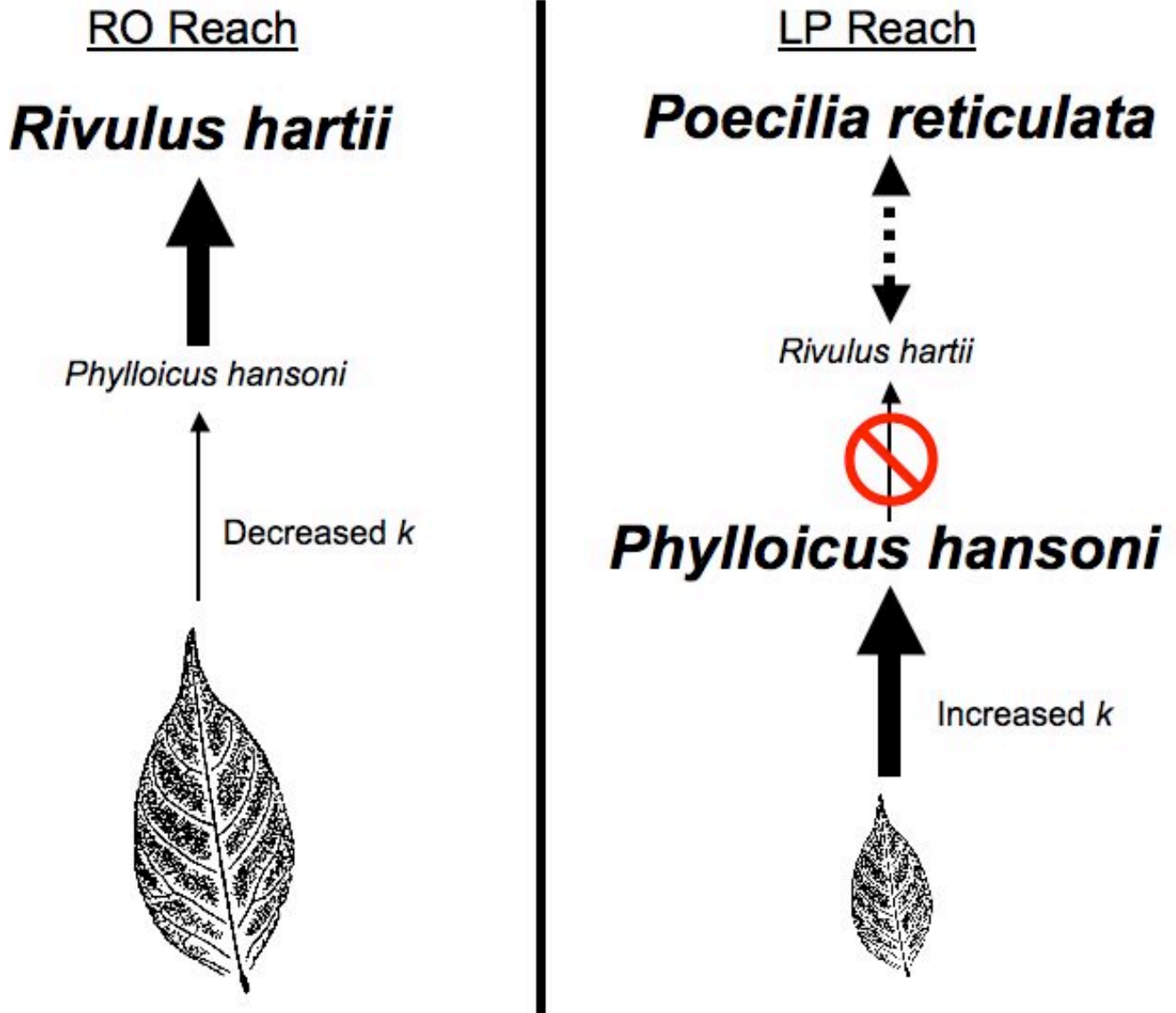


Figure 3.6: Conceptual diagram of detrital trophic cascade in our system. Solid arrows indicate movement up trophic levels while dashed arrows indicate interspecies competitive interference.

CHAPTER 4

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

Conclusions

In this study, we developed a method for isolating the top-down effects of macroconsumer taxa on benthic structure and function based on observations of taxa-specific diel activity patterns. We manipulated macroconsumer presence and absence *in situ* using electric exclusion treatments based upon these diel activity patterns. In Chapter 2, we successfully isolated the effects of guppies, quantifying their behavior as almost entirely diurnal. We also discovered that the diel behavior of the killifish *Rivulus* was context-dependent on guppy presence, with *Rivulus* demonstrating significantly higher nocturnal behavior in the reach with guppies. *Rivulus* and *Eudaniela* both displayed nocturnal behavior in both reaches, and consequently, their combined effects were quantified in both reaches, with naïve *Rivulus* and *Eudaniela* occurring in the **RE** reach (without guppies), and coevolved *Rivulus* and *Eudaniela* cohabitating with guppies in the **REP** reach. We next quantified the top-down effects of macroconsumers on algal accrual and standing crop, using ceramic tiles as a proxy for hard rock and cobble substrata commonly found distributed throughout the benthic environment in study pools. Successfully isolating the effects of guppies showed that guppies exerted strong and significant top-down effects on algae, reducing the rate of algal accrual and algal biomass (measured as a standing crop of chlorophyll *a*), and that the size of these effects was much

greater than the effects of both naïve and coevolved *Rivulus* and *Eudaniela*. In the absence of guppies, the combined effects of naïve *Rivulus* and *Eudaniela* significantly affected the rate of algal accrual, and we attributed this effect to be context-dependent on both natural variation between reaches and increases in the bioturbative effects of naïve *Rivulus*, which occur at significantly higher densities when guppies are absent. No differences in benthic invertebrate community (either abundance or biomass) existed between reaches, and none of the isolated macroconsumer effects significantly affected invertebrate communities, suggesting that direct top-down effects of macroconsumers, not grazer-mediated trophic cascades, controlled algal communities.

In Chapter 3 we evaluated the top-down effects of macroconsumers on the ecosystem process of leaf decomposition. We found that contrary to many ecosystem studies in tropical systems (Wootton and Oemke 1992, Rosemond et al. 1998, March et al. 2001, Nordhaus et al. 2006, Moss 2007), macroconsumers did not exert direct, top-down effects on leaf decomposition via foraging or shredding. Instead, we found that leaf decomposition was strongly controlled by the presence of a dominant larval shredding insect, the caddisfly *Phylloicus hansonii*. In the absence of guppies, the control treatment (which allowed unrestricted macroconsumer access) had significantly slower rates of leaf decomposition than other treatments, and also had significantly lower abundance and biomass of *Phylloicus*. We attributed these effects to strong top-down pressure on *Phylloicus* communities by the insectivorous killifish *Rivulus hartii*, which reaches very high population densities when guppies are absent. Summarily, *Rivulus* facilitated a trophic cascade via its direct effects on a dominant intermediate consumer, indirectly slowing the rate at which leaf litter decomposed. In the downstream reach, which contained a high-density population of low-predation adapted guppies, both *Phylloicus* abundance and biomass

was significantly higher in the control treatment than in the control treatment in the reach lacking guppies, and a marginally significant ($p = 0.11$) elevation in the decomposition rate of leaf litter was observed. These results strongly suggest that the presence of guppies decoupled a trophic cascade that was observed in a connected reach where guppies were absent, and that this decoupling is likely due to the concomitant decline of *Rivulus* population densities in the presence of guppies.

The results of this study strongly suggest that guppies are strong interactors in high-gradient, headwater tropical streams in Trinidad, and that their introduction and concomitant adaptation to a new environment can significantly affect multiple components of the structure and function of benthic ecosystems. *Rivulus* can exert context-dependent effects on ecosystem structure and function, as evidenced by their top-down effects on *Phylloicus* communities and algal accrual in the **RE** reach, though the significance of these top-down effects are lost in the presence of guppies. Furthermore, our results correlated strongly with the effects of guppies observed in previous mesocosm studies in Trinidad (Palkovacs et al. 2009, Bassar et al. 2010), and the *in situ* nature of our study's results can serve as a benchmark to evaluate the 'endpoint' of the effects of guppy evolution on ecosystem structure and function, allowing for comparative study of our results in the context of ongoing work evaluating interactions between evolutionary and ecosystem processes in recently introduced guppy populations in the same watershed.

Suggestions for future research

One potential area of future study is the nature of interspecies interactions between guppies and other macroconsumer taxa. We know that guppy presence is associated with lower densities of *Rivulus* relative to densities in *Rivulus*-only streams (Gilliam et al. 1993, Walsh and

Reznick 2008). These differences may be associated with intraguild predation, as *Rivulus* lay more eggs of smaller size in the presence of guppies and also have faster juvenile growth rates (Walsh and Reznick 2010). However, the nature of any behavioral plasticity in *Rivulus* populations as a result of guppy presence remains unknown. If *Rivulus* foraging behavior and habitat selectivity is context-dependent, and varies significantly in the presence of guppies, it may further explain the differences in ecosystem-level effects of *Rivulus* we attribute simply to differences in density.

We also find higher rates of *Eudaniela* visitation in reaches without guppies, suggesting that *Eudaniela* foraging behavior and potentially, population densities may also be context-dependent. Our knowledge of *Eudaniela* life history, population distributions, and population densities in the Guanapo watershed is extremely limited, save distribution data collected by Rostant (2005). Mark-recapture studies in reaches both with and without guppies would be extremely beneficial in determining whether *Eudaniela* population densities differ; similar densities between reaches would suggest that guppy presence alters behavior, making *Eudaniela* less “bold” and less likely to be observed. Though we did not find significant effects of *Eudaniela* on any measured ecosystem processes and properties in our study, mark-recapture in replicate pools would tell us whether *Eudaniela* densities are highly heterogeneous and habitat-specific or relatively evenly distributed throughout pools, allowing us to evaluate the strength of their top-down effects more effectively.

In Chapter 3, we showed that the presence of guppies decoupled a trophic cascade that existed in an upstream reach without guppies. While this study focused on the top-down effects of macroconsumers, bottom-up forces are also important in regulating ecosystem structure and function in streams. A previous study (Bassar et al. 2010) showed that guppies excrete nutrients

(N and P) at much higher rates than *Rivulus*. Furthermore, the effect of guppy phenotype is also significant, with guppies adapted to high-predation environments excreting significantly more nitrogen (Palkovacs et al. 2009). Microbial processes that first “condition” leaf litter (Barlocher and Kendrick 1975) and facilitate leaf softening and breakdown (Suberkropp and Klug 1976) have been hypothesized to be more important in leaf decomposition in tropical systems relative to temperate systems (Irons et al. 1994). Previous studies have linked elevated nutrient levels to increased rates of leaf decomposition, due to both stimulation of microbial decomposers and their associated processes (e.g. Meyer and Johnson 1983, Suberkropp and Chauvet 1995, Rosemond et al. 2002) and potential stimulation of shredding invertebrate secondary production (Robinson and Gessner 2000). It is possible that the introduction of guppies could increase decomposition rates of allochthonous litter; mesocosm studies using standardized leaf packs and both phenotypes of guppies could determine whether guppy excretion associated with introductions and/or invasions would significantly affect this important ecosystem process.

A final suggestion for future research expands into the concept of ecosystem structure and function in the light of anthropogenically-driven species loss. Our study sites in the Guanapo Valley lack decapod shrimp taxa due to the effects of pollution in the Caroni estuary (where our sites drain to the Gulf of Paria), which has completely extirpated these amphidromous crustaceans from our watershed (Rostant 2005). However, lotic systems draining the opposite slope of the Northern Range empty directly into the Caribbean Sea, and diverse, healthy communities of shrimps thrive in both low- and high-order streams and rivers (Rostant 2005). Our results showed no significant effects of *Eudaniela* on ecosystem structure or function; it appears that crabs do not fill the niche once occupied by shrimps in our system, since tropical shrimps have been shown to have very strong top-down effects on benthic ecosystem

processes and properties in streams across the Neotropics (e.g. Pringle and Blake 1994, Pringle 1996, Pringle and Hamazaki 1998, March et al. 2001, March et al. 2002). I hypothesize that in systems such as ours, with no shrimps and high densities of guppies, guppies have expanded to partially fill the niche once occupied by shrimps, as evidenced by strong top-down effects of guppies on algal communities, but that these species-poor systems are not functionally redundant in terms of the roles of macroconsumer taxa in regulating ecosystem structure and function. In order to adequately test these hypotheses, I propose that effect sizes of macroconsumers in streams with intact shrimp faunal assemblages be isolated and measured using the methods outlined in this thesis, and then compared to the effect sizes of macroconsumers in shrimp-free systems. Further work could make use of artificial mesocosm stream channels with combinations of shrimps, both LP and HP guppies (to examine effects of phenotypic divergence), and *Rivulus* to adequately separate out direct and indirect effects of these assemblages (e.g. nutrient cycling, organic matter transport). These studies would inform us of the potentially drastic implications of shrimp extirpation on stream ecosystem structure and function, and would also allow us to more completely understand how guppies, which evolve at ecologically-relevant time scales (Reznick et al. 1997, Fussmann et al. 2007), may or may not dampen the ecosystem-level effects of shrimp faunal extirpation as they invade and adapt to new habitats.

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