

DIRECT AND INDIRECT EFFECTS OF THE GUPPY, *POECILIA RETICULATA*, ON
THE STRUCTURE AND FUNCTION OF STREAM ECOSYSTEMS IN THE
NORTHERN RANGE MOUNTAINS OF TRINIDAD

by

TROY NATHANIEL SIMON

(Under the Direction of Catherine M. Pringle)

ABSTRACT

We are currently in the middle of the sixth mass extinction, thus predicting the ecological consequences of species loss from communities is a high priority research topic. Additionally, characterizing the ecosystem impact of macroconsumers has strong conservation implications, given the global trend in biodiversity loss toward higher trophic levels. The goal of this dissertation was to use Trinidadian guppies in the mountain streams of Northern Trinidad as a model system to explore several potentially important direct and indirect ecosystem effects of macroconsumers, which we have little understanding of in natural systems. This collection of studies utilizes both localized exclusion of macroconsumers using underwater electric fences within pools, as well as the natural reach-scale exclusion of guppies above and below barrier waterfalls to examine the ecosystem-level effects of macroconsumers.

Results show that local adaptation of guppy populations, to the absence of large piscivores in higher elevation streams, shifted their top-down effects from reducing macroinvertebrate standing stock biomass, to reducing primary producer standing stock

biomass. Additionally, the consumptive and non-consumptive effects guppies had similar but opposing effects on algal growth and biomass, which resulted in strong treatment effects from excluding guppies on primary algae. Finally, the loss of omnivorous guppies from a macroconsumer assemblage can alter the strength of top down effects of the remaining macroconsumer community on an important ecosystem-level process at both local- and landscape-scales. Together these studies expand our understanding of how macroconsumers alter ecosystem-level processes in natural systems.

INDEX WORDS: macroconsumer, guppy, *Poecilia reticulata*, streams, freshwater, local adaptation, direct and indirect effects, biomass, macroinvertebrates, algae, consumptive and non-consumptive effects, leaf decomposition, trophic cascades

DIRECT AND INDIRECT EFFECTS OF THE GUPPY, *POECILIA RETICULATA*, ON
THE STRUCTURE AND FUNCTION OF STREAM ECOSYSTEMS IN THE
NORTHERN RANGE MOUNTAINS OF TRINIDAD

by

TROY NATHANIEL SIMON

B.S., Florida State University, 2010

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2015

© 2015

Troy Nathaniel Simon

All Rights Reserved

DIRECT AND INDIRECT EFFECTS OF THE GUPPY, *POECILIA RETICULATA*, ON
THE STRUCTURE AND FUNCTION OF STREAM ECOSYSTEMS IN THE
NORTHERN RANGE MOUNTAINS OF TRINIDAD

by

TROY NATHANIEL SIMON

Major Professor: Catherine M. Pringle

Committee: Mary C. Freeman
James F. Gilliam
Amy D. Rosemond

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2015

DEDICATION

This dissertation is dedicated to my Wife, Gareth Crosby and to my Son,
Benjamin Oliver Simon.

ACKNOWLEDGEMENTS

I would not have been able to complete this document without the help of my advisor Catherine Pringle and the members of my committee Mary Freeman, James Gilliam, Amy Rosemond , and Richard Shefferson. I would also like to thank the members of the Pringle and Rosemond labs for their never-ending comments and encouragement. Additionally there are countless field and lab technicians for which this work would not have been possible without their huge effort. Finally and most importantly I would like to thank my Wife, Gareth Crosby and my Parents Dennis and Brooke Simon; without their constant encouragement and emotional support I would never have come close to finishing my degree.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	v
LIST OF TABLES	vii
LIST OF FIGURES	x
CHAPTER	
1 INTRODUCTION AND LITERATURE REVIEW	1
2 EXAMINING THE EFFECTS OF TRINIDADIAN GUPPIES ON THE STRUCTURE OF THE ECOSYSTEM RELATIVE TO SYMPATRIC MACROCONSUMERS USING AN <i>IN SITU</i> DENSITY MANIPULATION ...	6
3 LOCAL ADAPTATION IN TRINIDADIAN GUPPIES ALTERS ECOSYSTEM STRUCTURE <i>IN SITU</i> AT LOCAL- AND LANDSCAPE- SCALES.....	36
4 TRINIDADIAN GUPPIES DECOUPLE A DETRITUS-BASED TROPHIC CASCADE WHEN PRESENT IN MACROCONSUMER ASSEMBLAGES WITHIN THE MONTANE HEADWATER STREAMS OF NORTHERN TRINIDAD	67
5 CONCLUSIONS	103
6 REFERENCES	107
APPENDICES	
A CHATER 4.....	113

LIST OF TABLES

	Page
<p>Table 2.1: Mean estimate of physiochemical characteristics from pools across replicate streams (n=4) for each density manipulation treatment. END= Endler Stream, QRE2 and QRE6= tributaries of the Quare River, RDN= Ramdeen Stream. Physiochemical characteristics were measured one day after experiment was completed (April 13th 2012).....</p>	28
<p>Table 2.2: Guppy densities (individuals m⁻²) in pools across replicate streams (n=4) before (pre-) and after (post-) each density manipulation treatment was carried out. To account for migrations in and out of pools during our experiment, we also estimated post-manipulation densities upon experiment completion. The average density of guppies present in guppy Removal treatment pools during the experiment was used to calculate the post-manipulation density after the experiment. See Table 2.1 caption for stream names associated with abbreviations.....</p>	29
<p>Table 3.1: Physical and chemical parameters [mean±SE (n=7); range (min, max)] for paired reaches examined in the landscape-scale survey and the location of our local-scale experiment, the Caigual*. Physical and chemical parameters not measured are denoted by §. Physical and chemical parameters for landscape-scale study were measured one week before (April 13th-19th, 2011) biological sampling. Physical parameters for local-scale study were calculated one day after experiments were</p>	

completed for $G_{P1}KC$ and $G_{P2}KC$ reaches on 24 March 2009 and 25 May 2010, respectively.....60

Table 3.2: Bayesian model parameter estimates for each response variable measured in landscape-scale study including: mean and standard deviation (SD), upper (97.5%) and lower (2.5%) confidence limits (CL), and percent (%) effect size. To insure outliers within our dataset did not heavily bias our parameter estimates, we ran our models both with and without the top two outliers in for each response variable. We report parameter estimates highlighted in gray, and those that were well supported by our model are in bold.....61

Table 4.1: Density [No. m^{-2} (SE)] estimates of macroconsumers (killifish, crabs, and guppies) from the macroconsumer exclosure experiment. Macroconsumer density was estimated using day and night observations. Observations conducted for 10 hours per reach (n=20 observations per reach). Diurnal estimates based on pooled visitation from 24 hr. access and daytime access treatments, while Nocturnal estimates are based on 24 hr. access and nighttime access treatments. Bolded values represent significant results from Kruskal-Wallis rank sum tests ($P < 0.05$) between diurnal and nocturnal activity.....91

Table 4.2: Physiochemical characteristics [means \pm SE (min, max)] of pools from the landscape study measured on the final day of the experiment. Physical characteristics were measured in pools (n=18 replicate pools per reach type) across KCG (killifish, crab, and guppy) and KC (killifish and crab) reaches..91

Table 4.3: Abundance of macroconsumers (killifish, crabs, guppies) for the landscape study. Abundance estimated using catch per unit effort [CPUE (\pm SE)], for killifish

and guppies, and transect observations [individuals $m^{-1}(\pm SE)$] for crabs. CPUE estimates were calculated during the day using two baited minnow traps, once in each pool (n=18 replicate pools). Transect counts of crabs were conducted at night, across 100 m length of stream, once in each reach (n=6 replicate reaches). Bolded values represent significant differences ($P<0.05$) from one-way ANOVA between KCG and KC reaches for each species respectively.....92

LIST OF FIGURES

	Page
<p>Figure 2.1.: Location of study sites in the Caroni and Oropuche drainages of Trinidad's Northern Range (Inset A). Streams are coded by numbers (1) RDN= Ramdeen Stream, (2) END= Endler Stream, (3) QRE2 and (4) QRE6= tributaries of the Quare River</p>	32
<p>Figure 2.2: Schematic of experimental design for one block, experiment was blocked by stream (n=4). Experimental design isolated the ecosystem effects of one species of macroconsumer, the guppy <i>Poecilia reticulata</i>, from the remaining macroconsumer assemblage (including the killifish, <i>Anablepsoides hartii</i>, and the crab, <i>Eudaniela garmani</i>). Guppies were either removed from pools leaving only killifish and crabs (KC) present, removed and replaced at ambient (Low) density (KCG_{Low}), or removed and replaced at double ambient (high) density (KCG_{High}). To isolate the top-down effects of each macroconsumer community, underwater electric fences were placed in each pool to exclude macroconsumers from localized areas of the stream bottom</p>	33
<p>Figure 2.3: Mean (\pmSE) for the (a) biomass of total benthic invertebrate community (b) rate of algal accrual, (c) algal biomass, and percent (%) composition of (d) nitrogen (N) and (e) phosphorous (P) from experiment manipulating guppy density within pools across streams (n=4). Underwater electric frames manipulated the presence (light grey) and absence (dark grey) of all macroconsumers (killifish, crabs, guppies)</p>	

within pools from localized areas of the stream bottom. Split-plot statistical analysis investigated the interaction between macroconsumer community (*whole-plot*) and macroconsumer exclusion (*sub-plot*) treatments to test if the effect excluding macroconsumer depended on the presence of guppies within macroconsumer community. We found significant interactions effects for algal accrual rate and algal biomass, but no other ecosystem metrics. Additionally, the top-down effects of each macroconsumer community were tested using paired T-Tests for each enclosure and control treatments nested within pools, significant differences signified by * ($p < 0.05$); † ($p < 0.1$)... 34

Figure 2.4: Estimated percent (%) change caused by the per capita consumptive (dark grey), Non-consumptive (light grey), and net (black) effects guppies on invertebrates biomass (mg m^{-2}), Chl *a* accrual rate ($\text{mg m}^{-2}/ \text{day}$), Chl *a* biomass (mg m^{-2}), and percent (%) composition of phosphorous (P) and nitrogen (N) in biofilm. Per capita effects of guppies was estimated using linear mixed effects models, where guppy biomass was a continuous explanatory variable to estimate the biomass specific effect of guppies which scales our output to the effect increasing density by 1 individual m^{-2} . Positive values for percent change represent positive effects of guppies and negative values represent negative effects. Figures show 95% credible intervals (CI) around estimated effect sizes. Estimates where 95% CI do not include zero are signified by * 35

Figure 3.1: Map showing subwatersheds sampled for landscape-scale study [denoted by outlined watersheds, where either G_{P2} (1-4) or G_{P1} (5-8) guppies are located] and local-scale experiments (denoted by a dashed subwatershed) across the Caroni

drainage in Trinidad’s Northern Range mountains. The Caroni drainage contains large sections of high-density urban areas (diamond shaded area) and rural areas with mixed agriculture (hatch shaded area). Subwatersheds chosen for landscape study were located in watersheds with the largest tracts of intact-forested headwaters. Inset (B) illustrates paired reach design (above and below waterfalls) of landscape study, where reaches below barrier waterfalls contain guppies, killifish, and crabs ($G_{P1}KC$) and reaches above waterfalls contain only killifish and crabs (KC)...64

Figure 3.2: Mean (\pm SE) of natural log (LN) response ratio of ecosystem response variables in two local-scale experiments conducted in macroconsumer communities containing either (A) G_{P1} guppies, killifish, and crabs ($G_{P1}KC$), or (B) G_{P2} guppies, killifish, and crabs ($G_{P2}KC$). X-axis is the response variables measure during experiment including algal accrual rate ($\text{mg m}^{-2}/\text{day}$), algal biomass (mg m^{-2}), and invertebrate biomass (mg m^{-2}). Y-axis is the response ratios calculated as LN (response variable in control/response variable in macroconsumer exclusion), where positive values represent positive effects of macroconsumers and negative values represent negative effects. Statistical significance is signified by * ($p < 0.05$), ** ($p < 0.01$)...65

Figure 3.3: Mean \pm 95% Credible Intervals (CI) of percent (%) change caused by the presence of either G_{P1} or G_{P2} guppies relative to guppy-free reaches, or the effect of exchanging G_{P1} with G_{P2} guppies (Phenotype) on (A) algae biomass (mg m^{-2}) and (B) invertebrate biomass (mg m^{-2}) from landscape-scale study. Effects of guppies estimated using linear mixed effects models with random intercepts. Positive effect sizes represent positive effects of guppies and negative values represent negative

effects. Parameter estimates were considered to be statistically significant if the 95% credible interval of the estimate did not include zero, where significant parameter estimates indicated by an asterisk (*)......66

Figure 4.1: Conceptual diagram of detrital food web in streams consisting of paired reaches containing killifish, crabs, and guppies (KCG) downstream, and killifish and crabs (KC) upstream, where barrier waterfalls naturally limit upstream migration of guppies. *Phylloicus hansonii* is a dominant macroinvertebrate detrital shredder in these streams. Solid arrows indicate hypothesized energy movement up trophic levels while dashed arrows represents movement within trophic level.....96

Figure 4.2: Location of study sites in the Caroni drainage of Trinidad's Northern Range (Inset A) for macroconsumer exclosure experiment (1) and the landscape study (2a-f). Each study site consists of paired reaches (Inset B) containing killifish, crabs, and guppies (KCG) downstream, and killifish and crabs (KC) upstream. Guppies are naturally excluded from the upstream-most reaches by barrier waterfalls.....97

Figure 4.3: Mean (\pm SE) for (a,b) decay rate of *Cecropia peltata* leaves (% mass lost day⁻¹) and (c,d) *Phylloicus hansonii* resource-specific biomass (mg dry mass g⁻¹ *Cecropia*) for the exclosure experiment, where 24 hour treatments allow full macroconsumer access [control (C)] or completely exclude macroconsumers [electric (E)]. 12 hour treatments allow access to either diurnally active [day (D)] or nocturnally active [night (N)] macroconsumers. Experiment conducted in reaches containing either (a,c) killifish, crabs, and guppies (KCG), or (b,d) only killifish and crabs (KC) and replicated in pools (n=5 per reach). Letters between bars indicate

results of post-hoc Tukey-Kramer HSD tests, with different letters indicating significant within reach differences in pairs of means between treatments..... 98

Figure 4.4: Bivariate fit of decay rate for leaves of *Cecropia peltata* (% mass lost day⁻¹) and *Phylloicus hansonii* resource-specific biomass (mg dry mass g⁻¹ *Cecropia*) from the enclosure experiment. Experiment conducted in reaches containing either (a) killifish, crabs, and guppies (KCG), or (b) only killifish and crabs (KC) and replicated by diel treatments in pools (n=20 per reach). Statistical significance of correlation noted by * (P<0.05) and ** (P<0.01)..... 99

Figure 4.5: Mean (±SE) for (a) decay rate for *Cecropia peltata* leaves (% mass lost day⁻¹) and (b) *Phylloicus hansonii* resource-specific biomass (mg dry mass g⁻¹ *Cecropia*) for the landscape study. Landscape study conducted in streams consisting of a paired reach containing killifish, crabs, and guppies (KCG) downstream, and killifish and crabs (KC) upstream. Estimates of leaf decay were replicated across streams (n=6 streams per reach type). Letters between bars indicate results of one-way ANOVA, with different letters indicating significant differences in pairs of means between reaches..... 100

Figure 4.6: Bivariate fit of decay rate for *Cecropia peltata* leaves (% mass lost day⁻¹) and *Phylloicus hansonii* resource-specific biomass (mg dry mass g⁻¹ *Cecropia*) from the landscape study. Study conducted in streams consisting of a paired reach containing (a) killifish, crabs, and guppies (KCG) downstream, and killifish and (b) crabs (KC) upstream, and replicated in pools (n=18 pool per reach type). Statistical significance of correlation noted by * (P<0.05) and ** (P<0.01)..... 101

Figure 4.7: *Phylloicus hansonii* resource-specific biomass [mg dry mass g⁻¹ *Cecropia* (\pm SE)], in individual pools averaged over the 4 week landscape study. Landscape study consisting of a reach containing killifish, crabs, and guppies (KCG, pools 1-3) downstream, paired with a reach containing killifish and crabs (KC, pools 4-6) upstream. Estimates of *Phylloicus* biomass were replicated in pools nested within streams (n=6 streams per reach type). Individual pools were separated by at least 50 m and adjacent pools in KCG (pool 3) and KC (pool 4) reaches were separated by no more than 100 m. Analysis showed that changes in *Phylloicus* biomass are not gradual along a stream continuum between pools ($p>0.5$), but abrupt when macroconsumer assemblage changes (reach effect; $p<0.001$).....102

CHAPTER 1

INTRODUCTION AND LITURATURE REVIEW

We are currently in the middle of the sixth mass extinction (Wake and Vredenburg 2008). Biodiversity loss is predicted to disproportionately impact large-bodied consumers, relative to species in lower trophic levels (Petchey et al. 1999, Duffy 2002), therefore, predicting the consequences of macroconsumer loss is a high research priority in both terrestrial and aquatic systems (Sutherland et al. 2013). Here I define macroconsumers as species that are relatively large in their environment and therefore have relatively low predation risk as adults. Macroconsumers have been shown to influence ecosystem structure and function in a diversity of habitats worldwide (Estes et al. 2011, Schmitz et al. 2014).

The bulk of research investigating macroconsumers has focused on the effects of distinct top-consumers on lower trophic levels (Ripple et al. 2014). The removal or introduction of predators can trigger alternating changes in the relative populations of lower trophic levels, a phenomenon called a trophic cascade (Carpenter et al. 1985). Classic examples include decreased algal growth via increased herbivory from minnows following bass removal from streams (Power et al. 1985) and increases in elk populations and changes in the biomass of quaking aspen following wolf extirpation from Yellowstone National Park (Ripple et al. 2001). Food web theory predicts that trophic cascades are strongest in systems with simple linear food web structure and that the presence of omnivory can weaken otherwise strong cascades (Strong 1992, Polis and

Strong 1996). Because omnivores can have an effect on both prey populations and basal resources, their ecosystem effects are often less predictable (Polis and Strong 1996, Tanabe and Namba 2005). For example, the presence of omnivorous crayfish in experimental lakes increased zooplankton biomass by consuming fish eggs while simultaneously reducing the biomass of edible green algae through direct consumption (Dorn and Wojdak 2004). In order to better understand the potential cascading ecosystem effects of macroconsumer loss across systems, we must characterize the effects of removing both predatory and omnivorous consumers in systems without distinct top-consumers.

Research on the cascading effects of terrestrial macroconsumers has focused on relatively large-bodied species (Estes et al. 2011, Ripple et al. 2014). However, in aquatic ecosystems, such as headwater streams, small-bodied fish and crustaceans are often the top consumers. While experimental manipulations investigating the ecosystem effects of macroconsumers in terrestrial or larger aquatic systems can be logistically challenging, macroconsumer manipulations are more tractable in headwater streams, and can help to predict the effects of removing larger-bodied consumers in other systems. Beyond translating these effects to other systems, understanding the drivers of ecosystem function in headwater streams is significant, given their high prevalence across the landscape. Headwater streams contribute to local-scale ecosystem processes as well as global-scale process like the global carbon budget (Butman and Raymond 2011).

Trinidadian guppies have been a model system for evolutionary and ecological research for more than three decades (Magurran 2005). In the Northern Range Mountains of Trinidad, guppies are found across an elevational gradient (Liley and Seghers 1975).

In lower elevation reaches, guppy populations typically evolve under high predation risk from large piscivores, notably *Crenicichla* spp. and *Hoplias malabaricus* (Reznick and Endler 1982). Barrier waterfalls naturally limit the upstream migration of these large predators, such that guppies, the killifish, *Anablepsoides hartii*, and the freshwater crab, *Eudaniela garmani*, typically dominate reaches at higher elevations in the absence of large piscivores (Marshall et al. 2012). Guppies located in high-elevation streams (hereafter guppy *phenotype-1*, G_{P1}) display distinct genetic-based differences in life-history, color, morphology and behavior when compared to guppies from low-elevation streams (hereafter guppy *phenotype-2*, G_{P2}) (Magurran 2005).

Studying the effects of guppies on ecosystems in high elevation headwater streams in Trinidad is ideal because they contain natural controls for the ecosystem effects of guppies. Barrier waterfalls limit upstream colonization by guppies, such that only killifish and crabs are found in the uppermost reaches of streams (Gilliam et al. 1993, Walsh et al. 2011). Stream reaches with killifish, crabs, and guppies (KCG reaches) and those with only killifish and crabs (KC reaches) can be separated by a single waterfall and are often separated by less than 200 m. As a result, reaches with these different animal communities have similar physical habitat and environmental characteristics (Walsh and Reznick 2009) providing a natural control for the ecosystem effects of omnivorous guppies.

The goal of my dissertation is to use Trinidadian guppies as a model system to explore several potentially important direct and indirect effects of macroconsumers, which we have little understanding of in natural systems including: 1), the relative importance of consumptive and non-consumptive effects of macroconsumers on primary

producers, 2) the ecosystem consequences of macroconsumer local adaptation, and 3) the ecosystem effects of losing individual macroconsumer species from a system containing multiple macroconsumers species.

In Chapter 2, I focus on wild populations of guppies in high elevation, low predation risk streams, in order to investigate the importance of guppies for structuring the ecosystem of natural streams. We nested macroconsumer exclusion treatments, within the context of a guppy density manipulation to address two questions: (1) what are the top-down ecosystem effects of guppies relative to the remaining macroconsumer community *in situ*? And (2) how sensitive is the ecosystem to the abundance of guppies? We hypothesized that guppies would have unique top-down effects on algal accrual rates and biomass within the macroconsumer community, because while guppies have been documented to consume algae, killifish and crabs are more predatory and have not been shown to graze on algae. Additionally, we predict that while guppies in natural streams will have consumptive effects which are similar in direction, but lower magnitude to previous *ex situ* mesocosm, the non-consumptive effects of guppies observed in mesocosms will not be observed in natural streams due to increased environmental heterogeneity and increased water movement.

In Chapter 3, I address two questions: (1) what are the top-down ecosystem effects of two distinct, locally adapted populations of a fish consumer (the guppy, *Poecilia reticulata*) *in situ*? and (2) are the relative ecosystem effects of each phenotype distinct at a landscape-scale despite high environmental variability? To address these questions, I examine how trait variation (life history and diet selectivity), in two locally adapted populations (G_{P1} , G_{P2}) of Trinidadian guppy affects stream ecosystem properties

in situ, at a local-scale (one stream) and a larger landscape-scale (eight streams). Because consistent patterns in multiple other traits (life history, color, morphology, and behavior) have been documented in natural populations, I hypothesized that the different ecological effects of these two guppy phenotypes, previously observed in *ex situ* experiments, will also occur in nature despite high levels of environmental variation at local- and landscape- scales.

In Chapter 4, I examine how the loss of guppies from a system with multiple macroconsumers alters top-down effects on leaf litter decomposition – a major energy pathway in headwater streams. To address this question, we used both a mechanistic *in situ* enclosure experiment and a landscape-scale study to examine the cascading effects of macroconsumers on leaf decomposition in the context of the natural presence or absence of guppies, below and above waterfalls. We hypothesized that if the presence of any macroconsumer species reduced the biomass of insect shredders (particularly *Phylloicus*), then macroconsumers would indirectly decrease the rate of leaf litter decomposition. Additionally, because previous work has demonstrated that guppies and killifish are partial intraguild predators, we predicted that the documentation of a trophic cascade would be more likely to occur above waterfalls because the natural exclusion of guppies should enable greater killifish predation on invertebrate shredders, resulting in lower rates of leaf decomposition.

CHAPTER 2

EXAMINING THE EFFECTS OF TRINIDADIAN GUPPIES ON THE STRUCTURE OF THE ECOSYSTEM RELATIVE TO SYMPATRIC MACROCONSUMERS USING AN *IN SITU* DENSITY MANIPULATION¹

¹ Troy N. Simon, M.C. Freeman, A.S. Flecker, J.F. Gilliam, S.A. Thomas, J. Travis, D.N. Reznick, C.M. Pringle
To be submitted to *Freshwater Science*

Introduction

Human impacts on freshwater ecosystems are more likely to reduce the abundance of sensitive taxa rather than cause their extinction (Strayer 2010). Therefore, characterizing the ecosystem effects of changes in the abundance of individual species has strong conservation implications. A major challenge to understanding ecosystem effects caused by changes in abundance of individual species is distinguishing between effects of a single consumer species from a given consumer community, and quantifying these species-specific effects, *in situ*. Isolating species-specific effects in aquatic systems is often difficult because experimental exclusion techniques, such as cages, cannot distinguish between consumers of a similar size and the use of large *in situ* enclosures is often experimentally intractable. While some studies have overcome these challenges and effectively isolated species-specific effects (Taylor et al. 2006), fewer studies have isolated the effects of individual consumer species across a range of densities, *in situ*.

Populations of macroconsumers in the mountain streams of Trinidad provide an opportunity to investigate the ecosystem effects of an individual macroconsumer species, the guppy *Poecilia reticulata*, relative to the remaining community, which includes an insectivorous killifish (*Anablepsoides hartii*) and an omnivorous freshwater crab (*Eudaniela garmani*). This is an ideal study system as guppy populations can be effectively manipulated without the use of enclosures (Reznick et al. 2012). The use of enclosures is unnecessary for the following reasons: 1) guppies often occupy discrete pools, 2) guppies do not frequently migrate between pools during the dry season, and 3) pools are clear and lack vegetation, allowing researchers to effectively remove all guppies from a pool.

Trinidadian guppies are a model system for understanding interactions between evolutionary and ecosystem processes (an emerging field termed *eco-evolutionary dynamics*). In high-elevation streams, guppies (hereafter guppy *phenotype-1*, G_{P1}) experience lower predation pressure than guppies from low-elevation streams (hereafter guppy *phenotype-2*, G_{P2}), and display distinct genetic-based differences in life-history, color, morphology and behavior (Magurran 2005). Some of these traits can evolve rapidly, in as little as four years or eight generations (Reznick et al. 1997).

Previous *ex situ* experiments reported that guppy phenotypes have distinct consumptive and non-consumptive effects on ecosystem structure and function; consumptive being the effect of guppy predation on invertebrates and algae, and non-consumptive being changes in the physical environment of pools caused by guppies (e.g. sediment disturbance and increases in the availability of limiting nutrients). In *ex situ* common garden experiments G_{P1} guppies consumed significantly more algae and significantly fewer invertebrates than G_{P2} guppies (Palkovacs et al. 2009, Bassar et al. 2010). Corresponding to these differences in diet, mesocosms with G_{P1} guppies contained significantly lower algal biomass (Palkovacs et al. 2009, Bassar et al. 2010) and significantly higher biomass of larval midges [(Chironomidae:Diptera) the dominant invertebrate taxon] relative to mesocosms with G_{P2} guppies (Bassar et al. 2010). Non-consumptive effects of guppies included increases in the availability of ammonium (NH_4^+) via excretion, which stimulated gross primary production in mesocosms containing guppies (Bassar et al. 2010). Additionally, G_{P2} guppies were found to excrete ammonium (NH_4^+) at higher rates than G_{P1} guppies (Palkovacs et al. 2009, Bassar et al. 2010).

These studies strongly suggest that guppy evolution may alter the ecosystem, which, in turn, may cause further guppy evolution, resulting in a dynamic interaction between these two processes. In order to determine whether *eco-evolutionary dynamics* is a useful framework for understanding the forces that shape the structure of an ecosystem, as well as the evolutionary trajectories of resident consumers in this system (i.e. guppies), we must first determine if guppies significantly alter the ecosystem relative to other common macroconsumers present in natural streams.

Because previous *ex situ* mesocosm experiments greatly simplified food web diversity by not including predatory insects as well as other macroconsumers species naturally found with guppies, the general significance of the presence and abundance of guppies in structuring the ecosystem *in situ* remains unknown. While results from a previous *in situ* experiment suggested that guppies significantly altered algal accrual and biomass, their experimental design was not able to isolate the effects of guppies from that of the other macroconsumers present (Killifish and crabs) (Marshall et al. 2012).

Here we focus on wild populations of guppies in high-elevation, low-predation streams, representing a single phenotype of guppy (G_{PI} guppies), in order to investigate two questions: (1) what are the top-down ecosystem effects of guppies relative to the remaining macroconsumer community (killifish and crabs) *in situ*? And (2) how sensitive is the ecosystem to the abundance of guppies? To quantify the importance of guppy browsing on algal accrual and biomass, relative to the remaining macroconsumer community, we nested macroconsumer exclosures within pools where we categorically manipulated guppy density. To directly characterize the sensitivity of the ecosystem to the abundance of guppies we used continuously varying, pool-specific, guppy densities to

quantify the per capita effect of guppies on algal accrual rates and biomass in the context of our macroconsumer enclosure treatments including: (1) guppy effects in control frames (full access), which represents the *Net* response of the ecosystem to changes in guppy density, (2) guppy effects in enclosure treatments which represent the *Non-consumptive* ecosystem effects of changing guppy density, and (3) the treatment effects of guppies (i.e. the difference between control and enclosure treatments) which represent the top-down or *Consumptive* effects of changing guppy density.

We hypothesized that guppies would have unique top-down effects on algal accrual rates and biomass within the macroconsumer community, because, while guppies have been documented to consume algae, killifish and crabs are more predatory and have not been shown to graze on algae. Additionally, we predict that while guppies in natural streams will have consumptive effects which are similar in direction, but lower in magnitude to previous *ex situ* mesocosms, the non-consumptive effects of guppies observed in mesocosms will not be observed in natural streams due to increased environmental heterogeneity and increased water movement.

Methods

Study Sites

Research was carried out in the high-elevation streams draining the southern slope of Trinidad's Northern Range Mountains. Experiments were replicated across four streams, with two streams (QRE 2 and QRE 6) located in the headwaters of the Quare River that drains into the Oropouche River and two streams (END and RDN) in the headwaters of the Aripo and Arima Rivers respectively, which drain into the Caroni

River (Figure 2.1). The Oropouche and the Caroni are the two largest drainages on the island. We chose the above four streams because they contained reaches with distinct pool riffle structure and medium sized pools ranging from 5 – 20 m², which allowed us to, more easily manipulate the densities of guppies. Additionally, we chose streams that did not contain large piscivorous fish, thus guppies experienced limited predation pressure from killifish (*Anablepsoides hartii*), which predominantly consume aquatic invertebrates and terrestrial arthropods that fall into streams (Fraser et al. 1999) and only occasionally consume guppies (Reznick and Endler 1982). In addition to guppies and killifish, high-elevation streams contain the freshwater crab, *Eudaniela garmani*, which are opportunistic foragers on aquatic invertebrates, labile coarse detritus, and freshly fallen leaves and fruits (Marshall et al. 2012).

Experiments were conducted toward the end of the characteristic dry season (March 16th-April 13th 2012) to reduce the chances of high discharge events disturbing experiments. Streams chosen for this study drained heavily forested watersheds that provided dense canopy cover above pools. Canopy cover ranged from 5 – 44% openness (Table 2.1). Study reaches were approximately 120 m long and located at elevations from \approx 650 – 700 m a.s.l. Reaches were characterized by a distinct pool and riffle structure (which is exaggerated in the dry season), where stream benthic substrate was dominated by gravel and cobble, with some boulders and large woody debris at the edges of pools. Pools ranged greatly in size (4.63 to 14.65 m²), while mean depth was uniformly shallow (0.10 to 0.32 m) and water velocity relatively slow (0.03 to 0.10 m/s) (Table 2.1). Ambient nutrient availability also varied greatly among streams in both phosphorous

(2.45 to 40.50 $\mu\text{g L}^{-1}$) and nitrate + nitrite (52.48 to 231.45 $\mu\text{g L}^{-1}$) concentrations (Table 2.1).

Experimental Design

We manipulated the density of guppies in pools, across streams. The goal of the density manipulation was to produce a range in guppy densities (that could naturally occur between years due to storm events), and document their effect on algal accrual and biomass in pools. Guppy survival has been shown to decrease when the frequency and intensity of floods increases (Fitzpatrick et al. 2014). The 2011-12 rainy season that preceded our study included several large storm events, which were observed to depress guppy populations in multiple streams in Northern Trinidad (D.N.R unpublished data). Therefore, our experimental manipulation included an increase in guppy densities to reflect densities in years with less extreme discharge events. The resulting densities reflected what has been considered average low and high densities expected for guppy populations in the headwater stream of Northern Trinidad (Bassar et al. 2010).

In each replicate stream, three pools were selected that had defined riffles at the upstream and downstream end of each pool, which acted to reduce guppy migration between pools (Reznick et al. 2012). In headwater streams, guppies preferentially occupy pools and have relatively low migration rates from one pool to the next (Reznick et al. 2012). Pools were randomly assigned to three macroconsumer community treatments: (1) complete guppy removal leaving only killifish and crabs (KC) in pools, which were then checked every two days to record and remove any immigrant guppies; (2) pools where all guppies were removed and then added back at ambient densities leaving killifish, crabs, and guppies, which we considered a low guppy density treatment as stated above

(KCG_{Low}); and (3) pools where all guppies were removed and added back at double ambient density leaving killifish, crabs, and guppies at high densities (KCG_{High}).

Treatment pools were separated by at least 40 m to reduce the possible downstream effects of upstream manipulations.

Guppy densities were manipulated using previously described methods (Reznick et al. 2012). Guppies were collected from pools using butterfly nets and capture techniques that allowed us to avoid standing in the pool, thus limiting our disturbance of the benthic habitat. Captured guppies were immediately placed in buckets of stream water. Guppies were easily captured from clear pools, due to the lack of vegetation, and because they are attracted to the minor sediment disturbance caused by maneuvering nets. In the field and immediately after collection, guppies were lightly anaesthetized with MS-222, measured (standard length), placed in millimeter size classes for each sex, and weighed to the nearest milligram. We used 1 mm size classes ranging from 12 to 28 mm. Individuals <12mm were classified as immature. For *Low* and *High* density treatments, collected fish were returned to their original pools after, all were counted and weighed. In order to increase guppy density without changing the size and sex distribution of the original population, guppies added to the *High* density treatment were matched to the millimeter size categories (for each sex) recorded from the original guppy population of the given pool (methods detailed in Reznick et al. 2012). Finally, to account for migrations in and out of pools, we estimated post-manipulation densities, both before the experiment began and upon experiment completion, (when we again removed, counted, measured and weighed all guppies in each experimental pool).

Macroconsumer Exclusion

To isolate the top-down effects of macroconsumers (guppies, killifish, and crabs) on algae and invertebrates in each pool, an underwater electric enclosure technique was used (modified from Pringle and Blake 1994). A replicate pair of frames – one macroconsumer exclusion treatment (electrified) and one experimental control (non-electrified) – were nested within each guppy density treatment pool (n=12 total pools) (Figure 2.2). Electric frames excluded macroconsumers, but not invertebrates, from small (0.125 m²) benthic patches within each pool. Numerous previous studies have found that this design effectively excludes aquatic consumers across a range of taxa and geographic locations, while eliminating the experimental artifacts associated with enclosure cages (i.e. modifying flow and sedimentation regimes) (Greathouse et al. 2006, Connelly et al. 2008, Moulton et al. 2012).

Treatment frames were constructed of two concentric rectangles of 8-gauge solid copper wire, connected by plastic cable ties (outer rectangle 25 cm x 50 cm, inner 8 cm x 30 cm) (following Marshall et al. 2012). We used Speedrite 1000 fence chargers (Tru-Test Limited, Auckland, New Zealand) set on the low power and slow pulse settings. These settings resulted in energy output of about 1 joule at ≈ 2 sec intervals to power the exclusion frame. Control frames were identically constructed but not electrified. Treatment and control frames were placed in areas of pools with similar physical characteristics, where depth ranged from 10 – 20 cm and current velocity ranged from 0.0 – 0.03 m s⁻¹.

Algal biomass was measured over a four-week period on unglazed (ashed) ceramic tiles (25 cm²), nested within exclusion treatment and control frames. Tiles served as a proxy for hard benthic substrate and were retrieved three times during the first week,

and then weekly for the remainder of each experiment. Algal accrual rates were calculated using a linear fit across the entire 28-day experiment ($\text{mg Chl a m}^{-2} \text{ day}^{-1}$); day 28 biomass was sampled from a large tile (100 cm^2) in order to better characterize final standing stock biomass. Algal biomass was calculated using chlorophyll *a* fluorescence, measured with a Turner Aquafluor handheld fluorometer (Turner Designs, Inc., Sunnyvale, CA, USA) fitted with a chlorophyll *a*-specific wavelength channel, on replicated aliquots of extracted material scraped from tiles (detailed in Marshall et al. 2012). In addition to algal biomass, on day 28 we collected all material accrued on tiles, which were collected and dried onto glass fiber filters in order to measure relative nutrient content (on day 28). The biofilm nitrogen content (%N) was measured using a Carlo Erba 1500 CHN analyzer (Milan, Italy). Phosphorus (%P) content of the biofilm was determined using the plant dry ash/acid extraction method (Allen 1974) followed by colorimetric analysis of the extracted solution using an Alpkem Rapid Flow Analyzer 300 (Alpkem, College Station, TX, USA).

To determine the possible effects of macroconsumers on aquatic invertebrates we sampled invertebrates on a single large unglazed tile (100 cm^2 ; same tile material as algal biomass) on day 28 in order to quantify total invertebrate biomass. Invertebrate samples were dyed with Rose Bengal stain for 24 hours and sorted. All invertebrates $> 1 \text{ mm}$ were counted and identified to the lowest taxonomic resolution possible (usually genus, except for midge taxa) using taxonomic keys by Merritt et al. (2008) and Perez (1988). Length-mass (Benke et al. 1999, Becker et al. 2009) and volume-mass (Feller and Warwick 1988) regressions were used to generate biomass values for insects and non-insect invertebrates, respectively.

Statistical Analysis

Isolating the top-down effects of guppies relative to remaining macroconsumer community: This experiment followed a split-plot design where guppy density represented the whole-plot treatment ($n=3$) and macroconsumer exclusion was the sub-plot treatment ($n=2$) (Figure 2.3). Experimental treatments were blocked by stream ($n=4$) in order to account for differences between streams, among experimental treatments (e.g. ambient nutrient availability or biofilm community composition). Significance testing was conducted with an analysis of variance (ANOVA) using two factors (macroconsumer community and exclusion treatment) with three and two levels respectively: (1) killifish and crabs guppies removed (KC), (2) killifish, crabs, and guppies at low density (KCG_{Low}), (3) killifish, crabs, and guppies at low density (KCG_{High}) and (1) macroconsumer exclusion and (2) control. Significant effects of guppies versus the rest of the macroconsumer community (killifish and crabs) on each response variable (invertebrate biomass, algal accrual rate, algal biomass, and biofilm %N and %P) were determined by analyzing the interaction between the whole-plot treatment (guppy density) and the sub-plot treatment (exclusion). A significant effect of exclusion would show that macroconsumers affected a given response variable regardless of the density of guppies. The effect of macroconsumer community is not a treatment effect of interest, because it combines the effect of excluding and allowing macroconsumers access to frames, therefore results will not be discussed. Additionally, to determine the significance of the top-down effects of each macroconsumer community used Paired T-Tests for each exclusion and control treatments nested within pools. In order to meet the assumption of

normality required by ANOVA, all data was natural-log transformed before statistical analysis.

Estimation of per capita effects of guppies: We used a mixed effects linear regression to estimate the effects of continuously varying guppy density across pools. To account for possible changes in guppy density across the experimental period, we used the average of the total density measured at the beginning and end of the experiment. The response variables examined by linear regression were, treatment-frame specific algal accrual rate ($\text{mg Chl a m}^{-2} \text{ day}^{-1}$) and day-28 biomass (mg Chl a m^{-2}), invertebrate biomass (mg DM m^{-2}), and biofilm percent (%) composition of N and P. We applied natural-log transformations to each response variable. Our analysis nested macroconsumer enclosure and control treatments within streams to account for algal differences among streams, not explained by covariates (modeled as a random effect on the intercept). Pool-specific guppy densities were used as covariates to estimate the per capita effect of guppies, which scaled the output to the effect, increasing density by 1 individual m^{-2} . Additionally our model was crossed with either macroconsumer access (controls) or macroconsumer exclusion, to estimate, respectively, net and non-consumptive effects of guppies across pools on each ecosystem response variable. The resulting regression equations took the form:

$$\text{Response}_{ij} = \alpha_i + \beta_{\text{Net}} * \text{Guppy_Density}_j * \text{Control}_j + \beta_{\text{Non-consumptive}} * \text{Guppy_Density}_j * \text{Exclusion}_j + \text{residual_error}$$

where Response_{ij} was the frame-specific measure for the response variable, α_i was the stream-specific intercept, Guppy_Density_j was the guppy density associated with each treatment frame, and Control_j and Exclusion_j were set to 1, respectively, for control and

exclusion frames and to 0 otherwise. The terms β_{net} and $\beta_{\text{Non-consumptive}}$ were the estimated net and non-consumptive guppy effects. The guppy top-down consumptive effect (or treatment effect) was estimated as the difference between β_{net} and $\beta_{\text{Non-consumptive}}$.

To facilitate simple estimation of net, consumptive, and non-consumptive guppy effects and associated uncertainty, we fit the linear models using a Bayesian framework. Priors for parameter values were specified as normal distributions with a mean of 0 and a precision of 0.001. Priors for standard deviations of the stream effect and residuals were specified as uniform distributions between 0 and 100. The model derived a posterior distribution for the top-down effects of guppies (means and 95% credible intervals) as the difference between net (exclusion control frames) and non-consumptive (exclusion treatment frames) effects. We ran three independent Markov chain Monte Carlo (MCMC) chains of 20000 iterations with a thinning rate of 2 and burn-in of 1000. Convergence was assessed using the Rhat potential scale reduction factor (Gelman 2007). Model fitting was implemented in JAGS v. 3.2 through R package *R2jags* (Plummer 2003). We considered parameter estimates to be statistically significant if the 95% credible interval of the estimate did not include zero.

Results

Guppy density manipulation

Our categorical experimental manipulations of guppy populations effectively altered the densities of guppies in pools (Table 2.2) and mirrored the expected mean high and low densities of guppies reported in previous studies (Bassar et al. 2010). Densities

of guppies in our removal killifish and crab (KC) only treatments were reduced on average from 9.04 individuals m^{-2} before our manipulation to 0.17 individuals m^{-2} during the course of our experiment. Guppy densities in our killifish, crab, and low guppy density treatment (KCG_{Low}) treatments guppies were on average 7.09 individuals m^{-2} at the start of the experiment and increased to 8.41 individuals m^{-2} by the end of our experiment. In our killifish, crab, and high guppy density treatment (KCG_{High}) treatments, we increased densities on average from 7.82 individuals m^{-2} to 14.77 individuals m^{-2} , and as expected, densities decreased during the course of the experiment to 12.54 individuals m^{-2} .

Isolating the top-down effects of guppies relative to remaining macroconsumer community

The split plot ANOVA analysis showed that guppies were the only macroconsumer to affect algal accrual rate and algal biomass. However, while guppy browsing on tiles negatively affected algal accrual rate and biomass, guppies did not consistently affect invertebrate biomass or %N and %P of tile material. We found a significant interaction between macroconsumer exclusion and guppy density treatments on algal accrual ($F_{2,12}=4.34, p<0.05$) and algal biomass ($F_{2,12}=5.79, p<0.05$) (Figure 2.3b,c), but not on %N and %P, or invertebrate biomass (Figure 2.3a,d,e). In KC pools where guppies were removed, algal accrual rate and biomass was similar whether remaining macroconsumers (killifish and crabs) were excluded or allowed access to tiles ($p>0.5$; t-test), suggesting that killifish and crabs did not strongly affect algal accrual or biomass. However, algal accrual rate and biomass tended to be 2.5- and 3- fold higher [non-significantly ($p<0.1$)], respectively when macroconsumers were excluded in

KCG_{Low} treatments and approximately 3- and 4- fold higher ($p < 0.05$; t-test), respectively when macroconsumers were excluded in the KCG_{High} treatments.

Additionally, the effect of excluding macroconsumers without accounting for guppy density significantly reduced algal accrual ($F_{1,12}=6.72, p < 0.05$), %N ($F_{1,12}=8.24, p < 0.05$) and %P ($F_{1,12}=14.09, p < 0.01$) of accrued tile material, and had no effect on algal biomass or invertebrate biomass. No significant block effects were detected among streams for all response variables except %P ($F_{3,6}=15.38, p < 0.01$). Finally we found no significant effect for macroconsumer community regardless of enclosure treatment ($p > 0.1$) for any response variables, however this was not a treatment effect of interest, because it does not take into account effect of excluding macroconsumers.

Estimation of per capita effects of guppies

Regression models showed that although guppies had strong negative top-down *consumptive* effects on algal accrual and biomass, a nearly equal and opposing positive *non-consumptive* effect of guppy excretion on algae accrual rate and biomass resulted in negative but non-significant (with 95% CI overlapping zero) *net* per capita effect of guppies on algal accrual rate and biomass (Figure 2.4). The per capita consumptive effects of guppies significantly (95% credible intervals not overlapping zero) reduced algal accrual rates by 10.2% and biomass by 8.4%, while non-consumptive effects simultaneously increased accrual rates by 7.5% and biomass by 7.6%. *Net* per capita effects of guppies tended to be negative, however, there was large uncertainty surrounding this estimate (95% credible interval overlapping zero -10.1% and 3.2% and -8.4% and 5.8%, respectively). Additionally, the consumptive effects of increasing guppy density reduced %P on accrued tile material by 2.8% and %N by 0.9%, while non-

consumptive effects increased %P by 3.5% and %N by 0.9% on material accrued on tiles, resulting in no significant net effects of guppy biomass on either %N or %P. Finally models showed no significant effects (net, consumptive, or non-consumptive) of increasing guppy biomass on total invertebrate biomass across replicate pools (Figure 2.4).

Discussion

Effects of guppies relative to remaining macroconsumer community

We found strong evidence that guppy grazing of biofilms dominated the top-down effects of macroconsumers on algal accrual rate and algal biomass consistently across four streams in Northern Trinidad. While excluding macroconsumers from tiles had no treatment effect on algal accrual and biomass when guppies were removed, macroconsumer communities with low and high densities of guppies tended to reduce algal accrual and biomass, however these effects were only strongly significant at high guppy density. Additionally, we found no evidence for top-down control of invertebrates by any macroconsumer present in this system. These findings are similar to conclusions from previous *in situ* experiments in this system, which reported no top-down effects on invertebrates and negative top-down effects on algal accrual biomass in stream reaches with killifish, crabs, and guppies (Marshall et al. 2012). Moreover, we found negative per capita, top-down effects of guppies, which further supports our findings that guppies are a key macroconsumer in this system. Given that life history evolution in guppies results in different population growth rates and densities, these results suggest that eco-evolutionary dynamics could be a useful framework for understanding both what shapes

the structure of the ecosystem as well as what shapes the evolutionary trajectories of resident consumers (i.e. guppies).

Several studies have examined the potential for one species of macroconsumer to dominate a particular ecosystem function relative to other resident macroconsumers. For example, field experiments isolating the effects of the armored catfish, *Prochilodus mariae*, from a diverse fish community in a tropical river, strongly suggested that there was no functional redundancy for the large detritivor at either the patch scale (Flecker 1996) or the reach scale (Taylor et al. 2006). Similarly, Connelly et al. (2008) found that tadpoles dominated the top-down effects on algal populations and that sympatric fish or macroinvertebrate did not fill this functional role after tadpoles were extirpated by disease.

The above studies provide strong evidence for a lack of functional redundancy of key consumers within communities; however, several studies have shown that multiple species can hold similar functional roles. For example, Pringle and Hamazaki (1998) found that both shrimp and fish species had negative top-down effects on several measures of stream ecosystem structure including algal and invertebrate biomass. Similarly, Schofield et al. (2008) predicted that the extirpation of obligate benthic insectivores would release benthic macroinvertebrate from top-down control, yet counter to this prediction they found that the remaining fish community maintained top-down control after the loss of the specialist species. Together, these results suggest that predicting the functional redundancy of a species within a community is often difficult and does not merely depend on the diversity of species or of the presence of species that appear to be specialists.

Our results suggested that guppies hold a unique role within macroconsumer communities especially when found at high densities. While we did not find strongly significant effects of guppies at low densities, this was likely caused by our relatively small sample size (four replicate streams). Given that the effects of guppies at low densities are consistent with effects reported in previous *in situ* experiments, it is likely that these results would be significant with increased replication. Of the four pools sampled for the low-density treatment, three had strong negative effects, while one had positive effects, which was likely due to a sloughing event that reduced the biomass of algae remaining on the tiles in exclosures. Moreover, a previous *in situ* study found experimental evidence that guppies, at densities even lower than our low-density treatment, had significant negative top-down effects on algal accrual and biomass (Marshall et al. 2012).

Per Capita Effects of Guppies

Counter to our prediction, in addition to a negative per capita consumptive effect of guppies on algal accrual and biomass we found positive per capita non-consumptive effects of guppies. Knoll et al. (2009) and Capps et al. (2014) found that grazing by armored catfish (*Ancistrus triradiatus* and Loricariidae: *Pterygoplichthys*, respectively) depressed algal biomass, while catfish-mediated nutrient excretion enhanced algal biomass within grazing exclosures. We report similar consumptive and non-consumptive effects of guppy density on algal accrual and biomass and several lines of evidence suggest that non-consumptive effects were a consequence of guppy excretion stimulating algal biomass accrual. First, we found that the non-consumptive effect of increasing guppy density increased %P and %N of material accruing on tiles with algae, which

strongly suggests that in the absence of grazing, increasing the density of guppies increased nutrient availability on tiles. Second, a previous *ex situ* experiment found that the presence of guppies in mesocosms increased ambient N and P flux in the water column, again suggesting guppies increased nutrient availability (Bassar et al. 2010). Third, previous *in situ* work has shown that excretion by guppies can contribute 30% of biofilm nitrogen demand, suggesting that guppy excretion may be an important component of nutrient cycling in natural streams (Marshall *in prep*).

Sterner (1986) showed that zooplankton remineralisation of nitrogen (N) and phosphorous (P) stimulated algal reproductive rates as strongly as increased mortality from zooplankton grazing, resulting in no net effect of zooplankton on phytoplankton in the water column of a pond. Similarly, our results suggest that both guppy excretion and consumption had strong effects on algae, which resulted in reduced net per capita effects of guppies on algal biomass accruing on benthic tiles in a headwater stream. This result was surprising and counter to our prediction that the net effects of increasing guppy density on algal accrual and biomass in pools would be strongly negative due to large consumptive effects. However, we caution against interpreting this non-significant effect to mean there is no net effects of guppies on algal biomass in this system. Instead we suggest that the trend toward negative net effects of guppies on algae would be stronger with increased replication, which would reduce the uncertainty surrounding our estimate. Additionally, it is possible that algal biomass was too coarse of an ecosystem response for the effects of guppy grazing on primary producers given our limited replication.

While we found large variability surrounding the net per capita effects of guppies on algal biomass accrual, guppy browsing on tiles could have resulted in more consistent

effects on biomass-specific algal primary productivity and algal community structure. For example, tadpole grazing on benthic algae in a tropical stream has been shown to increase biomass-specific net primary production by removing less productive algal cells (Connelly et al. 2008). Additionally, previous *ex situ* studies in our system reported that guppy grazing in mesocosms stimulated biomass-specific gross primary production (Bassar et al. 2010). Therefore, further research is needed to examine these effects on biomass-specific primary productivity and algal community composition in order to fully characterize the net per capita effects of guppies on primary producers.

We wish to note that although we found significant per capita top-down effects of guppies on %N and %P using linear models, we did not detect a treatment effect of adding guppies to macroconsumer communities on N and P in our split-plot ANOVA analysis. However, the disjoint in the results of our two statistical analyses may not be surprising given that the effect of consumers on N and P content are likely to be directly linked to actual guppy densities rather than a categorical manipulation of high or low densities. Additionally our inability to detect significant effects using our ANOVA analysis may again have been a consequence of low replication. Of the 12 pools sampled, the 3 largest positive effects of excluding macroconsumers on %N and %P were found in pools containing Ambient or >Ambient guppy densities, providing some evidence that the presence of guppies can alter biofilm nutrient content.

Conclusions

Few studies have isolated the species-specific ecosystem effects of a macroconsumer community in the context of an unconstrained, *in situ*, density manipulation. Not only did this approach allow us to compare the relative top-down

effects of guppies to that of killifish and crabs, but also estimate the per capita ecosystem effects of guppies. By estimating the per capita effects of guppies we found that guppies can also simultaneously stimulate algal biomass accrual and that the opposing consumptive and non-consumptive effects can decrease the overall net effects of guppies on algal accrual and biomass. Moreover, these results have strong implications for our understanding of the interactions between ecological and evolutionary interactions in this model system. We found that the effects of one phenotype of guppy (G_{P1} guppy) are similar in both *ex situ* mesocosm and *in situ* experiments, which strongly suggests that the ecosystem effects of trait evolution observed in mesocosms could occur in natural streams.

Acknowledgements

We thank T. Mcdevitt-Galles for his help during field sampling; this work would not have been possible without his heroic effort. We thank K. Murray, C. Nguyen, A. Snellgrove, and E. White for their help processing samples in the lab. We thank P. Bumpers, D. Manning and the members of the Pringle Lab for their extensive manuscript comments. We thank the Water and Sewage Authority of Trinidad and Tobago, the University of the West Indies at St. Augustine, and the Asa Wright Nature Centre for permission to access various sites and for use of facilities. The authors appreciate the help and support of Jogie Ramlal and the Ramlal family. This work was supported by a National Science Foundation Frontiers in Integrative Biological Research (FIBR) Grant to DNR and others (NSF-FIBR, DEB-0623632) and the NSF Graduate Research Fellowship program (fellowship to TNS).

Table 2.1: Mean estimate of physiochemical characteristics from pools across replicate streams (n=4) for each density manipulation treatment. END= Ender Stream, QRE2 and QRE6= tributaries of the Quare River, RDN= Ramdeen Stream. Physiochemical characteristics were measured one day after experiment was completed (April 13th 2012).

Macroconsumer Community	Stream	Physiochemical Characteristics					
		Area (m ²)	Canopy Openness	Depth (m)	Velocity (m/s)	P (µg L ⁻¹)	NO ₃ ⁻ + NO ₂ ⁻ (µg L ⁻¹)
KC	END	8.74	30.42	0.18	0.05	2.45	150.98
	QRE2	9.74	8.49	0.32	0.07	3.58	55.64
	QRE6	7.15	7.73	0.15	0.03	2.04	52.48
	RDN	4.63	10.19	0.15	0.04	40.50	231.55
	Mean (SE)	7.56 (1.11)	14.21 (5.43)	0.20 (0.04)	0.05 (0.01)	12.14 (9.46)	122.66 (42.89)
KCG _{Low}	END	6.75	17.51	0.23	0.08	5.11	125.73
	QRE2	12.66	14.43	0.17	0.04	3.17	59.50
	QRE6	11.64	6.24	0.14	0.05	2.45	57.00
	RDN	5.59	7.45	0.23	0.06	26.98	198.25
	Mean (SE)	9.16 (1.76)	11.41 (2.72)	0.19 (0.02)	0.06 (0.01)	9.43 (5.88)	110.12 (33.41)
KCG _{High}	END	9.03	44.13	0.13	0.10	2.86	113.73
	QRE2	14.65	7.13	0.10	0.05	6.95	60.40
	QRE6	5.16	13.87	0.10	0.03	5.72	64.91
	RDN	12.86	5.20	0.12	0.06	25.03	166.71
	Mean (SE)	10.42 (2.11)	17.58 (9.04)	0.11 (0.01)	0.06 (0.01)	10.14 (5.04)	101.44 (24.88)

Table 2.2: Guppy densities (individuals m⁻²) in pools across replicate streams (n=4) before (pre-) and after (post-) each density manipulation treatment was carried out. To account for migrations in and out of pools during our experiment, we also estimated post-manipulation densities upon experiment completion. The average density of guppies present in guppy Removal treatment pools during the experiment was used to calculate the post-manipulation density after the experiment. See Table 2.1 caption for stream names associated with abbreviations.

Macroconsumer Community	Stream	Guppy Density (indiv. m ⁻²) [Mean (SE)]		
		Pre-Manipulation	Experiment Start	Experiment End
KC	END	6.63	0.00	0.22
	QRE2	3.69	0.00	0.14
	QRE6	8.53	0.00	0.11
	RDN	17.30	0.00	0.20
	Mean (SE)	9.04 (2.93)	0.00 (0.00)	0.17 (0.02)
KCG _{Low}	END	4.74	4.74	6.52
	QRE2	5.61	5.61	6.08
	QRE6	8.34	8.34	8.51
	RDN	9.66	9.66	12.53
	Mean (SE)	7.09 (1.15)	7.09 (1.15)	8.41 (1.59)
KCG _{High}	END	7.65	15.29	8.19
	QRE2	5.46	10.31	8.14
	QRE6	3.49	6.20	18.66
	RDN	14.69	27.29	15.29
	Mean (SE)	7.82 (2.44)	14.77 (4.57)	12.54 (2.26)

Figure Legends

Figure 2.1: Location of study sites in the Caroni and Oropuche drainages of Trinidad's Northern Range (Inset A). Streams are coded by numbers (1) RDN= Ramdeen Stream, (2) END= Ender Stream, (3) QRE2 and (4) QRE6= tributaries of the Quare River.

Figure 2.2: Schematic of experimental design for one block, experiment was blocked by stream (n=4). Experimental design isolated the ecosystem effects of one species of macroconsumer, the guppy *Poecilia reticulata*, from the remaining macroconsumer assemblage (including the killifish, *Anablepsoides hartii*, and the crab, *Eudaniela garmani*). Guppies were either removed from pools leaving only killifish and crabs (KC) present, removed and replaced at ambient (Low) density (KCG_{Low}), or removed and replaced at double ambient (high) density (KCG_{High}). To isolate the top-down effects of each macroconsumer community, underwater electric fences were placed in each pool to exclude macroconsumers from localized areas of the stream bottom.

Figure 2.3: Mean (\pm SE) for the (a) biomass of total benthic invertebrate community (b) rate of algal accrual, (c) algal biomass, and percent (%) composition of (d) nitrogen (N) and (e) phosphorous (P) from experiment manipulating guppy density within pools across streams (n=4). Underwater electric frames manipulated the presence (light grey) and absence (dark grey) of all macroconsumers (killifish, crabs, guppies) within pools from localized areas of

the stream bottom. Split-plot statistical analysis investigated the interaction between macroconsumer community (*whole-plot*) and macroconsumer exclusion (*sub-plot*) treatments to test if the effect excluding macroconsumer depended on the presence of guppies within macroconsumer community. We found significant interactions effects for algal accrual rate and algal biomass, but no other ecosystem metrics. Additionally, the top-down effects of each macroconsumer community were tested using paired T-Tests for each enclosure and control treatments nested within pools, significant differences signified by * ($p < 0.05$); † ($p < 0.1$).

Figure 2.4: Estimated percent (%) change caused by the per capita consumptive (dark grey), Non-consumptive (light grey), and net (black) effects guppies on invertebrates biomass (mg m^{-2}), Chl *a* accrual rate ($\text{mg m}^{-2}/\text{day}$), Chl *a* biomass (mg m^{-2}), and percent (%) composition of phosphorous (P) and nitrogen (N) in biofilm. Per capita effects of guppies was estimated using linear mixed effects models, where guppy biomass was a continuous explanatory variable to estimate the biomass specific effect of guppies which scales our output to the effect increasing density by 1 individual m^{-2} . Positive values for percent change represent positive effects of guppies and negative values represent negative effects. Figures show 95% credible intervals (CI) around estimated effect sizes. Estimates where 95% CI do not include zero are signified by *.

Figure 2.1

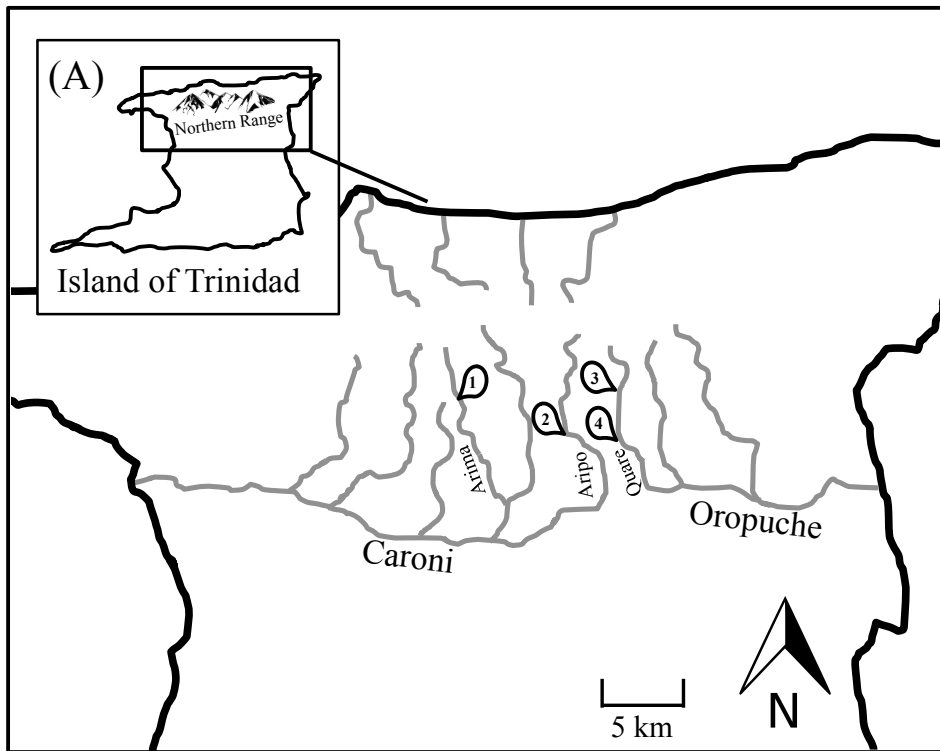


Figure 2.2

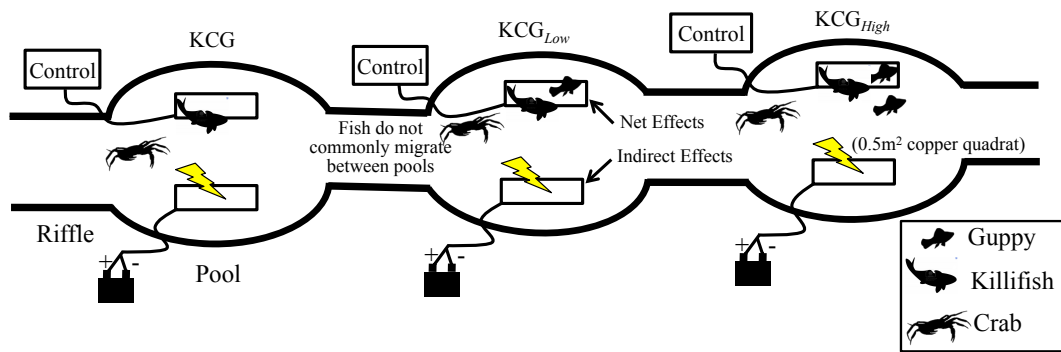


Figure 2.3

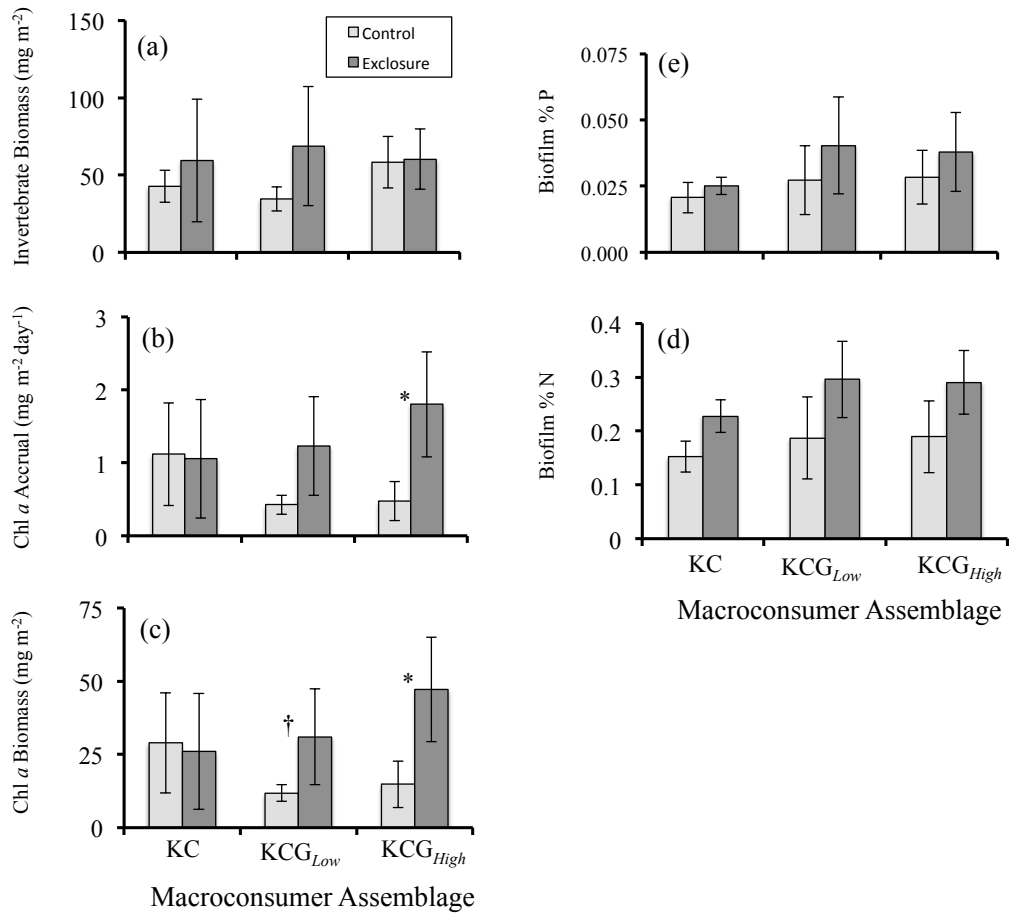
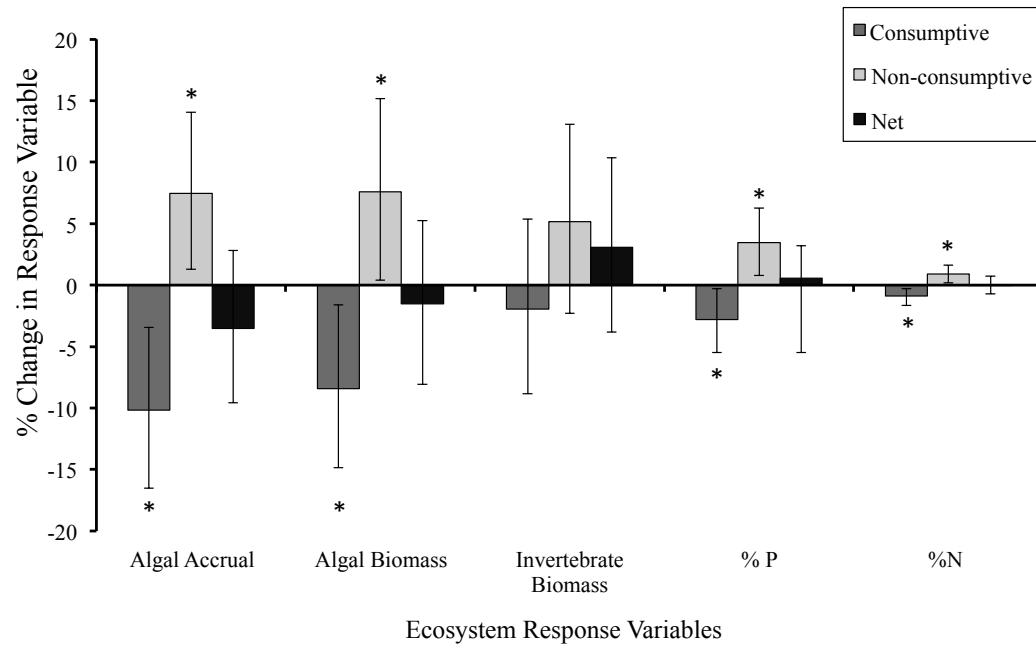


Figure 2.4:



CHAPTER 3

LOCAL ADAPTATION IN TRINIDADIAN GUPPIES ALTERS ECOSYSTEM STRUCTURE *IN SITU* AT LOCAL- AND LANDSCAPE- SCALES²

² Troy N. Simon, M.C. Freeman, A.J. Binderup, M.C. Marshall, R.D. Bassar, A.S. Flecker, J.F. Gilliam, S.A. Thomas, J. Travis, D.N. Reznick, C.M. Pringle
To be submitted to *Ecology Letters*

Abstract

In this paper we address two questions: (1) what are the top-down ecosystem effects of two distinct, locally adapted populations of a fish consumer (the guppy, *Poecilia reticulata*) *in situ*? and (2) are the relative ecosystem effects of each phenotype distinct at a landscape-scale despite high environmental variability? First, we experimentally assessed how each guppy phenotype (abbreviated here as G_{P1} , G_{P2}) affected algal primary producers and invertebrate consumers *in situ*, in paired reaches of one headwater stream. We then measured algal and invertebrate biomass in paired reaches of seven additional streams within the larger drainage that differ in terms of the presence of G_{P1} or G_{P2} guppies.

Experiments demonstrated that each guppy phenotype had significant top-down effects on different aspects of the ecosystem. G_{P1} guppies significantly reduced algal accrual rates by 18% (SE \pm 6%) and biomass by 52% (\pm 31%), and had highly variable, non-significant effects on invertebrate biomass, which, on average, reduced biomass by 49% (\pm 470%). G_{P2} guppies significantly reduced biomass of invertebrates by 84% (\pm 74%), but not algae accrual or biomass; on average algal accrual rates were reduced by 4% (\pm 11%), and biomass by 8% (\pm 10%).

In our expanded landscape-scale assessment we found similar negative effects of G_{P1} and G_{P2} guppies on algal and invertebrate biomass, respectively, yet our ability to distinguish between the effects of each phenotype was much stronger for algal biomass relative to invertebrate biomass. We found that the effect of replacing G_{P2} with G_{P1} guppies resulted in a significant reduction in algal biomass [Mean (Lower and Upper 95% Credible Intervals) -34% (-53%, -12%)]; however, we did not detect a significant

phenotype effect on invertebrate biomass despite a strong trend toward a positive effect (43% (-10%, 114%). Additionally, we found that the effect of exchanging guppy phenotypes on algal biomass was comparable to the effect of increasing canopy openness by one standard deviation of the values measured across the landscape (equivalent to 40% of the observed variation). These findings demonstrate that divergent local adaptation can be ecologically significant relative to an important environmental driver at the landscape-level.

Introduction

G.E. Hutchinson's classic essay *The Ecological Theater and the Evolutionary Play* (1965) provides a conceptual framework for the field of evolutionary ecology. Hutchinson used a metaphor to illustrate how ecology drives evolution: the "ecological theater" provides the "stage design" (niche), to which the "evolutionary play" tailors the "actors" (organisms). Recent experimental evidence has also shown that the "evolutionary play" can affect the "ecological theater" – altering populations, communities, and ecosystems (Schoener 2011). This alternative perspective, whereby ecological and evolutionary forces interact in contemporary time has been termed *eco-evolutionary dynamics* (Schoener 2011).

Despite much empirical evidence that evolution can influence ecological processes *ex situ* (Yoshida et al. 2003, Bassar et al. 2010) or in common garden field experiments (Turcotte et al. 2011, Agrawal et al. 2013), a major challenge in assessing the general significance of eco-evolutionary dynamics is testing whether the evolution-to-ecology pathway is frequent and strong enough to regulate communities and ecosystems

in natural ecosystems (Schoener 2011). Few studies have reported evidence of evolutionary change altering communities and ecosystems in nature because the ecological effects of locally adapted phenotypes are often difficult to isolate in comparable environments in contemporary time (but see Post et al. 2008, Urban 2013). Isolating these effects is particularly challenging given that complex species interactions and environmental variation may diminish the ecological consequences of evolutionary change *in situ*.

The well-documented case of rapid evolution by the small freshwater guppy, *Poecilia reticulata*, provides an opportunity to test the ecosystem consequences of evolutionary change in a complex natural system. In the mountain streams of Trinidad's Northern Range, two locally adapted phenotypes of guppy have been described from localities that differ in predation pressure (Reznick and Endler 1982). Guppies located in high-elevation streams (hereafter guppy *phenotype-1*, G_{P1}) experience lower predation pressure than guppies from low-elevation streams (hereafter guppy *phenotype-2*, G_{P2}), and display distinct genetic-based differences in life-history, color, morphology and behavior (Magurran 2005). Some of these traits can evolve rapidly in natural streams, within as little as four years or eight generations (Reznick et al. 1997).

While the effects of each guppy phenotype on ecosystem structure and function have been well-documented *ex situ* (Palkovacs et al. 2009, Bassar et al. 2010, Bassar et al. 2012), the strength of these effects *in situ* is uncertain. In *ex situ* common garden experiments G_{P1} guppies consumed significantly more algae and significantly fewer invertebrates than G_{P2} guppies (Palkovacs et al. 2009, Bassar et al. 2010). Corresponding to these differences in diet, mesocosms with G_{P1} guppies contained significantly lower

algal biomass (Palkovacs et al. 2009, Bassar et al. 2010) and significantly higher biomass of larval midges [(Chironomidae:Diptera) the dominant invertebrate taxon] relative to mesocosms with G_{P2} guppies (Bassar et al. 2010). Additionally, an *in situ* manipulative experiment has suggested that some top-down effects of guppies observed in *ex situ* experiments are reflected in natural streams containing G_{P1} guppies (Marshall et al. 2012, Simon *In Prep*), yet conclusive evidence that the two phenotypes have differing and persistent ecosystem effects in natural streams has yet to be documented.

Here we address two questions: (1) what are the top-down ecosystem effects of two distinct, locally adapted populations of a fish consumer (the guppy, *Poecilia reticulata*) *in situ*? and (2) are the relative ecosystem effects of each phenotype distinct at a landscape-scale despite high environmental variability? To address these questions, we examine how trait variation (life history and diet selectivity) in two locally adapted populations (G_{P1} , G_{P2}) of Trinidadian guppy affects stream ecosystem properties *in situ*, at a local-scale (one stream) and a larger landscape-scale (eight streams). Because consistent patterns in multiple other traits (life history, color, morphology, and behavior) have been documented in natural populations, we hypothesize that the different ecological effects of these two guppy phenotypes, previously observed in *ex situ* experiments, will also occur in natural streams despite high levels of environmental variation at local- and landscape- scales. Specifically, we predict that: (1) at the local-scale, G_{P1} guppies will significantly reduce algal standing stocks and algal accrual rates but not invertebrate biomass, while G_{P2} guppies will significantly reduce invertebrate biomass, but not algal biomass or algal accrual rates; (2) at the landscape-scale, stream reaches characterized by either G_{P1} or G_{P2} guppies will reflect patterns in the relative

biomass of algal and invertebrate biomass observed in local-scale experiments despite higher levels of environmental variation; and (3) relative to previous *ex situ* mesocosm experiments (Palkovacs et al. 2009, Bassar et al. 2010), the effects of each guppy phenotype on algal and invertebrate biomass will be similar in direction, but lower in magnitude in natural streams.

Methods

Study Site

Our studies were carried out within the Caroni drainage on the southern slope of Trinidad's Northern Range Mountains. The Caroni is the largest drainage in Trinidad (\approx 60,000 ha) and is located in the southwestern half of the Northern Range Mountains (Figure 3.1). Rivers draining into the Caroni are characterized by fish communities that are separated by intermittent waterfalls, resulting in a consistent pattern of declining fish diversity with increasing elevation. In lower elevation reaches, guppy populations (G_{P2} guppies) typically evolve under high predation risk from large piscivores, notably *Crenicichla* spp. and *Hoplias malabaricus* (Reznick and Endler 1982). Barrier waterfalls restrict the access of fish species, such that guppies and the killifish, *Anablepsoides hartii*, typically dominate reaches at higher elevations in the absence of large piscivores. In these upper reaches, guppy populations (G_{P1} guppies) have evolved under low-predation risk from killifish, which only occasionally consume guppies and primarily forage on terrestrial ants and larval aquatic midges (Fraser et al. 1999). Killifish can disperse over land and bypass barriers during wet periods and are commonly the only fish species present in the most upstream reaches of streams. These isolated, killifish-only

reaches are ideal for tracking the evolution of small populations of G_{P2} guppies that have been experimentally introduced from lower elevations into low-predation risk environments (Reznick et al. 1990).

Local-Scale Experiments

As part of a *Frontiers in Integrative Biological Research* (FIBR) project funded by the National Science Foundation (2007-2011), populations of G_{P2} guppies from low-elevation streams were introduced upstream into four high-elevation focal study streams (Pennisi 2012). To isolate the ecosystem effects of each guppy phenotype, we experimentally manipulated the presence and absence of G_{P2} and G_{P1} guppies in an upstream and downstream reach of one of the focal study streams [the Caigual (Figure 3.1)]. The downstream reach of the Caigual contains a natural population of G_{P1} guppies, killifish, crabs ($G_{P1}KC$) and the upstream reach contains introduced G_{P2} guppies and native populations of killifish and crabs ($G_{P2}KC$). We conducted our first experiment (22 February – 23 March, 2009) in the downstream $G_{P1}KC$ reach of the Caigual, prior to the introduction of G_{P2} guppies upstream to preclude any potential confounding of native G_{P1} guppy effects with effects produced by introducing G_{P2} guppies or their offspring moving downstream. Our second experiment (26 April - 24 May 2010) was conducted in the $G_{P2}KC$ reach one year after G_{P2} guppies were introduced, in order to allow the small introduced guppy population (38 individuals) to expand and include first and second-generation individuals (generation time \approx 5 months).

Both experiments were conducted in the dry season when stream discharge was low (except for occasional storms) to reduce the confounding effects of seasonal variation in stream discharge between experiments. The Caigual drains a heavily forested

watershed (≈ 170 ha) that provided abundant canopy cover over both the $G_{P1}KC$ and $G_{P2}KC$ reaches (Table 3.1). Study reaches (200 m long) were separated by ~ 200 m and located at elevations from $\approx 650 - 700$ m a.s.l. Both reaches were characterized by a distinct pool and riffle structure, where stream benthic substrate was dominated by gravel and cobble, with some boulders and large woody debris at the edges of pools. Pools in the $G_{P1}KC$ reach were slightly deeper with faster moving water than pools in the $G_{P2}KC$ reach (Table 3.1).

To isolate the top-down effects of macroconsumers (guppies, killifish, and crabs), on algae and invertebrates, we used an underwater electric enclosure technique (modified from Pringle and Blake 1994). A number of previous studies have found this design to work effectively in excluding aquatic consumers across a range of taxa and geographic locations (Greathouse et al. 2006, Connelly et al. 2008, Moulton et al. 2012). Five replicate pairs of macroconsumer exclusion (electrified frames) and control (non-electrified frames) treatments were nested within each of five pools in each of the two study reaches (200 m) of the Caigual focal stream. Electric frames excluded macroconsumers from small (0.125 m^2) benthic patches within each pool and pools were located ≈ 40 m apart within each study reach.

Treatment frames were constructed of two concentric rectangles of 8-gauge solid copper wire, connected by plastic cable ties (outer rectangle 25 cm x 50 cm, inner 8 cm x 30 cm) (following Marshall et al. 2012). We used Speedrite 1000 fence chargers (Tru-Test Limited, Auckland, New Zealand) set on the low power and slow pulse settings. These settings result in energy output of about 1 joule at ≈ 2 -sec intervals to power the exclusion frame. Control frames were identically constructed but not electrified.

To confirm observations from previous experiments that guppies were the dominant macroconsumer visiting control frames in the Caigual (Marshall et al. 2012), we estimated the density of macroconsumers (guppies, killifish, and crabs) over timed observation periods. Each control treatment frame was observed for one hour, spread across four observation periods per experiment: two diurnal and two nocturnal. We recorded the number of each macroconsumer present (0.125 m^2) using visual point counts once a minute over a 15 min period ($n=15$ point counts). Before making observations we waited quietly for 5 min to allow macroconsumers to resume normal activity. Density of each species was estimated as an average over the 15-point counts (following Reznick et al. 2001).

Algal biomass was measured over a four-week period on unglazed (ashed) ceramic tiles (25 cm^2) nested within control and exclusion treatments. Tiles served as a proxy for hard benthic substrate and were retrieved three times during the first week, and then weekly for the remainder of each experiment. Peak algal biomass was measured on day 21 and day 28 in $G_{P1}KC$ and $G_{P2}KC$ reaches, respectively, to assess algal standing stocks prior to sloughing in the $G_{P1}KC$ reach and after it had recovered from a storm event that occurred on day 15 in the $G_{P2}KC$ reach. Additionally due to the storm event in the $G_{P2}KC$ reach on day 15 we calculated algal accrual rates across the first 14 days of rapid growth. Accrual rates were calculated using a linear fit of the algal biomass accrued on days 0, 3, 7, and 14. Algal biomass was calculated using chlorophyll *a* fluorescence measured with a Turner Aquafluor handheld fluorometer (Turner Designs, Inc., Sunnyvale, CA, USA) fitted with a chlorophyll *a*-specific wavelength channel, on

replicated aliquots of extracted material scraped from tiles (detailed in Marshall et al. 2012).

Invertebrates were sampled on day 28 with a stovepipe core of the stream bottom within each treatment frame. We sampled one core (102.6 cm²) per treatment frame, equivalent to 16% of the quadrat area not affected by other sampling substrates (tiles). Samples were dyed with Rose Bengal stain for 24 hr and sorted, and all invertebrates > 1 mm were counted and identified using taxonomic keys by Merritt et al. (2008) and Perez (1988). Length-mass (Benke et al. 1999, Becker et al. 2009) and volume-mass (Feller and Warwick 1988) regressions were used to generate biomass values for insects and non-insect invertebrates, respectively. Because previous studies found that guppy diets contain mostly small aquatic insects (Bassar et al. 2010), we removed predatory aquatic invertebrates (which tended to be the largest invertebrates) from our statistical analysis to better isolate the top-down effects of guppy foraging behavior on invertebrates (Bassar et al. 2012).

Landscape-Scale Study

To put results of our local-scale experiments in the context of environmental variability at a larger landscape-scale, we expanded our studies to include seven additional streams draining watersheds within the larger Caroni River drainage (\approx 14,053 ha) for a total of eight study streams (Figure 3.1). We measured algal and invertebrate biomass in each of the eight streams, with paired upstream (guppy-free) and downstream (guppy-dominated) reaches isolated by barrier waterfalls. Streams dominated by natural populations of G_{PI} guppies were located in the Aripo, Guanapo, Arima, and Tacarigua,

while four subwatersheds of the Guanapo were dominated by experimentally introduced G_{P2} guppies (Figure 3.1).

All study streams were sampled during one week in the dry season at base flow (April 20th-27th, 2011). In each of the eight study streams we sampled the first seven pools up- and down- stream of barrier waterfalls where stream gradient was < 7% (avoiding cascades of waterfalls). Elevation was similar among subwatersheds, ranging from 600 – 750 m a.s.l, but many physiochemical variables varied greatly among sites (Table 3.1): discharge ($Q \text{ m}^3 \text{ s}^{-1}$) ranged from 0.33 - 2.65 $\text{m}^3 \text{ s}^{-1}$; total N ($\text{NO}_2 + \text{NO}_3$) ranged from 126 - 314 $\mu\text{g L}^{-1}$ and soluble reactive phosphorous (SRP) ranged from 0.87 - 25.95 $\mu\text{g SRP L}^{-1}$. Our water chemistry measurements were conducted once at the downstream end of each reach; however, our values of SRP and total N in the G_{P2} introduction reaches are consistent with annual means reported in previous studies (Kohler et al. 2012). All sites contained heavily forested watersheds that ranged from \approx 53 - 170 ha in drainage area, with mean canopy openness ranging from 3.4 - 24 %. Similar to the Caigual focal study stream (where we ran local-scale experiments), all study reaches were characterized by a distinct pool and riffle structure, with stream benthic substrate dominated by gravel and cobble, and some boulders and large woody debris at the edges of pools. Pool morphology was highly variable within and between sites (Table 3.1), with pool area ranging from 1.8 - 28.2 m^2 , mean depth ranging from 0.06 - 0.27 m, and mean velocity ranging from 0.01 - 0.05 m s^{-1} .

Fish abundance was quantified by observations (guppies) and baited trapping (killifish) in pools. We visually estimated guppy density, similar to that used in local-scale experiments, within a standardized quadrat area (1 m^2) during the day in the seven

downstream (guppy dominated) pools of each stream. Guppy density was estimated as an average 16-point counts (once every 45 seconds for 12 min). To insure that killifish abundance was similar between paired upstream and downstream reaches, a single baited trap was placed in each pool (n=14 per stream) for 5 min. All captured killifish were counted and promptly returned to a pool yielding a catch per unit effort (CPUE; Seber 1982). Crabs were never observed in quadrats during observation periods or in baited minnow traps used for CPUE of killifish; however, they were present at low densities in all streams sampled (T.N.S personal observation).

Algal standing stock biomass was calculated by measuring chlorophyll *a* collected from natural substrates on the stream bottom using a suction sampler (4.823 cm²) modified from Loeb (1981). Five Loeb samples were combined for each of the seven pools per reach into a single sample bag and analyzed for chlorophyll *a* using methods described earlier. Invertebrates were collected from the stream bottom of pools using two separate stovepipe cores (102.6 cm²) combined into a single sample bag for each of the seven pools per reach and measured and identified using methods described earlier. Biomass for non-predatory taxa were processed and prepared for statistical analysis in the same manner as reported above for local scale experiments.

Statistical Analysis

Local-Scale Experiments: The magnitude of top-down control was estimated by calculating a single value for the treatment effects of macroconsumers between each control and exclosure treatment replicate (n=5 pairs). Magnitude of the effects were calculated as natural log response ratios for statistical analysis in order for data to meet assumptions of normality (Hedges et al. 1999).

$$\ln\left(\frac{\text{Variable}_{control}}{\text{Variable}_{exclosure}}\right)$$

One-sample t-tests were used to determine if the effect size of macroconsumers was statistically different from 0 for each experiment. All statistical analyses were conducted using R (R Version 3.0.1).

Landscape-Scale Study: We used mixed effects linear regression to estimate the fixed effects of guppy phenotype (either LP or HP) and percent canopy openness on standing stock biomass of algae and macroinvertebrates, while stream (n=8) was included as a random effect. We included canopy openness in our models because light availability in particular has been identified as a significant driver of algae and invertebrate biomass in this system (Heatherly in prep; Kohler et al. 2012; El Sabaawi et al. 2015). Our analysis nested pools within streams in order to estimate the size and uncertainty of each guppy phenotype effect on algae or invertebrate biomass relative to guppy-free locations, while accounting for canopy openness (standardized to a mean of 0 and standard deviation of 1), a possible interaction between each phenotype and canopy openness, and differences among streams not explained by covariates (modeled as a random effect on the intercept). Additionally, we used natural log transformations to normally distribute our response variables (algae and invertebrate biomass).

We fit linear models of algae and invertebrate biomass using a Bayesian framework, which allowed us to estimate the net effect on algae or invertebrate biomass of exchanging HP with LP guppies (LP – HP = Phenotype Effect) and the associated uncertainty in this estimate. Priors for effect sizes were specified as normal distributions

with a mean of 0 and a precision of 0.001. Priors for standard deviations of the stream effect and residuals were specified as uniform distributions between 0 and 100. We ran three independent Markov chain Monte Carlo (MCMC) chains of 20000 iterations with a thinning rate of 2 and burn-in of 1000. Convergence was assessed using the Rhat potential scale reduction factor (Gelman 2007). Model fitting was implemented in JAGS v. 3.2 through R package R2jags (Plummer 2003). We considered parameter estimates to be statistically significant if the 95% credible interval of the estimate did not include zero. To insure that our parameter estimates were not biased by statistical outliers, we ran our models both with and without the top two outliers identified in each dataset (algae or invertebrate biomass). Outliers in our dataset were identified using Cook's distance regression diagnostic (Cook 1977).

Results

Local-Scale Experiments

No macroconsumers were observed within enclosure treatments except when electricity was briefly turned off to collect samples. Observations confirmed that guppies were the most numerically abundant macroconsumer to visit control treatments in both experiments. Guppy densities were on average at least 8x that of other macroconsumer taxa observed in control treatments. Guppies were active exclusively during daytime observations in both $G_{P1}KC$ (8.3 ± 2.60 [mean \pm SE] $G_{P1} m^{-2}$) and $G_{P2} KC$ (18.2 ± 7.72 introduced $G_{P2} m^{-2}$) study reaches. Killifish maintained low densities in $G_{P1}KC$ (daytime 0.37 ± 0.20 Killifish m^{-2} ; nighttime 0.80 ± 0.28 Killifish m^{-2}) and $G_{P2}KC$ (daytime 1.76 ± 1.47 Killifish m^{-2} ; nighttime 1.12 ± 0.60 Killifish m^{-2}) study reaches. Crabs also maintained low densities in both $G_{P1}KC$ (daytime 0.27 ± 0.12 Crab m^{-2} ; nighttime

0.16±0.11 Crab m⁻²) and G_{P2}KC (daytime 0.88±0.78 Crab m⁻²; nighttime 2.56±1.32 Crab m⁻²) study reaches.

In the G_{P1}KC reach, macroconsumers consistently reduced algal accrual rates by 18% (SE±6%)(*p*=0.03; Figure 3.2a) and biomass by 52% (±31%)(*p*=0.05; Figure 3.2a) in control vs. macroconsumer enclosure treatments, whereas macroconsumers had both positive and negative effects on invertebrate biomass, on average reducing biomass by 49% (±470%)(*p*=0.72; Figure 3.2a). In the G_{P2}KC reach, macroconsumers had both positive and negative effects on algae, on average algal accrual rates were reduced by 4% (±11%)(*p*=0.73; Figure 3.2b) and biomass by 8% (±10%)(*p*=0.42; Figure 3.2b), whereas macroconsumers consistently reduced invertebrate biomass, on average by 84% (±74%)(*p*=0.03; Figure 3.2b) in the control vs. macroconsumer enclosure treatments.

Landscape-Scale Study

Guppies were abundant across all eight subwatersheds in reaches below barrier waterfalls: (4.5±0.68 [mean±SE] for G_{P1} guppies; 5.3±0.67 Guppies m⁻² for G_{P2} guppies). Guppies were never observed in reaches above waterfalls. Killifish abundance (CPUE) was similar between paired reaches above and below barrier waterfalls in subwatersheds containing G_{P1} (above 5.96±1.19 Killifish CPUE; below 5.29±0.96 Killifish CPUE) or G_{P2} guppies (above 3.66±0.72 Killifish CPUE; below 4.00±1.24 Killifish CPUE).

The top outliers in the algae dataset did not bias parameter estimates from our models and therefore we discuss the model output from the full dataset (Table 3.2). Models showed a significant positive correlation between canopy openness and algal biomass, with algal biomass increasing, by 29% when canopy openness was increased by 1 standard deviation unit (Table 3.2). Additionally, we found no support for interactive

effects between canopy openness and the presence of either G_{P1} or G_{P2} guppies on algal standing stocks (Table 3.2). Models did show a significant negative correlation between the presence of G_{P1} guppies and algal biomass [-24% (-40%, -6%); mean (lower, upper 95% CI)] relative to paired upstream guppy-free reaches (Figure 3.3a). Additionally, models showed a positive, but highly variable correlation between the presence of G_{P2} guppies and algal biomass [17% (-6%, 44%)], relative to paired upstream guppy-free reaches (Figure 3.3a). Additionally, we found that the effect of replacing G_{P2} with G_{P1} guppies resulted in a significant reduction in algal biomass [-34% (-53%, -12%)].

The top outliers in our invertebrate dataset biased the parameter estimates from our model, so we report model outputs with the top two outliers removed (Table 3.2). Models including and excluding the top two outliers found strong negative correlations between the presence of G_{P2} guppies and invertebrate biomass, although there was no support for a positive interaction of G_{P2} guppy presence and canopy openness when outliers were removed from the model (Table 3.2).

Models showed a significant positive correlation between canopy openness and invertebrate biomass, with invertebrate biomass increasing on average by 53% when canopy openness was increased by 1 standard deviation unit (Table 3.2). Models also showed a significant negative correlation between the presence of G_{P2} guppies and invertebrate biomass [-31% (-52%, -6%)], relative to paired upstream guppy-free reaches (Figure 3.3b). Additionally, models showed a negative, but highly variable correlation between the presence of G_{P1} guppies and invertebrate biomass [-4% (-33%, 32%)], relative to paired upstream guppy-free reaches (Figure 3.3b). Finally, we found that the effect of replacing G_{P2} with G_{P1} guppies on invertebrate biomass was skewed toward a

positive effect (43% (-10%, 114%); however, we did not detect a significant effect likely due to the large variability surrounding the effects of each phenotype on invertebrate biomass (Figure 3.3b).

Discussion

Our study provides strong evidence that two phenotypes of the Trinidadian guppy, which represent the outcome of genetic adaptation to different predatory environments, have different effects on stream ecosystem properties despite natural levels of environmental variation at local- and landscape- scales. Even though our *in situ* local-scale experiments were conducted in separate years and the two focal experimental reaches varied in four of the five environmental variables measured, we found that macroconsumer communities dominated by G_{PI} guppies had different treatment effects on algal accrual rate and algal and invertebrate biomass than communities in an upstream reach of the same stream dominated by G_{PI} guppies. While we recognize that the relative effects of macroconsumer communities cannot be directly compared given that our experiments were confounded by both time and location, it is important to note that experimental differences between control and enclosure treatments in each reach were consistent with previous common garden mesocosm experiments (Palkovacs et al. 2009, Bassar et al. 2010). Additionally, Marshall et al. (2012) reported strikingly similar top-down effects of macroconsumer communities dominated by G_{PI} guppies on algal (significant negative effect) and invertebrate (lack of effects) biomass in the same reach ($G_{PI}KC$) of our experimental stream one year prior to our study (2008). Moreover, Simon (*In Prep*) reported similar top-down effects in an *in situ* experiment isolating the effects

of G_{P1} guppies across four streams on the southern slope of the Northern Range mountains. The above lines of evidence strongly suggest that trait differences between guppy phenotypes (diet selectivity and life history), rather than any concomitant differences in the time or location of respective experiments, were the mechanism for differences in treatment effects between our experimental reaches. Moreover, landscape-scale patterns in the relative biomass of algae and invertebrates in stream reaches containing either G_{P1} or G_{P2} guppies reflected the divergent effects of guppy phenotypes observed in local-scale experiments, despite landscape-scale levels of environmental variation.

At the landscape-scale, we found increased variability in all five of the physical characteristics measured in our local-scale experiments (Table 3.1). While the effects of G_{P1} and G_{P2} guppies, relative to upstream controls across eight streams, reflected treatment effects from local scale experiment, we could only detect a significant effect of exchanging guppy phenotypes on algal biomass. Our inability to detect a significant phenotypic effect on invertebrates could have been caused by high environmental variability. Of those physical characteristics measured, canopy openness in particular has been identified as a significant driver of algae and invertebrate biomass in this system (Heatherly in prep; Kohler et al. 2012); therefore we could reasonably expect that patterns caused by variation in canopy openness to obscure the influence of guppy phenotype, if its effects on invertebrate biomass are comparatively small. Additionally, given that G_{P1} guppies have been found to be generalist browsers (consuming algae and some invertebrates) relative to G_{P2} guppies (Palkovacs et al. 2009, Bassar et al. 2010) it is

not surprising that it is more difficult to detect a phenotype effect on invertebrates relative to algae.

Differences in diet flexibility between guppy phenotypes is highlighted by the variability in the effects of each phenotype on their less desired prey type; the effects of G_{P1} guppies on invertebrates, while non-significant, varied from positive and negative at both local- and landscape- scales, while the effects of G_{P2} guppies on algae were much less variable tending to be either zero or positive at local- and landscape- scales, respectively.

While distinguishing between the effects of each phenotype on invertebrates may be difficult to detect, phenotypic effects of algal biomass were much stronger. The effect of exchanging G_{P2} for G_{P1} guppies was similar to the effect of increasing canopy openness by one standard deviation (40% of the observed variation in canopy across 112 pools) on these variables across our 16 study reaches (Table 3.2). This result from natural streams supports the findings reported by El-Sabaawi et al. (2015) from artificial stream channels where light and phenotype were examined in a factorial experiment. Moreover, despite 3x higher ambient concentrations of soluble reactive phosphorous (SRP) in our G_{P2} guppy streams (including paired upstream guppy-free reaches), our model still showed strong evidence that G_{P1} guppies reduced algae biomass and G_{P2} guppies tended to increase algal biomass relative to upstream guppy free reaches. Therefore, our findings suggest that divergent effects of guppy phenotypes on algal biomass are not only persistent at the landscape-scale, but are also ecologically significant relative to known, bottom-up drivers (light and nutrients) of ecosystem structure and function.

A caveat to our landscape-study is that it was conducted in the only river system in Trinidad's Northern Range that does not contain shrimp populations. Shrimp have been shown to have a strong influence on stream ecosystems of other Caribbean islands (Pringle et al. 1999, Greathouse et al. 2006), therefore it remains unclear whether the influence of guppy local adaptation on ecosystem structure will be similar in drainages containing dense populations of shrimp.

We predicted that results from our *in situ* studies would match the direction of phenotypic effects reported in previous *ex situ* mesocosm experiments, but we expected the magnitude of the effects observed in natural systems to be comparatively small. In contrast, however, we found that while the effects of guppies (regardless of phenotype) on algal biomass were greater in previous *ex situ* compared to our *in situ* work, the relative difference in effects of G_{P1} and G_{P2} guppies on algal and invertebrate biomass was conserved between experimental approaches. In mesocosms, the presence of guppies (mean of both phenotypes relative to fishless controls) reduced algae and larval midge biomass by 400% and 70% respectively, compared to mostly <50% reductions of these biomass pools in our *in situ* studies. However, similar to our findings at the landscape scale, detecting the effects of exchanging guppy phenotypes in previous mesocosm experiments was much stronger for algal than invertebrate biomass. Both Palkovacs et al. (2009) and Bassar et al. (2010) found highly significant effects of guppy phenotype on algal biomass (\approx 50% difference in both *ex situ* experiments and 34% difference in landscape study), while only Bassar et al. (2010) found an effect on invertebrate biomass that was only marginally significant. Two previous studies (Palkovacs and Post 2009, Urban 2013) have shown that mesocosm experiments can predict some of the ecological

effects of local adaptation observed *in situ* but often overestimate the magnitude of those effects. Therefore, while mesocosms may predict general trends, estimating the *realized* ecological consequences of local adaptation may require measuring the strength of the evolution-to-ecology pathway in natural systems, where environmental heterogeneity may alter the ecosystem effects of evolution in nature.

Density is a key demographic parameter that can impact both the ecological effects and evolution of Trinidadian guppies (Bassar et al. 2010, Reznick et al. 2012). It is important to note that while guppy density was controlled for in previous mesocosm experiments, density was not controlled for in our unmanaged *in situ* field studies. In our landscape study guppy densities were similar for each phenotype; however, in our local-scale experiments, G_{P2} guppies were, on average, twice as dense as G_{P1} guppies. It is conceivable then, that the differences in the top-down effects of each phenotype observed in the local scale experiment were the result of density rather than differences in diet or life history. Several lines of evidence, however, suggest that differences in density would not have changed the direction of the effects of each guppy phenotype on algal and invertebrate biomass. A previous *ex situ* mesocosm experiment reported that doubling guppy density reduced algal biomass, regardless of phenotype, however, despite densities of G_{P2} guppies being double that of G_{P1} guppies in our experiment, only G_{P1} guppies significantly reduced algal biomass, which provides strong evidence that guppy phenotypes have divergent effects on algal biomass. Additionally, Simon (*In Prep*) found that G_{P1} guppies had no top-down effects on invertebrates, at either low or high guppy densities, in an *in situ* experiment across four streams. Therefore, if G_{P1} guppy densities were increased to match

that of G_{P2} guppies in our local-scale experiment it is not likely that guppies would reduce invertebrate biomass.

To our knowledge, our study is the first to report experimental evidence of the ecosystem consequences of local adaptation in an unmanaged natural system. Two previous studies, however, have provided strong evidence that local adaptation of a single consumer species can alter the structure of communities in observational studies at the landscape-scale: (1) Post and colleagues (2008) reported that diet selectivity in migratory vs. non-migratory alewives caused divergent zooplankton communities in 19 natural lakes across coastal Connecticut; and (2) Urban (2013) reported that foraging rates of spotted salamander populations varied with the abundance of an intraguild predator (marbled salamander), which in turn altered zooplankton diversity in 14 natural ponds across Totoket Mountain in Connecticut. Here we report similar effects of foraging traits (diet selectivity) in two locally adapted guppy phenotypes in 16 stream reaches across the Caroni drainage in Trinidad's Northern Range Mountains. Similarities among these studies highlight an emergent theme addressing the effects of local adaptation on communities and ecosystems in nature. Because the differences in resource use of guppies affect aspects of ecosystem structure, our results expand the breadth of effects attributed to local adaptation of populations within the landscape, beyond community-level responses.

Conclusions

In support of our hypothesis, our results show a strong linkage between local adaptation and ecosystem structure, despite high natural levels of environmental variation. Moreover, our study is the first to our knowledge to report evidence that the

ecosystem consequences of local adaptation are consistent local- and landscape- scales and are also ecologically significant relative to known, bottom-up drivers of ecosystem structure and function. While our results strongly suggest that divergent local adaptation can result in different ecosystem effects of each guppy phenotype on algal and invertebrate biomass at local- and landscape- scales, our ability to distinguish between the effects of each phenotype was much stronger for algal biomass relative to invertebrate biomass due to reduced variability in top-down effects.

Acknowledgements

We thank T. Mcdevitt-Galles, W. Roberts, M. Rautenberg, A. Bratt, T. Schipper, and E. Nash for their help during field sampling. We thank K. Murray, C. Nguyen, A. Snellgrove, and E. White for their help processing samples in the lab. We thank R. Shefferson and the members of the Pringle Lab for their extensive manuscript comments. We thank the Water and Sewage Authority of Trinidad and Tobago, the University of the West Indies at St. Augustine, and the Asa Wright Nature Centre for permission to access various sites and for use of facilities. The authors appreciate the help and support of Ronnie Hernandez and the staff of William Beebe Tropical Research Station. This work was supported by a National Science Foundation Frontiers in Integrative Biological Research (FIBR) Grant to DNR and others (NSF-FIBR, DEB-0623632) and the NSF Graduate Research Fellowship program (fellowship to TNS).

Table 3.1: Physical and chemical parameters [mean±SE (n=7); range (min, max)] for paired reaches examined in the landscape-scale survey and the location of our local-scale experiment, the Caigual*. Physical and chemical parameters not measured are denoted by §. Physical and chemical parameters for landscape-scale study were measured one week before (April 13th-19th, 2011) biological sampling. Physical parameters for local-scale study were calculated one day after experiments were completed for G_{P1}KC and G_{P2}KC reaches on 24 March 2009 and 25 May 2010, respectively.

River	Reach	Q (m ³ s ⁻¹)	Canopy openness (%)	Pool Area (m ²)	Pool Depth (m)	Pool Velocity (m s ⁻¹)	SRP (µg L ⁻¹)	TIN (µg L ⁻¹)
Arima	G _{P1} KC	1.32	18.91±3.13 (6.50, 25.74)	5.28±1.06 (1.80, 9.14)	0.15±0.02 (0.08, 0.23)	0.04±0.02 (0.00, 0.14)	1.60	314.96
	KC	1.02	7.87±1.24 (3.90, 13.52)	11.23±1.12 (6.62, 16.12)	0.21±0.03 (0.12, 0.33)	0.01±0.01 (0.01, 0.03)	1.08	280.54
Aripo	G _{P1} KC	0.88	24.07±2.87 (11.70, 35.62)	6.39±1.43 (3.07, 12.53)	0.13±0.01 (0.09, 0.17)	0.04±0.01 (0.01, 0.12)	3.24	126.11
	KC	0.98	7.50±1.67 (2.86, 15.86)	11.75±0.83 (8.68, 14.63)	0.25±0.03 (0.13, 0.37)	0.02±0.00 (0.01, 0.03)	0.87	147.17
Guanapo	G _{P1} KC	1.21	12.93±3.07 (1.04, 24.70)	5.83±1.16 (2.43, 9.67)	0.17±0.02 (0.09, 0.24)	0.04±0.01 (0.01, 0.08)	5.99	248.19
	KC	1.77	7.87±1.84 (2.86, 14.82)	6.25±0.69 (4.16, 8.52)	0.18±0.01 (0.10, 0.22)	0.01±0.00 (0.00, 0.04)	5.95	224.73
Tacarigua	G _{P1} KC	0.35	3.45±0.81 (1.30, 6.50)	9.38±3.87 (2.65, 28.20)	0.15±0.02 (0.10, 0.20)	0.03±0.00 (0.01, 0.04)	14.60	222.72
	KC	0.33	3.38±0.34 (2.08, 4.42)	5.94±1.12 (1.99, 10.79)	0.16±0.01 (0.12, 0.23)	0.03±0.01 (0.01, 0.11)	8.65	215.33
Guanapo (Ciagual)	G _{P2} KC	1.39	8.95±2.82 (1.30, 22.88)	7.26±1.75 (3.68, 14.14)	0.18±0.03 (0.07, 0.26)	0.03±0.00 (0.02, 0.05)	6.49	169.24
	KC	1.21	15.30±2.94 (7.02, 29.38)	10.98±1.61 (7.20, 19.24)	0.25±0.01 (0.21, 0.32)	0.02±0.00 (0.01, 0.04)	12.43	163.16
Guanapo (Lower Lalaja)	G _{P2} KC	2.32	9.03±2.52 (1.56, 15.86)	5.73±1.48 (2.03, 13.96)	0.14±0.02 (0.08, 0.24)	0.02±0.01 (0.01, 0.04)	25.95	239.22
	KC	2.21	5.27±0.91 (1.30, 8.32)	6.77±0.86 (3.11, 10.12)	0.15±0.01 (0.09, 0.21)	0.01±0.01 (0.00, 0.02)	23.79	249.13
Guanapo (Taylor)	G _{P2} KC	0.99	21.93±1.27 (17.42, 26.26)	6.98±1.11 (4.57, 12.78)	0.11±0.01 (0.06, 0.16)	0.05±0.01 (0.02, 0.07)	17.30	190.45
	KC	1.03	8.69±1.05 (6.24, 14.30)	11.88±1.27 (5.03, 15.15)	0.27±0.02 (0.18, 0.32)	0.01±0.00 (0.01, 0.02)	19.46	197.62
Guanapo (Upper Lalaja)	G _{P2} KC	2.16	16.42±2.72 (8.32, 28.86)	6.33±0.97 (2.52, 8.90)	0.16±0.02 (0.10, 0.23)	0.04±0.01 (0.01, 0.09)	13.52	247.92
	KC	2.65	7.80±1.17 (4.94, 12.74)	10.81±1.59 (5.36, 16.55)	0.19±0.03 (0.12, 0.31)	0.04±0.01 (0.01, 0.08)	9.73	258.61
Guanapo* (Ciagual)	G _{P1} KC	1.35	25±3.98 (3.38, 32.31)	9.56±2.14 (6.25, 11.68)	0.24±0.06 (0.11, 0.28)	0.07±0.03 (0.02, 0.09)	§	§
	G _{P2} KC	1.30	15.74±3.44 (1.56, 24.70)	8.95±2.57 (4.59, 9.65)	0.17±0.03 (0.12, 0.23)	0.03±0.01 (0.01, 0.07)	§	§

Table 3.2: Bayesian model parameter estimates for each response variable measured in landscape-scale study including: mean and standard deviation (SD), upper (97.5%) and lower (2.5%) confidence limits (CL), and percent (%) effect size. To insure outliers within our dataset did not heavily bias our parameter estimates, we ran our models both with and without the top two outliers in for each response variable. We report parameter estimates highlighted in gray, and those that were well supported by our model are in bold.

Response Variable	Parameter	Full Dataset			Outliers Removed		
		Estimate (SD)	Lower, Upper CL	% Change of Response	Estimate (SD)	Lower, Upper CL	% Change of Response
Algae							
	Intercept	4.54 (0.17)	4.19, 4.88	n.a.	4.54 (0.16)	4.23, 4.84	n.a.
	G_{p1} Guppy	-0.29 (0.11)	-0.51, -0.07	-24.5%	-0.22 (0.11)	-0.44, -0.01	-19.1%
	G_{p2} Guppy	0.15 (0.11)	-0.07, 0.36	16.6%	0.14 (0.11)	-0.06, 0.35	16.1%
	Phenotype	-0.44 (0.16)	-0.74, -0.13	-34.4%	-0.36 (0.15)	-0.66, -0.06	-29.6%
	Canopy Openness	0.21 (0.06)	0.08, 0.33	28.8%	0.20 (0.06)	0.08, 0.32	28.3%
	G_{p1} x Canopy	-0.04 (0.13)	-0.30, 0.23	-2.9%	-0.04 (0.13)	-0.29, 0.21	-3.0%
	G_{p2} x Canopy	-0.09 (0.10)	-0.27, 0.10	-7.8%	-0.08 (0.09)	-0.26, 0.10	-7.5%
	Intercept Random effects	0.43 (0.17)	0.22, 0.85	n.a.	0.39 (0.15)	0.20, 0.76	n.a.
	Residual	0.39 (0.03)	0.34, 0.45	n.a.	0.38 (0.03)	0.33, 0.44	n.a.
Invertebrate							
	Intercept	-0.31 (0.13)	-0.57, -0.05	n.a.	-0.22 (0.13)	-0.47, 0.03	n.a.
	G_{p1} Guppy	0.03 (0.19)	-0.34, 0.39	4.8%	-0.06 (0.17)	-0.40, 0.28	4.2%
	G_{p2} Guppy	-0.29 (0.19)	-0.65, 0.07	-23.9%	-0.39 (0.17)	-0.73, -0.06	-31.5%
	Phenotype	0.32 (0.24)	-0.17, 0.78	41.6%	0.33 (0.22)	-0.11, 0.76	43.1%
	Canopy Openness	0.22 (0.10)	0.03, 0.42	32.0%	0.35 (0.10)	0.16, 0.54	52.8%
	G_{p1} x Canopy	0.08 (0.23)	-0.37, 0.52	10.4%	-0.05 (0.21)	-0.46, 0.37	-2.5%
	G_{p2} x Canopy	0.26 (0.34)	-0.07, 0.60	31.9%	0.14 (0.16)	-0.17, 0.45	16.4%
	Random effects on Intercept	0.21 (0.14)	0.02, 0.55	n.a.	0.22 (0.14)	0.02, 0.54	n.a.
	Residual	0.72 (0.05)	0.63, 0.83	n.a.	0.66 (0.05)	0.57, 0.76	n.a.

Figure Legends

Figure 3.1: Map showing subwatersheds sampled for landscape-scale study [denoted by outlined watersheds, where either G_{P2} (1-4) or G_{P1} (5-8) guppies are located] and local-scale experiments (denoted by a dashed subwatershed) across the Caroni drainage in Trinidad's Northern Range mountains. The Caroni drainage contains large sections of high-density urban areas (diamond shaded area) and rural areas with mixed agriculture (hatch shaded area). Subwatersheds chosen for landscape study were located in watersheds with the largest tracts of intact-forested headwaters. Inset (B) illustrates paired reach design (above and below waterfalls) of landscape study, where reaches below barrier waterfalls contain guppies, killifish, and crabs ($G_{P1}KC$) and reaches above waterfalls contain only killifish and crabs (KC).

Figure 3.2: Mean (\pm SE) of natural log (LN) response ratio of ecosystem response variables in two local-scale experiments conducted in macroconsumer communities containing either (A) G_{P1} guppies, killifish, and crabs ($G_{P1}KC$), or (B) G_{P2} guppies, killifish, and crabs ($G_{P2}KC$). X-axis is the response variables measure during experiment including algal accrual rate ($\text{mg m}^{-2}/\text{day}$), algal biomass (mg m^{-2}), and invertebrate biomass (mg m^{-2}). Y-axis is the response ratios calculated as LN (response variable in control/response variable in macroconsumer exclusion), where positive values represent positive effects of

macroconsumers and negative values represent negative effects. Statistical significance is signified by * ($p < 0.05$), ** ($p < 0.01$).

Figure 3.3: Mean \pm 95% Credible Intervals (CI) of percent (%) change caused by the presence of either G_{P1} or G_{P2} guppies relative to guppy-free reaches, or the effect of exchanging G_{P1} with G_{P2} guppies (Phenotype) on (A) algae biomass (mg m^{-2}) and (B) invertebrate biomass (mg m^{-2}) from landscape-scale study. Effects of guppies estimated using linear mixed effects models with random intercepts. Positive effect sizes represent positive effects of guppies and negative values represent negative effects. Parameter estimates were considered to be statistically significant if the 95% credible interval of the estimate did not include zero, where significant parameter estimates indicated by an asterisk (*).

Figure 3.1

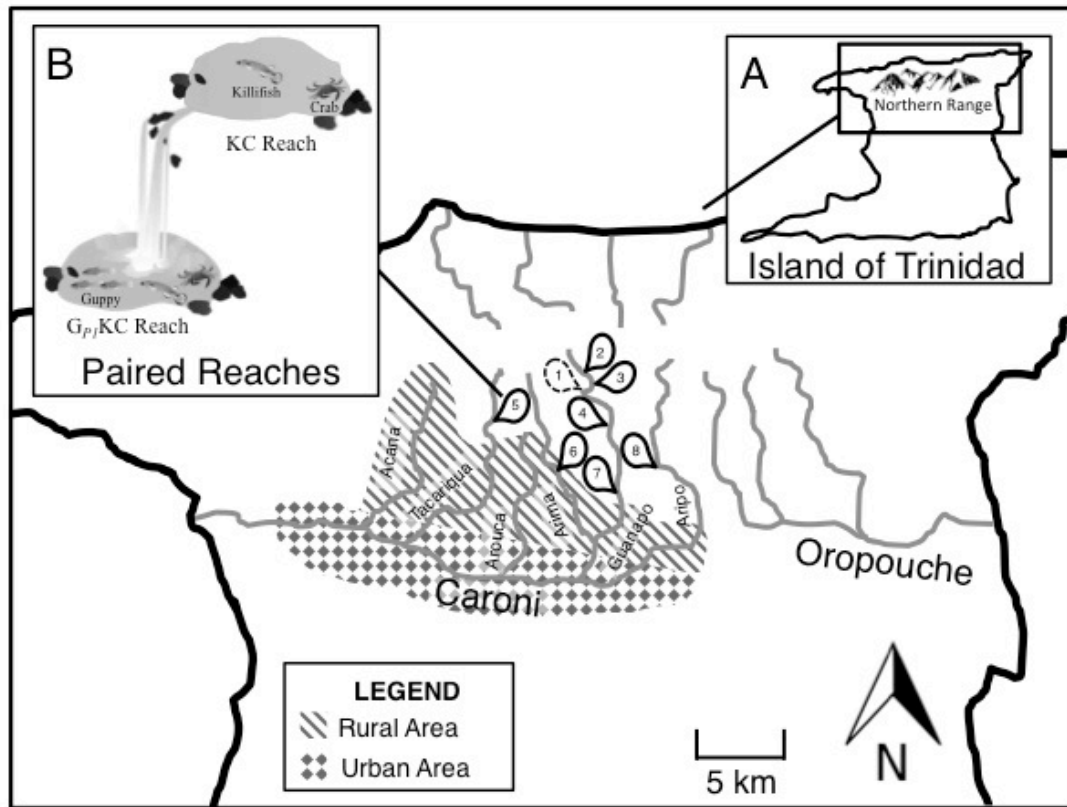


Figure 3.2

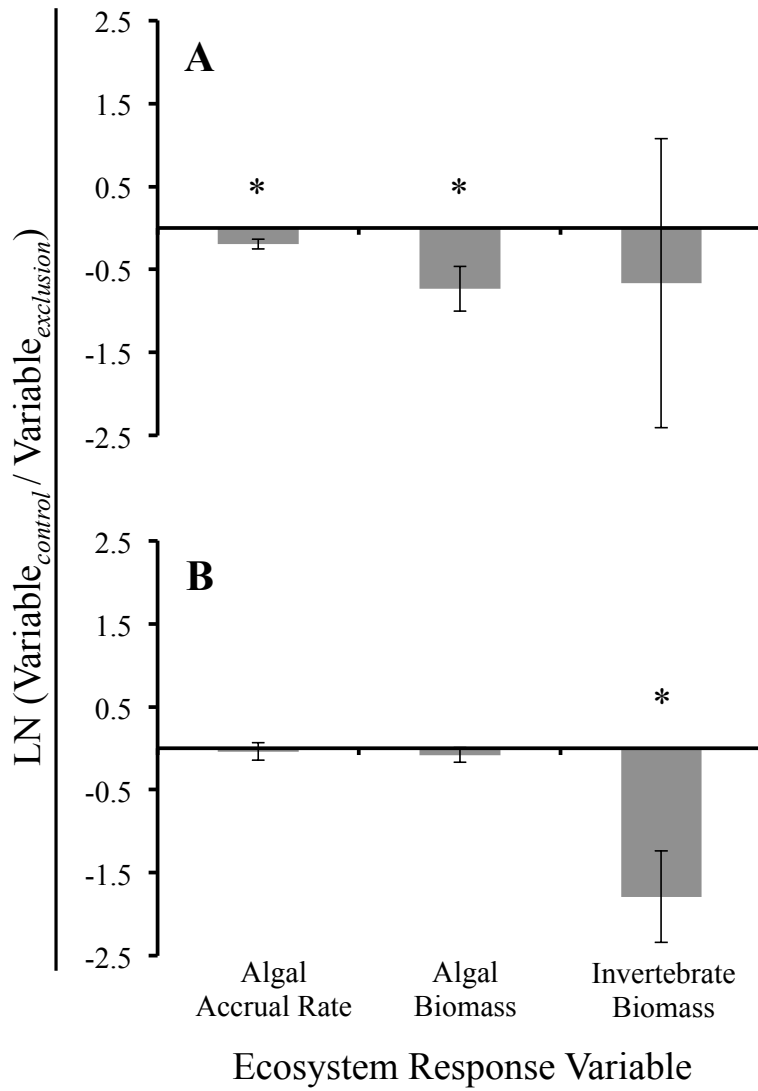
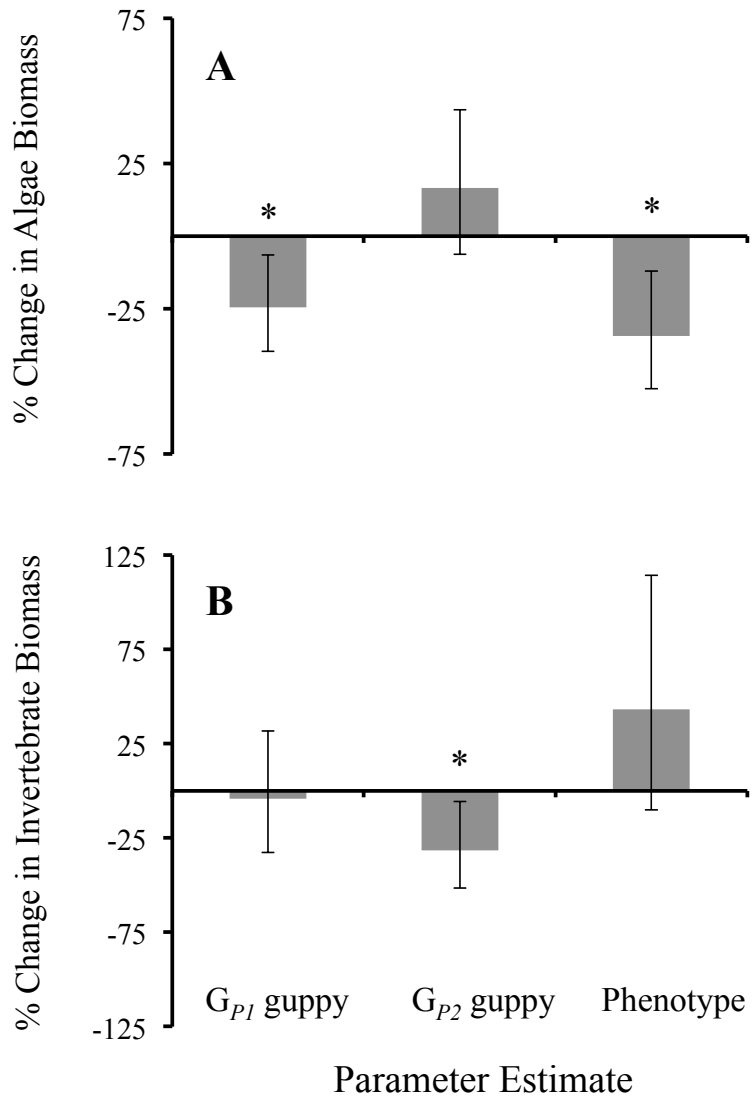


Figure 3.3



CHAPTER 4

TRINIDADIAN GUPPIES DECOUPLE A DETRITUS-BASED TROPHIC CASCADE
WHEN PRESENT IN MACROCONSUMER ASSEMBLAGES WITHIN THE
MONTANE HEADWATER STREAMS OF NORTHERN TRINIDAD³

³ Troy N. Simon, A.J. Binderup, A.S. Flecker, J.F. Gilliam, S.A. Thomas, J. Travis, D.N. Reznick, C.M. Pringle
To be submitted to *Ecology*

Abstract

The loss of dominant macroconsumer species can have strong cascading ecosystem effects. However, the ecosystem-level effects of consumer loss from systems that lack dominant macroconsumers remains unclear. Here, we investigated how the natural presence or absence of an omnivorous guppy, *Poecilia reticulata*, in a macroconsumer assemblage including a generalist predator (the killifish, *Anablepsoides hartii*) and an omnivore (the freshwater crab, *Eudaniela garmani*), alters the top-down effects of the macroconsumer assemblage on leaf litter decomposition. We used an *in situ* enclosure experiment in one stream and a landscape-scale study to quantify the potential effects of macroconsumers on insect shredders and leaf decomposition rates in the context of the presence or absence of guppies below and above natural barrier waterfalls, respectively.

We found strong evidence that killifish caused a detritus-based trophic cascade, but only when guppies were absent above waterfalls. Above waterfalls, where guppies were absent, experimental enclosures isolating the effects of nocturnal and diurnal consumers suggest that killifish rather than crab reduced the biomass of a dominant insect shredder (Trichoptera: *Phylloicus hansonii*) 4-fold ($p < 0.001$) and reduced leaf decomposition rates by 40% ($p < 0.01$). Below waterfalls, where guppies were present, macroconsumers had no observable top-down effects on *P. hansonii* biomass ($p > 0.5$) or leaf decomposition rates ($p > 0.5$). In support of our experimental findings, our landscape scale study found 25-fold higher biomass of *P. hansonii* ($p < 0.001$) and 3.5-fold higher leaf decomposition rates ($p < 0.05$) when guppies were present relative to where they were absent above barriers.

Reduced killifish density in the presence of guppies is a likely mechanism for reduced predation of *P. hansonii* below barriers. Previous studies in this system reported that guppy predation on larval killifish and predicted that the presence of guppies would reduce killifish density. In support of this prediction, we found a 40% and 400% reduction in the density of killifish in presence of guppies in our enclosure experiment and landscape study, respectively. Our results strongly suggest that the presence of intraguild predation between guppies and killifish decoupled a detritus-based trophic cascade at both local- and landscape- scales.

Introduction

The loss of macroconsumers can have landscape-scale effects on communities and ecosystems in both aquatic and terrestrial systems worldwide (Estes et al. 2011). Much research investigating macroconsumers has focused on how predators alter community and ecosystem properties via trophic cascades, whereby predators indirectly affect lower trophic levels by altering the density or activity of intermediate consumer species. Textbook examples of trophic cascades include increases in elk populations and changes in the biomass of quaking aspen following wolf extirpation from Yellowstone Park (Ripple et al. 2001) or increases in primary production via indirect reductions of herbivorous zooplankton in lakes following the removal of piscivorous largemouth bass (Carpenter et al. 1987). Although much research on the ecosystem effects of macroconsumers has focused on the direct and cascading ecosystem effects of dominant species, the effect of losing less dominant consumer species from systems with multiple macroconsumers remains unclear. Given that biodiversity loss is predicted to

disproportionally impact large-bodied consumers (Petchey et al. 1999, Duffy 2002), predicting the consequences of macroconsumer loss remains a high research priority (Sutherland et al. 2013).

Predicting the ecosystem consequences of losing an individual macroconsumer species from a system containing multiple macroconsumers often requires understanding the functional relationships among consumer species. For example, if predatory macroconsumers compete for shared prey or engage in intraguild predation, then removing one species could increase prey consumption by the second species (Polis et al. 1989, Huang and Sih 1991, Finke and Denno 2002), which can then have indirect effects on lower trophic levels (e.g. basal resources)(Finke and Denno 2005). However, predicting the cascading effects of macroconsumer loss from a system containing both predators and omnivores is difficult because omnivores can affect both prey populations and basal resources and therefore may reduce the strength of trophic cascades on basal resources (Polis and Strong 1996, Bruno and O'Connor 2005). In order to better understand the potential cascading ecosystem effects of macroconsumer losses across systems, we must quantify the effects of removing individual species from systems containing functionally diverse macroconsumer assemblages (e.g. including both predators and omnivores).

We examined the ecosystem consequences of the presence or absence of an omnivore from a macroconsumer assemblage containing both strict predators and generalist omnivores. We define macroconsumers as species that are relatively large in their environment and have low predation risk as adults. In the headwater streams of northern Trinidad, two distinct macroconsumer assemblages, separated by barrier

waterfalls, are characterized by the presence or absence of the guppy, *Poecilia reticulata*. Killifish (*Anablepsoides hartii*), crabs (*Eudaniela garmani*), and guppies dominate macroconsumer communities below waterfalls. However, waterfalls limit upstream colonization by guppies, such that only killifish and crabs are found in the uppermost reaches of streams. Because detrital processing is a dominant energy pathway in many forested headwater streams (Vannote et al. 1980, Wallace et al. 1997), we addressed the top-down effects of macroconsumer communities on leaf decomposition rates.

Previous research in this system has shown that macroconsumer communities directly increase the decomposition rates of soft, palatable, black stick leaves (Marshall et al., 2012). However, no studies have attempted to isolate possible indirect cascading effects of macroconsumers (e.g. consumption of leaf shredding invertebrates) within these communities. Isolating the effects of macroconsumer predation on detrital processing is simplified in the streams of Northern Trinidad compared to many mainland streams because a single species, the caddisfly, *Phylloicus hansonii* (Flint Jr 1996), is the dominant insect shredder (Botosaneanu and Sakal 1992). Thus, if any macroconsumer species consumes *Phylloicus hansonii* (hereafter referred to as *Phylloicus*), then a trophic cascade on leaf decomposition rates may occur (Figure 4.1). Although each macroconsumer species in this system consumes aquatic invertebrates, conclusive evidence that any species significantly impacts invertebrate biomass in high elevation streams has yet to be documented. Gut content analysis shows that killifish are generalist predators of aquatic invertebrates and terrestrial arthropods that fall into streams (Fraser et al. 1999), while Crabs and guppies are omnivores (Marshall et al. 2012). Guppies consume mostly algae and fine detritus, as well as some invertebrates and aquatic

consumers (Zandonà et al. 2011), and crabs are opportunistic foragers of labile coarse detritus, and freshly fallen leaves and fruits (T.N.S personal observation). Additionally, when found together, guppies and killifish are reported to be partial intraguild predators, where guppies consume larval killifish and killifish occasionally consume adult guppies (Fraser and Lamphere 2013).

Here, we assessed differences in the top-down effects of macroconsumers on leaf decomposition rates in the context of the presence or absence of omnivorous guppies from a system containing multiple macroconsumers. We used both an *in situ* enclosure experiment and a landscape-scale study to examine the potential cascading effects of macroconsumer assemblages above and below barrier waterfalls. We hypothesized that if the presence of any macroconsumer species reduced the biomass of insect shredders (particularly *Phylloicus*), then macroconsumers would indirectly decrease the rate of leaf litter decomposition. Additionally, because previous work has demonstrated that guppies and killifish are partial intraguild predators, we predicted that the documentation of a trophic cascade would be more likely to occur above waterfalls because the natural exclusion of guppies should enable greater killifish predation on invertebrate shredders, resulting in lower rates of leaf decomposition. Moreover, because crabs can consume both macroinvertebrates and coarse organic matter, their exclusion could potentially accelerate or decelerate leaf decomposition rate. Given that the activity of some macroconsumers in this system varied along a diel cycle (Marshall et al. 2012), we also included 12hr diel exclusion treatments to better isolate the effects of diurnally active consumers (killifish and or guppies) from nocturnally active consumers (crabs and killifish).

Methods

Study Site

Our studies were carried out within the Caroni drainage on the southwestern slope of the Northern Range Mountains in Trinidad ($10^{\circ} 39'N$ $61^{\circ}18' W$). The Caroni is the largest drainage in Trinidad ($\approx 60,000$ ha) (Figure 4.2). Rivers draining the Northern Range Mountains and into the Caroni are characterized by declining fish diversity with increasing elevation. In the high elevation reaches of these streams, killifish, crabs, and guppies characterize macroconsumer communities. Macroconsumers in this system vary in size, where guppies and killifish have a maximum total length of 45mm and 100mm (Fraser and Lamphere 2013), and crabs have a maximum carapace width of 110 mm (Maitland 2003). Killifish and crabs can disperse over land and bypass barrier waterfalls during wet periods and are commonly the only macroconsumer species present in the most upstream reaches of streams. Stream reaches with killifish, crabs, and guppies (KCG reaches) and those with only killifish and crabs (KC reaches) can be characterized by a single waterfall and are often separated by less than 200 m. As a result, stream reaches with these communities have similar physical habitat and environmental characteristics, while maintaining distinctly different consumer assemblages (Walsh and Reznick 2009).

Exclusion Experiment

Experimental Design: We conducted experiments in two reaches of the Caigual River a third order tributary of the Guanapo River (Figure 4.2). Experiments were conducted during the dry season when stream discharge was low [KC (February 22nd – March 23rd 2009) and KCG (April 16th – May 13th 2009)] to reduce the confounding

effects of seasonal variation in stream discharge between experiments. Temporally separated experiments were necessary due to logistical limitations, and we assumed stable populations of macroconsumers between reaches due to stable base flows with no storm events, which could have ‘reset’ the benthic environment between experimental periods. Previous studies in the Caigual River (Gilliam et al. 1993) found that in addition to killifish and guppies, there were very low numbers of the Pimelodid catfish, *Rhamdia quelen*, however no individuals were observed in experimental pools. The Caigual drains a heavily forested watershed (≈ 170 ha) that provided abundant canopy cover in the KCG (25% canopy openness) and KC (11.3% canopy openness) reaches. Study reaches (200 m long) were isolated by a large barrier waterfall and separated by ~ 200 m (located at elevations from $\approx 650 - 700$ m a.s.l.). Reaches were characterized by a distinct pool and riffle structure, where stream benthic substrate was dominated by gravel and cobble, with some boulders and large woody debris at the edges of pools. Pools in the KCG reach were similar in depth with slightly slower water velocity than pools in the upstream KC reach (22.9 vs. 25.5 cm depth and 0.23 vs. 0.33 m s⁻¹).

To isolate the top-down effects of macroconsumers (guppies, killifish, and crabs) on leaf decomposition rates, we used an underwater electric enclosure technique (modified from Pringle and Blake 1994), where macroconsumer are either allowed access (non-electrified control) or excluded (electrified exclusion treatment). Electric enclosures have not been found to exclude any aquatic invertebrates in this system (Marshall et al. 2012). A number of previous studies have found this design to work effectively in excluding aquatic macroconsumers across a range of taxa and geographic locations (Greathouse et al. 2006, Connelly et al. 2008, Moulton et al. 2012). In order to better

characterize the potentially opposing effects of crabs from killifish and guppies on leaf decomposition, we also included 12hr diel exclusion treatments to better isolate the effects of diurnally active consumers (killifish and or guppies) from nocturnally active consumers (crabs and killifish). Replicate sets of macroconsumer treatments (24h access, 24h exclusion, 12h daytime exclusion, 12h nighttime exclusion) were deployed in five separate pools across each of the two study reaches (200 m) of the Caigual, resulting in 20 total frames per reach. Electric frames excluded macroconsumers from small (0.125 m^2) benthic patches within each pool. Pools were located $\approx 40 \text{ m}$ apart within each study reach.

Treatment frames were constructed of two concentric rectangles of 8-gauge solid copper wire, connected by plastic cable ties (outer rectangle 25 cm x 50 cm, inner 8 cm x 30 cm) (following Marshall et al. 2012). We used Speedrite 1000 fence chargers (Tru-Test Limited, Auckland, New Zealand) set on the low power and slow pulse settings. These setting result in energy output of about 1 joule at ≈ 2 -sec intervals to power the exclusion frame. Control frames were identically constructed but not electrified.

Macroconsumer observations: To confirm observations from previous experiments of macroconsumer activity patterns (Marshall et al. 2012), we estimated the density of macroconsumers over timed observation periods. We made observations of each macroconsumer access treatment (control and non-electrified 12hr treatment) for 1hr, spread across four 15 min observation periods per experiment: two-diurnal and two-nocturnal (10 total observation hours per reach). Nocturnal observations were made on relatively clear nights with the moon in either first or last quarter phase. We recorded the number of each macroconsumer species present using visual point counts once a minute

over a 15 min period (n=15 point counts) for each treatment. Before making observations we waited 5 min to allow macroconsumers to resume normal activity. Density of each species was estimated as an average number of individuals observed across point counts divided by the frame area (0.125 m²) (following Reznick et al. 2001).

Leaf Decomposition: We used packs of recently fallen leaves from the guarumo tree, *Cecropia peltata* (hereafter referred to as *Cecropia*), leaves to assess the effects of macroconsumers on leaf litter decomposition rates. *Cecropia* is a common Neotropical tree genus found in riparian areas across Central and South America and throughout many of the islands of the Caribbean Basin (Rickson 1977) including Trinidad. *Cecropia* leaves tend to be high in nutrients and low in secondary compounds (Zou et al. 1995). Leaves were oven-dried at 40°C for at least 3 days and then grouped in batches of 5 g, weighed to the nearest 0.01 g and clipped together at the petiole using a binder clip to allow access by larger shredding consumers (e.g. crabs). Leaf packs were attached to the copper wire quadrats using plastic zip ties, and one pack from each frame was collected after 4, 8, 15, 22, and 29 days by placing a 63µm mesh net placed downstream of the pack while removing it from the quadrat to avoid loss of invertebrates or leaf material. Retrieved leaf packs were rinsed over a 250-µm sieve and all leaf particles were placed in pre-weighed paper bags and the >1mm size fraction of invertebrates was saved for quantification of taxa. Bags were dried at 40°C for at least 3 days and weighed to the nearest 0.01 g. The average dry mass lost in handling packs was subtracted from the initial dry mass of leaf packs. We calculated the percent of leaf dry mass remaining using initial and final measurements.

Leaf Invertebrates: Invertebrate samples were dyed with Rose Bengal stain for 24

hr and sorted; all invertebrates >1mm were identified to the lowest taxonomic resolution possible (usually genus, except for midge taxa) using taxonomic keys by Merritt et al. (2008) and Perez (1988). All invertebrates >1mm were also assigned to functional feeding groups, as described by Merritt et al. (2008) and de Carvalho and Uieda (2009). We used length-mass regressions (Benke et al. 1999, Becker et al. 2009) to estimate invertebrate biomass in each leaf pack, which was then divided by the final leaf mass of the pack on the corresponding sample date. We focused only on the biomass of invertebrates in the shredding functional feeding group for our statistical analysis in order to better isolate the cascading effects of macroconsumers on leaf decomposition.

Landscape Study

To determine how the natural exclusion of guppies above and below waterfalls altered leaf decomposition rates at the landscape scale, we expanded our studies to six additional streams draining subwatersheds within the larger Caroni River drainage (\approx 14,053 ha; Figure 4.2). We measured leaf decomposition rates and the biomass of shredding invertebrates in each of the six streams, with paired upstream (killifish and crab) and downstream (killifish, crab, and guppy) reaches separated by waterfalls.

Leaf packs were deployed and collected in all streams concurrently under baseflow conditions toward the end of the dry season (April 23rd – May 21st, 2014). In each of the six study streams we placed *Cecropia* leaf packs in the first three pools (separated by >50 m) up- and down- stream of waterfalls. Additionally, paired reaches within streams were separated by no more than 200 m, and elevation was similar among subwatersheds, ranging from 600 – 800 m a.s.l. All sites contained heavily forested subwatersheds that ranged from \approx 53 - 105 ha in drainage area. Similar to the Caigual

subwatershed from the exclosure experiment, all study reaches were characterized by a distinct pool and riffle structure, with stream benthic substrate dominated by gravel and cobble, and some boulders and large woody debris at the edges of pools. In each pool we estimated percent canopy openness, percent leaf cover (visually estimated for entire pool), and areal biomass of leaf litter from randomized transects (0.125 m wide) across pools. We also measured depth, percent saturation of dissolved oxygen and water temperature using a YSI Pro2030 (Yellow Springs, Ohio) at the downstream end of each pool, and current velocity near leaf packs using a Marsh McBirney Flo-Mate 2000 (Loveland, Colorado).

Macroconsumer Abundance: Fish abundance (killifish and guppies) was estimated by placing two baited minnow traps in each pool ($n=6$ per stream) for 10 min. All captured fish were counted and promptly returned to a pool yielding a catch per unit effort (CPUE; Seber 1982). Total catch in each of the two traps in a pool were combined for CPUE calculation. Crab abundance was estimated using nocturnal transect counts across a 100 m stream-reach. One transect count was conducted in each of the two reaches per stream ($n=12$ total transects), yielding number of crabs per m of stream length.

Leaf Decomposition and Leaf Shredding Invertebrates: The deployment, collection, and calculation of mass loss for leaf packs followed methods described for the exclosure experiment. All invertebrates >1 mm in the shredding functional feeding guild were counted and length-mass regressions (Benke et al. 1999, Becker et al. 2009) were used to generate total shredder biomass values for each leaf pack.

Statistical Analysis

Exclosure Experiment: Because experiments in KCG and KC reaches were spatially and temporally isolated from each other we compared relative treatment effects within reaches only. Macroconsumer density data from diurnal and nocturnal observations within each reach were compared using Friedman's non parametric test to investigate the effects of partial access (12 hr exclusion) vs. full access (control) treatments on macroconsumer density estimates (i.e., full access and daytime access observed during the diurnal period, and full access and nighttime access during the nocturnal period); with a random block effect of replicate pool. If statistical significance was detected ($\alpha < 0.05$), pairwise comparisons were made with Kruskal-Wallis rank sum tests. The rate of decomposition (% mass lost day⁻¹) was determined using a linear fit because mass-loss through time was not strongly exponential. Differences in leaf decomposition rate between all treatments were first examined using a one-way ANOVA where treatment was a fixed effect and pool was a random block effect. Significant treatment effects led to examination of pairwise differences in decay rates using Tukey-Kramer HSD *post-hoc* tests. Differences in the mean normalized biomass of invertebrate shredders (mg invertebrate dry mass / mg leaf material remaining) between treatments were also compared using one-way ANOVA and Tukey-Kramer HSDs. Leaf decomposition rate and invertebrate biomass data were natural log transformed to meet the assumptions of normality. Leaf decomposition rates and shredder biomass across all treatments (including controls) were examined for bivariate correlations in each reach.

Landscape-Study: Macroconsumer CPUE data between reaches were compared using one-way ANOVA where reach was the fixed effect and stream was the random block effect. The rate of decomposition (% mass lost day⁻¹) was determined using a linear

fit because mass-loss through time was not strongly exponential. Leaf decomposition rates between reaches was compared using one-way ANOVA where reach was the fixed effect and stream was the random block effect. Differences in the mean normalized biomass of invertebrate shredders above and below waterfalls were also compared using one-way ANOVA. Leaf decomposition and invertebrate biomass and abundance data were natural log transformed to meet the assumptions of normality. Additionally, we used a two-way ANOVA to analyze whether changes in shredder biomass were gradual along a stream continuum (i.e., an effect of pool position, with pools numbered 1-6 in order from upstream to downstream) or abrupt corresponding to barrier waterfalls (loss of guppies), where pool and reach were fixed effects and stream was a random block effect. Leaf decomposition rates and shredder biomass were examined for bivariate correlations. All statistical analyses were conducted using R (R Version 3.0.1).

Results

Macroconsumer Exclosure Experiment

Macroconsumer Observations: No macroconsumers were observed in either the 24hr exclusion treatments or when the 12hr exclusions treatments were electrified. Additionally there were no significant differences between visits to treatments in the same diel period (i.e., full access and daytime access observed during the diurnal period, and full access and nighttime access during the nocturnal period); therefore, observation treatments from the same diel period were pooled (Table 4.1).

In the KCG (guppy present) reach, guppies were strictly diurnally active (7.31 ± 1.49 [mean \pm SE] individuals m^{-2}). Killifish were active both day and night, yet we

observed significantly more killifish during the night in both KCG ($p=0.03$; daytime 0.35 ± 0.12 individuals m^{-2} ; nighttime 0.84 ± 0.18 individuals m^{-2}) and KC reaches ($p=0.02$; daytime 0.56 ± 0.33 individuals m^{-2} ; nighttime 1.36 ± 0.52 individuals m^{-2}). Crabs were not observed more at night than during the day in the KCG reach ($p=0.35$; daytime 0.16 ± 0.07 individuals m^{-2} ; nighttime 0.08 ± 0.06 individuals m^{-2}) but were observed significantly more at night in the KC reach ($p=0.04$; daytime 0.16 ± 0.13 individuals m^{-2} ; nighttime 1.89 ± 0.60 individuals m^{-2}).

Leaf Decomposition Rate: In the downstream KCG reach we found no effect of macroconsumers on leaf decomposition rate in any of the macroconsumer manipulation treatments. Decomposition rates of *Cecropia* in control treatments (full access) were similar to treatments allowing access only during the day or night, or no access ($F_{3,16}=0.023$, $p=0.85$; Figure 4.3a).

The presence of killifish and crabs reduced the decomposition rate of *Cecropia* in the upstream KC reach ($F_{3,16}=6.67$, $p=0.004$; Figure 4.3a). Decomposition rates were reduced by 40% in control frames relative to 24hr. and 12hr. exclusion ($p < 0.03$; Figure 4.3b). Additionally, our results suggest that killifish rather than crabs likely reduced decomposition rates, because decomposition rates were similar in treatments excluding either diurnal (Killifish; 12hr. daytime exclusion) or nocturnal (Killifish+Crabs; 12hr. nighttime exclusion) macroconsumers ($p>0.1$).

Invertebrate community and leaf decomposition: We found 18 different macroinvertebrate taxa on *Cecropia* leaves, and similar to previous studies in the region (Botosaneanu and Sakal 1992), the only leaf shredding macroinvertebrate was the caddisfly *Phylloicus hansonii* (supplemental Table 4.1). In control treatments (full

macroconsumer access) we found 2-fold higher total biomass of macroinvertebrates >1mm in the downstream KCG (guppy present) reach (374.20 mg) compared to the upstream KC (guppy-free) reach (178.73 mg) reach. This difference in biomass was driven primarily by increased biomass of *Phylloicus* in KCG (251.61 mg; 67% of total invertebrate biomass) compared to KC (21.89 mg; 12% of total invertebrate biomass) reaches (Appendix Table 1). We found a significant positive correlation between *Phylloicus* biomass and *Cecropia* litter decomposition in the downstream KCG reach ($R^2=0.41$, $p=0.002$; Figure 4.4a) where *Phylloicus* biomass was high, but not the upstream KC reach ($p=0.37$; Figure 4.4b) where *Phylloicus* biomass was low.

Phylloicus Biomass: We found no effect of macroconsumers on *Phylloicus* biomass in any of the macroconsumer manipulation treatments in the downstream KCG reach ($F_{3,16}=0.149$, $p=0.94$; Figure 4.3c).

In the upstream KC reach, we found that macroconsumers, when allowed full access, reduced *Phylloicus* biomass on average by 4-fold, relative to 12hr. and 24hr. exclusion treatments ($F_{3,16}=9.25$, $p=0.001$; Figure 4.3d). Additionally, we note that experimental results on *Phylloicus* abundance are parallel to the results on *Phylloicus* biomass in both experimental reaches (Data not presented here). Additionally, our results suggest that killifish rather than crabs likely reduced *Phylloicus* biomass, because biomass was similar in treatments excluding either diurnal (Killifish; 12hr. daytime exclusion) or nocturnal (Killifish+Crabs; 12hr. nighttime exclusion) macroconsumers ($p>0.1$).

Landscape Study

Physiochemical characteristics: The physiochemical characteristics of pools downstream of waterfalls in the KCG (guppy present) reaches were similar to those upstream of waterfalls in the KC (guppy-free) reaches (Table 4.2). The percent canopy openness was low, while percent leaf litter cover in pools and standing stocks of course particulate organic matter were relatively high (Table 4.2). Pools were generally shallow with low current velocity (Table 4.2). Finally, the percent saturation of dissolved oxygen and water temperatures were also similar between pools above and below waterfalls (Table 4.2).

Macroconsumer Density/Abundance: In the KCG reaches we captured approximately 3-fold more individual guppies than killifish in baited traps (Table 4.3). Additionally, killifish CPUE abundance was approximately 4-fold higher in the KC reach relative to the downstream KCG reach ($F_{1,5}=27.97, p=0.003$) (Table 4.3). Finally, we found similar abundances of crabs in our transect surveys of KCG and KC reaches ($F_{1,5}=0.78, p=0.42$) (Table 4.3).

Dynamics between Leaf Decomposition and Phylloicus biomass: *Cecropia* leaves decayed 3.5-fold faster in the KCG reaches (below waterfalls) as compared to the KC reaches (above waterfalls), ($F_{1,5}=9.85, p=0.03$; Figure 4.5a). *Phylloicus* biomass was 25-fold higher in KCG reaches compared to the KC reaches ($F_{1,5}=17.19, p=0.009$; Figure 4.5b), corresponding to differences in litter decay rates. Finally, we found a significant positive correlation between *Phylloicus* biomass and litter decomposition rates in the KCG reach where biomass was high ($R^2=0.57, p=0.01$; Figure 4.6a), but not in the KC reach where biomass was low ($p=0.61$; Figure 4.6b).

Stream Continuum Analysis Of Phylloicus Biomass: *Phylloicus* biomass was statistically higher between KCG and KC reaches ($p < 0.001$), but not between pools nested across streams ($p > 0.10$) (Figure 4.7). There was no trend toward gradual changes in *Phylloicus* biomass along a stream continuum, as biomass dropped dramatically above waterfalls.

Discussion

In support of our hypothesis, we found strong evidence that macroconsumers caused a detrital-based trophic cascade by reducing *Phylloicus* biomass, yet only in stream reaches where guppies were absent. In our enclosure experiments we found that macroconsumers had no observable top-down effects on leaf decay or *Phylloicus* biomass in the presence of guppies (KCG reach). However, in the absence of guppies (KC reach) macroconsumers reduced leaf decomposition rates and depressed the biomass of the macroinvertebrate shredder. Moreover, in six streams across the Caroni drainage we found lower leaf decay rates and lower *Phylloicus* biomass in stream reaches where guppies were naturally excluded above waterfalls relative to where they were present below barriers.

In contrast to our findings of a trophic cascade in the upstream KC reach, Marshall and others (2012) reported that macroconsumers directly increased leaf decay rates in the upstream KC but not the downstream KCG reach. They concluded that these differences might have been caused by differences in crab activity between reaches. Differences in the quality of leaf species used between our and the Marshall et al. (2012) study may help explain the conflicting results. Marshall et al. (2012) used soft, palatable,

black stick leaves (100% decomposed in two weeks) that crabs were reported to directly consume, while we used less fragile and palatable *Cecropia* leaves (~50% mass lost in 29 days), which we never observed crabs to consume. The use of *Cecropia* leaves allowed us to investigate the affect of macroinvertebrate shredding, because unlike black stick leaves, invertebrate shredders are able to colonize *Cecropia* leaves before they decompose. These results suggest that when omnivorous macroconsumers are present the pathway of top-down effects (direct vs. indirect) on leaf decomposition may depend on the quality of litter used in experiments.

Several lines of evidence suggest that killifish predation on *Phylloicus* increased in the absence of guppies (i.e., in the upstream KC reach). We found similar biomass of *Phylloicus* in the macroconsumer daytime and nighttime exclosure treatments, suggesting that killifish rather than nocturnally active crabs were the primary predators on the caddisfly. Additionally, killifish were observed to readily consume *Phylloicus* larvae; if *Phylloicus* were dropped into the stream (by a stream-side observer), larvae were quickly pulled from their case and consumed by killifish (T.N.S personal observation). Moreover, gut content analysis of killifish from four different streams in northern Trinidad confirmed this observation – dissections of 30 killifish showed that ~30% of individuals contained *Phylloicus* in their stomach contents (Murray *In Prep*).

Theory predicts that the presence of an intraguild predator can reduce prey risk (Polis et al. 1989) and recent empirical research supports this prediction. In a meta-analysis of 50 experiments manipulating predator diversity, Schmitz (2007) reported that 70% of field experiments found that increasing predator diversity weakened prey suppression. Intraguild predation has frequently been cited as a mechanism for the effects

of increasing predator diversity dampening the strength of trophic cascades (Finke and Denno 2005; Bruno and Cardinale 2008; Sanders et al., 2011; Sitvarin and Rysprtra 2014). While cascading ecosystem effects of intraguild predation have been observed in terrestrial systems no studies to our knowledge have been conducted in aquatic systems. In a terrestrial example, Karp and Daily (2014) showed that intraguild predation between bats and spiders reduced herbivore suppression leading to increased grazing on coffee plants in Costa Rica. Similarly, in our study several lines of evidence suggest that the presence of guppies decoupled the cascading effects of killifish on leaf decay. First, Fraser and Lamphere (2013) reported that guppies consumed larval killifish, which caused reductions of killifish recruitment in a multi-year, whole-stream guppy introduction experiment. Second, in support of Fraser and Lamphere (2013) conclusions we found that killifish densities were reduced in the presence of guppies (~40% increase). Moreover, previous studies from our experimental exclusion stream (Marshall et al., 2012; Gilliam et al., 1993), as well as other similar streams (Walsh and Reznick 2009, Walsh et al. 2011), reported that killifish densities were more than three times as dense in KC compared to KCG reaches, further indicating that the presence of guppies is linked to reduced killifish densities. Moreover, although the differences in top-down effects observed in our exclusion experiment could have been caused by other, unmeasured environmental differences between the upstream and downstream experimental reaches, results from our landscape study suggest that the absence of guppies rather than environmental context strengthened a detrital-based trophic cascade across the Caroni drainage.

In support of our prediction that the absence of guppies strengthened cascading effects on leaf decomposition, we found 29-fold higher biomass of *Phylloicus* and 3.5-fold lower leaf decay rates in reaches where guppies were naturally absent above waterfalls relative to where they were present below barriers in six streams across the Caroni drainage. Similar to previous studies (Gilliam et al. 1993, Walsh and Reznick 2009) in this system, we found that while physiochemical characteristics were similar between upstream and downstream reaches across all six streams, killifish abundance was significantly higher (4-fold) in KC relative to KCG reaches. Additionally, we found 40-fold lower abundance but similar mean length of *Phylloicus* in KC relative to KCG reaches suggesting that observed differences in *Phylloicus* biomass were not the result of size selective predation (Appendix Table 2). Furthermore, we found strong statistical evidence that the presence or absence of guppies rather than gradual changes along a stream continuum (across 6 pools sampled in a stream) explained differences in *Phylloicus* biomass between reaches. We note, however, that the effect size of guppy absence on leaf decomposition and *Phylloicus* biomass were much larger (2- and 10- fold greater respectively) in our landscape-study relative to differences observed between reaches in our enclosure experiment. There are multiple possibilities for why this difference occurred, but one possible reason may have been the results of the historic drought that preceded our landscape study. The combination of stable flows (no scouring floods) and increased leaf input (T.N.S Personal Observation) likely allowed *Phylloicus* populations to flourish in downstream KCG reaches where they experience reduced predation risk. Therefore, the cascading effects of killifish on leaf decomposition could

be mediated by climate patterns, such that the strength of cascading effects may be dependent on the length and intensity of the dry season.

While the indirect effects of macroconsumers on algal-driven “green food webs” have been well characterized (reviewed by Estes et al. 2011), less is known about the indirect effects of consumers on detritus-based “brown food webs” (Moore et al. 2004, Wu et al. 2011). Those studies that have reported detrital-based trophic cascades have predominantly been documented within temperate streams. For example, by preying on leaf-consuming detritivores, stonefly predators indirectly reduced leaf decay rates in alpine streams (Obernbörfer 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, S Greig and R McIntosh 2006, Woodward et al. 2008). Boyero and others (2008), in the only detritus-based trophic cascade to our knowledge observed in tropical streams, reported that predatory rainbowfish in Australia significantly reduced the breakdown rate of leaf material by inhibiting activity of all shredding macroinvertebrates. Similarly, we found strong evidence that predatory killifish caused a detritus-based trophic cascade by reducing the biomass of an insect shredder in Neotropical mountain streams. However, in contrast to the above examples, we found these cascading effects depended on the presence or absence of a sympatric omnivorous consumer (guppies).

While research has consistently shown strong indirect ecosystem effects of losing dominant top consumers at the landscape-scale (Estes et al. 2011), we found increasing the number of macroconsumers in a stream community decoupled a detritus-based trophic cascade at both local- and landscape-scales. Moreover, our findings suggest that

characterizing the functional relationships among sympatric consumers will be important in predicting the community and ecosystem effects of losing individual macroconsumer species from systems containing multiple macroconsumers.

Acknowledgements

We thank C. Morris, S. Collins, K. MacNeill, J. Zagarola, and S. Grabinski for field assistance, and C. Morris, K. MacNeill, J. Zagarola, C. Braman, J. Bielenberg, A. Donnelly, T. Stratmann, and M. Chamblee for lab assistance. We thank P. Bumpers, D. Manning, and K. Farrell and the members of the Pringle Lab for their extensive manuscript comments. We thank the Water and Sewage Authority of Trinidad and Tobago, the University of the West Indies at St. Augustine, and the Asa Wright Nature Centre for permission to access various sites and for use of facilities. The authors appreciate the help and support of Ronnie Hernandez and the staff of William Beebe Tropical Research Station. Additionally we appreciate the help and support of Jogie Ramlal and the Ramlal family. This work was supported by a National Science Foundation Frontiers in Integrative Biological Research (FIBR) Grant to DNR and others (NSF-FIBR, DEB-0623632) and the NSF Graduate Research Fellowship program (fellowship to TNS).

Table 4.1: Density [No. m⁻² (SE)] estimates of macroconsumers (killifish, crabs, and guppies) from the macroconsumer enclosure experiment. Macroconsumer density was estimated using day and night observations. Observations conducted for 10 hours per reach (n=20 observations per reach). Diurnal estimates based on pooled visitation from 24 hr. access and daytime access treatments, while Nocturnal estimates are based on 24 hr. access and nighttime access treatments. Bolded values represent significant results from Kruskal-Wallis rank sum tests (P<0.05) between diurnal and nocturnal activity.

Reach	Observation Period	Macroconsumer Density		
		Killifish	Crab	Guppy
KCG	Daytime	0.35 (0.12)	0.16 (0.07)	7.31 (1.49)
	Nighttime	0.84 (0.18)	0.08 (0.06)	0.00 (0.00)
KC	Daytime	0.56 (0.33)	0.16 (0.13)	Not Present
	Nighttime	1.36 (0.52)	1.89 (0.60)	.

Table 4.2: Physiochemical characteristics [means±SE (min, max)] of pools from the landscape study measured on the final day of the experiment. Physical characteristics were measured in pools (n=18 replicate pools per reach type) across KCG (killifish, crab, and guppy) and KC (killifish and crab) reaches.

Reach	Physiochemical Characteristics						
	Canopy openness (%)	CBOM (g/m ²)	Depth (cm)	DO (% saturation L ⁻¹)	Leaf Cover (%)	Temp (°C)	Velocity (m/s)
KCG	4.25±1.41 (1.3, 15.86)	112.93±21.46 (23.52, 394.86)	13.78±1.09 (8.0, 18.0)	79.9±43.16 (66.9, 89.9)	46.94±8.08 (15, 80)	23.78±0.16 (23.3, 24.4)	0.0049±0.001 (0.00, 0.03)
KC	4.64±1.13 (0.26, 12.48)	161.7±25.71 (48.80, 480.25)	14.22±1.01 (10.5, 18.5)	81.64±3.30 (56.9, 91.7)	43.61±8.04 (15, 80)	23.69±0.18 (23.1, 24.4)	0.0054±0.001 (0.00, 0.07)

Table 4.3: Abundance of macroconsumers (killifish, crabs, guppies) for the landscape study. Abundance estimated using catch per unit effort [CPUE (\pm SE)], for killifish and guppies, and transect observations [individuals m^{-1} (\pm SE)] for crabs. CPUE estimates were calculated during the day using two baited minnow traps, once in each pool (n=18 replicate pools). Transect counts of crabs were conducted at night, across 100 m length of stream, once in each reach (n=6 replicate reaches). Bolded values represent significant differences ($P<0.05$) from one-way ANOVA between KCG and KC reaches for each species respectively.

Reach	Macroconsumer Abundance		
	Killifish (CPUE)	Crab (Indiv. m^{-1})	Guppy (CPUE)
KCG	8.83 (2.81)	3.32 (1.38)	23.00 (9.57)
KC	30.61 (6.98)	3.09 (1.35)	N.A.

Figure legends

Figure 4.1: Conceptual diagram of detrital food web in streams consisting of paired reaches containing killifish, crabs, and guppies (KCG) downstream, and killifish and crabs (KC) upstream, where barrier waterfalls naturally limit upstream migration of guppies. *Phylloicus hansonii* is a dominant macroinvertebrate detrital shredder in these streams. Solid arrows indicate hypothesized energy movement up trophic levels while dashed arrows represents movement within trophic level.

Figure 4.2: Location of study sites in the Caroni drainage of Trinidad's Northern Range (Inset A) for macroconsumer exclosure experiment (1) and the landscape study (2a-f). Each study site consists of paired reaches (Inset B) containing killifish, crabs, and guppies (KCG) downstream, and killifish and crabs (KC) upstream. Guppies are naturally excluded from the upstream-most reaches by barrier waterfalls.

Figure 4.3: Mean (\pm SE) for (a,b) decay rate of *Cecropia peltata* leaves (% mass lost day⁻¹) and (c,d) *Phylloicus hansonii* resource-specific biomass (mg dry mass g⁻¹ *Cecropia*) for the exclosure experiment, where 24 hour treatments allow full macroconsumer access [control (C)] or completely exclude macroconsumers [electric (E)]. 12 hour treatments allow access to either diurnally active [day (D)] or nocturnally active [night (N)] macroconsumers. Experiment conducted in reaches containing either (a,c) killifish, crabs, and guppies (KCG), or (b,d) only killifish and crabs (KC) and replicated in pools (n=5 per reach). Letters between

bars indicate results of post-hoc Tukey-Kramer HSD tests, with different letters indicating significant within reach differences in pairs of means between treatments.

Figure 4.4: Bivariate fit of decay rate for leaves of *Cecropia peltata* (% mass lost day⁻¹) and *Phylloicus hansonii* resource-specific biomass (mg dry mass g⁻¹ *Cecropia*) from the enclosure experiment. Experiment conducted in reaches containing either (a) killifish, crabs, and guppies (KCG), or (b) only killifish and crabs (KC) and replicated by diel treatments in pools (n=20 per reach). Statistical significance of correlation noted by * (P<0.05) and ** (P<0.01).

Figure 4.5: Mean (\pm SE) for (a) decay rate for *Cecropia peltata* leaves (% mass lost day⁻¹) and (b) *Phylloicus hansonii* resource-specific biomass (mg dry mass g⁻¹ *Cecropia*) for the landscape study. Landscape study conducted in streams consisting of a paired reach containing killifish, crabs, and guppies (KCG) downstream, and killifish and crabs (KC) upstream. Estimates of leaf decay were replicated across streams (n=6 streams per reach type). Letters between bars indicate results of one-way ANOVA, with different letters indicating significant differences in pairs of means between reaches.

Figure 6: Bivariate fit of decay rate for *Cecropia peltata* leaves (% mass lost day⁻¹) and *Phylloicus hansonii* resource-specific biomass (mg dry mass g⁻¹ *Cecropia*) from the landscape study. Study conducted in streams consisting of a paired reach containing (a) killifish, crabs, and guppies (KCG) downstream, and killifish and (b) crabs (KC) upstream, and replicated in pools (n=18 pool per reach type). Statistical significance of correlation noted by * (P<0.05) and ** (P<0.01).

Figure 4.7: *Phylloicus hansonii* resource-specific biomass [mg dry mass g⁻¹ *Cecropia* (\pm SE)], in individual pools averaged over the 4 week landscape study. Landscape study consisting of a reach containing killifish, crabs, and guppies (KCG, pools 1-3) downstream, paired with a reach containing killifish and crabs (KC, pools 4-6) upstream. Estimates of *Phylloicus* biomass were replicated in pools nested within streams (n=6 streams per reach type). Individual pools were separated by at least 50 m and adjacent pools in KCG (pool 3) and KC (pool 4) reaches were separated by no more than 100 m. Analysis showed that changes in *Phylloicus* biomass are not gradual along a stream continuum between pools ($p>0.5$), but abrupt when macroconsumer assemblage changes (reach effect; $p<0.001$).

Figure 4.1

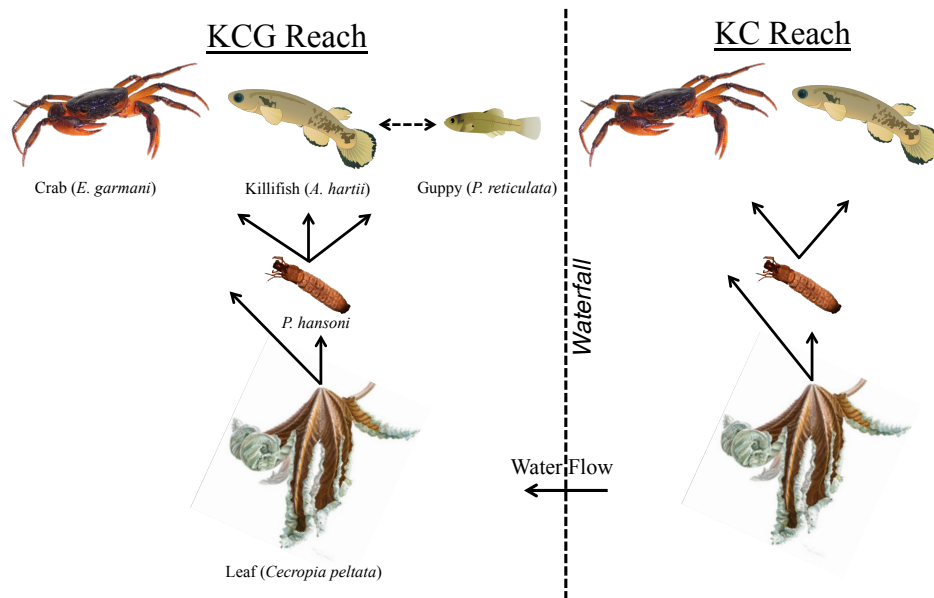


Figure 4.2

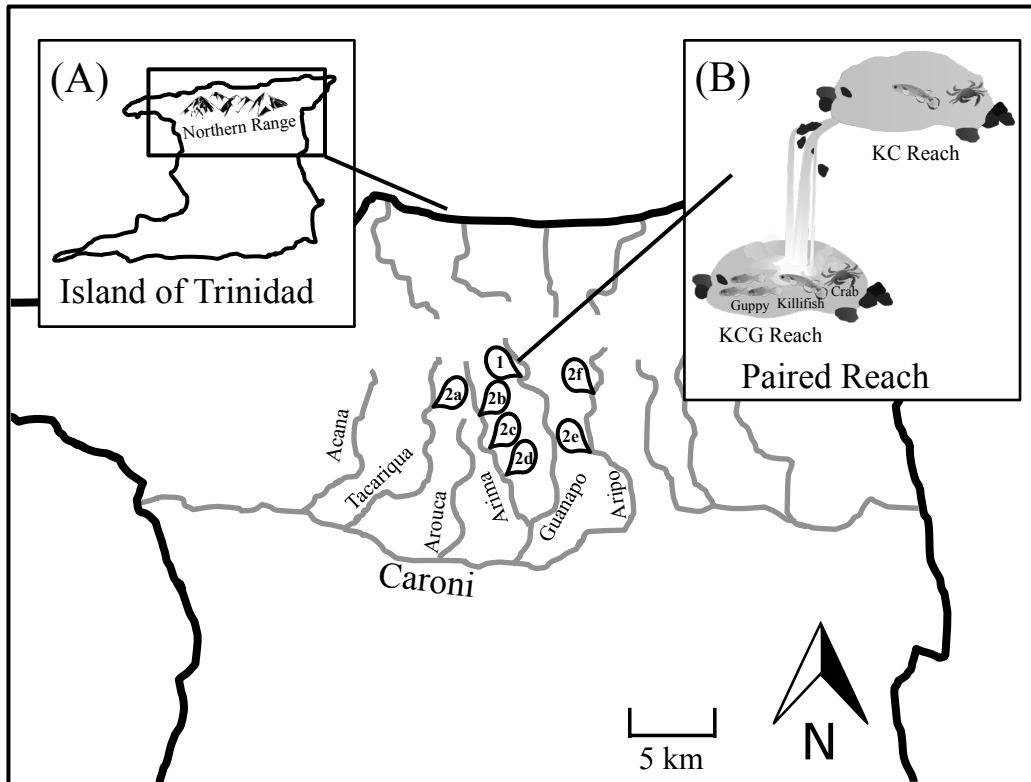


Figure 4.3

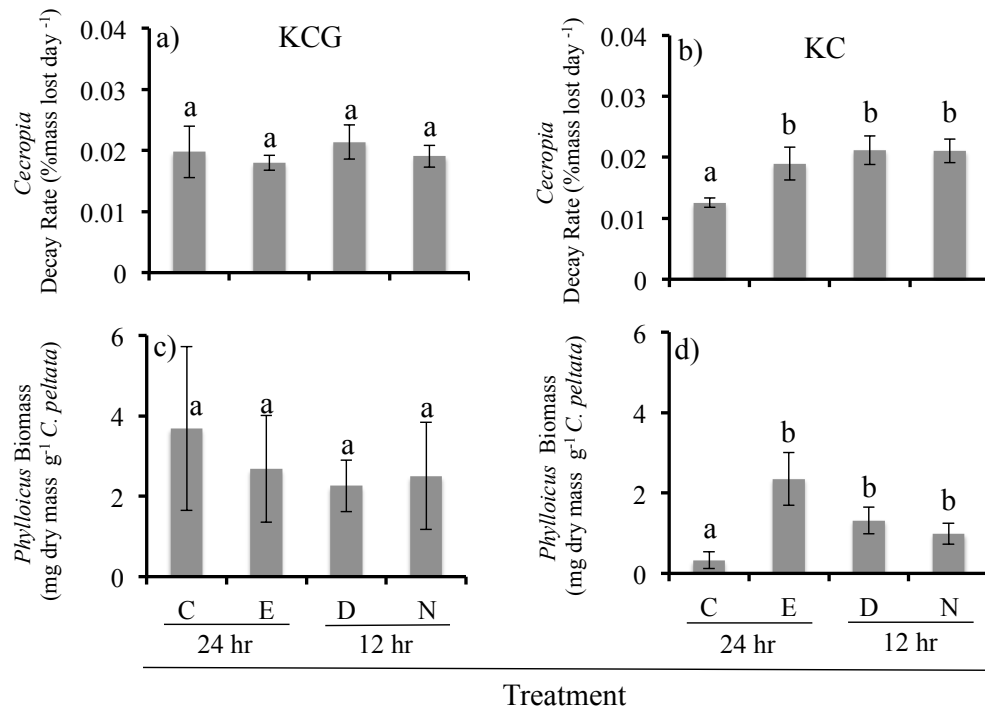


Figure 4.4

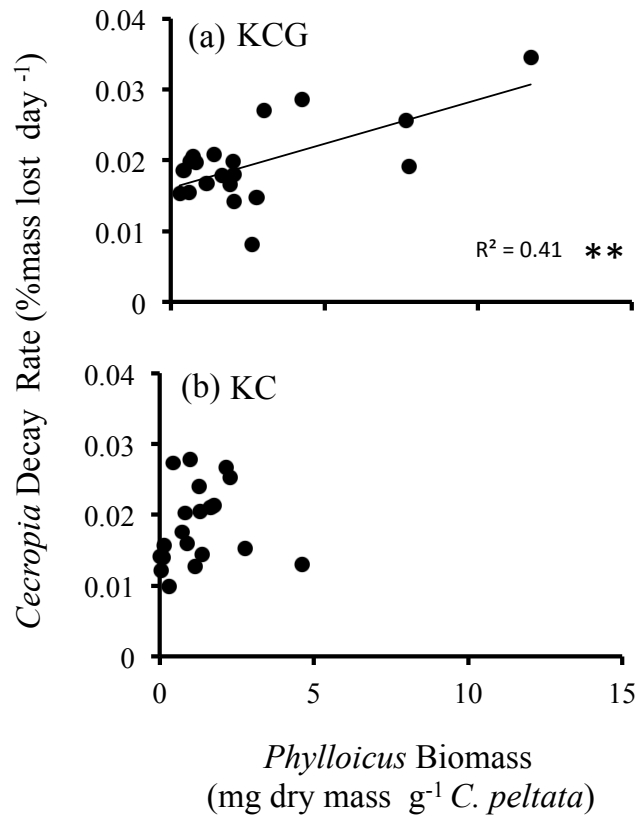


Figure 4.5

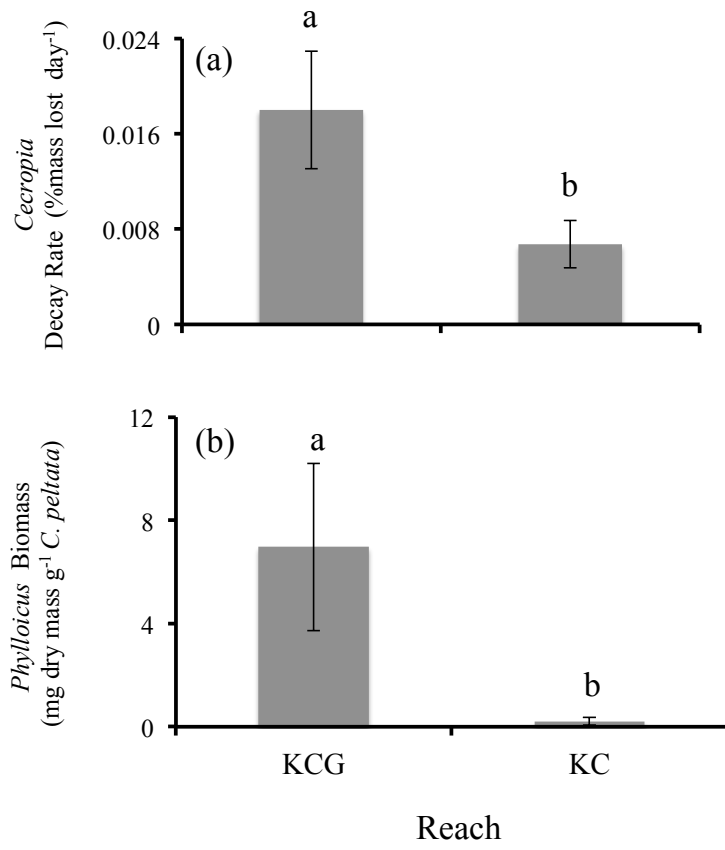


Figure 4.6

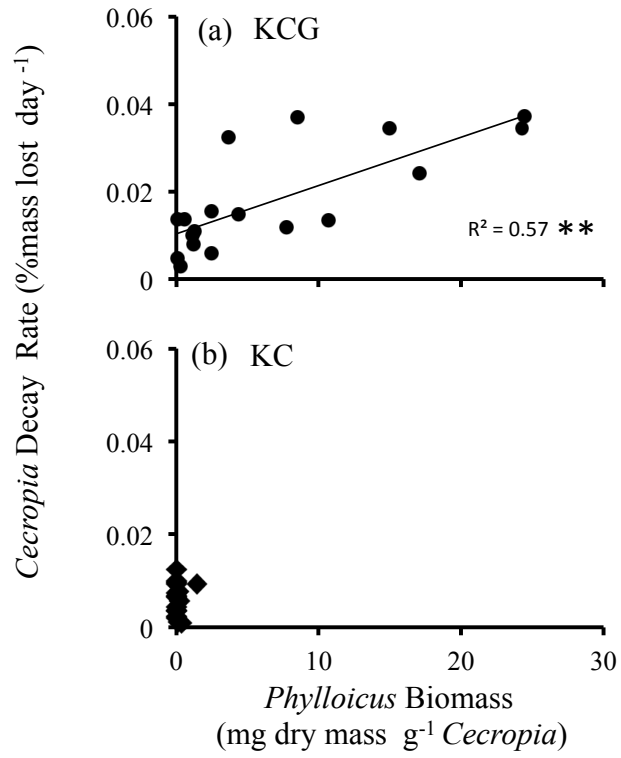
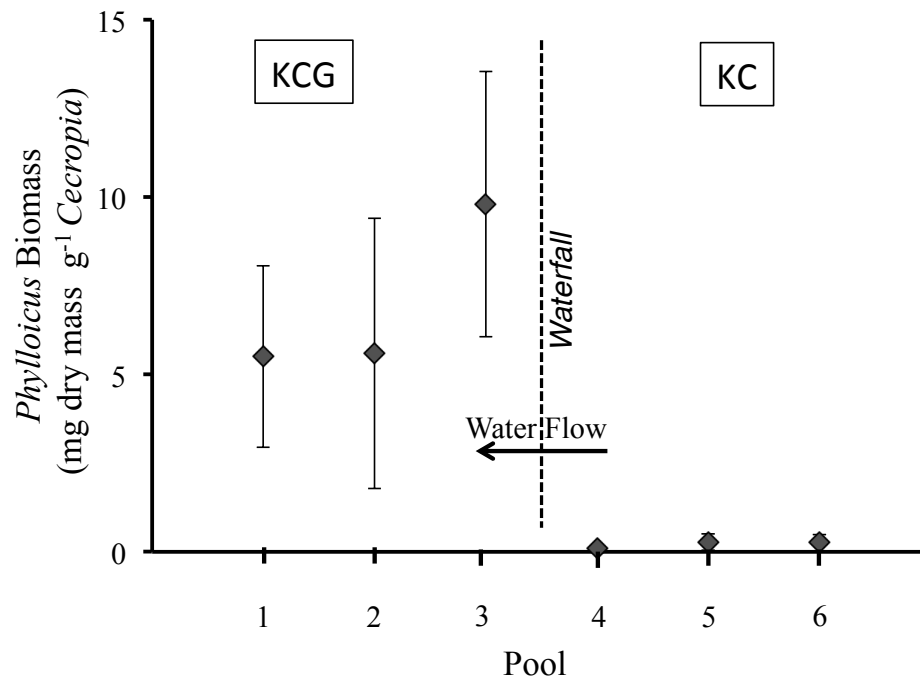


Figure 4.7



CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

General Conclusions

This dissertation explored direct and indirect ecosystem effects of macroconsumers in aquatic community dynamics, using Trinidadian guppies in the mountain streams of Northern Trinidad as a model system. The findings of this dissertation broadly suggest that in order to understand the ecosystem consequences of macroconsumers more fully, future research must consider the effects of intraspecific variation, macroconsumer consumption and excretion, and the functional relationships among sympatric macroconsumers. These results have strong conservation implications given the global trend of biodiversity loss in higher trophic levels (Duffy 2002, Petchey et al. 2004).

In Chapter 2, I explored the relative importance of macroconsumer consumptive and non-consumptive effects for primary producer growth and biomass. We found strong evidence that browsing by guppies dominated the top-down effects of macroconsumers on algal accrual rate and algal biomass consistently across four streams in Northern Trinidad. Additionally I found that the net effects of guppies, from high elevation streams, on algal growth and biomass, was not the result of a simple top-down consumptive effect, but likely the combination of large and opposing top-down and

bottom-up effects. Surprisingly, these results suggest that opposing consumptive and non-consumptive effects reduced the observed net effects of guppies.

In Chapter 3, I examined the ecosystem consequences of macroconsumer local adaptation *in situ*. The results of this study show that the local adaptation of guppy populations, to the loss of large piscivorous fish in higher elevation streams, shifted their top-down ecosystem effects from reducing macroinvertebrate standing stock biomass to reducing primary producer standing stock biomass. Moreover, I found that the differential effects of guppy phenotypes on primary producers were similar in magnitude to that of light availability, an important abiotic driver of ecosystems.

Finally in Chapter 4, I investigated the top-down effects of macroconsumers on leaf decomposition rates in the context of the natural presence or absence of omnivorous guppies from a system containing multiple macroconsumers. Our results demonstrate that the loss of an omnivorous consumer from a macroconsumer assemblage can alter the strength of top down effects of the remaining macroconsumer community on an important ecosystem-level process at both local- and landscape-scales.

Recommendations For Future Research

My findings are based on studies conducted during the dry season. In Trinidadian streams, like those most temperate and tropical streams, the strongest interaction strength between biota is likely to occur during baseflow conditions experienced during the dry season. This is partly due to the reduced water volume in the dry season, which increases the density of consumers, but also because repeated storm events, during the wet season, often ‘reset’ the benthic environment and may greatly reduce the biomass of biota.

Therefore, to fully characterize the effects of macroconsumers on ecosystem structure and function, future research should examine effects of macroconsumers in both the wet and the dry season. If resources were infinite, I would suggest a continuous electric enclosure study to examine the top-down and bottom-up effects of macroconsumers across multiple years. This would allow investigating if macroconsumer ecosystem effects are context dependent and elucidate possible interactive effects of macroconsumers and abiotic disturbances like floods. Pringle and Homazaki (1997), reported evidence that the presence of omnivorous fish stabilized the benthic environment, thereby reducing the effects of a storm event.

In Trinidad, I predict that transition periods between wet and dry season could be a particularly key time to investigate interactions between macroconsumer and abiotic events. For example, in a dry period after a prolonged phase of flooding, macroinvertebrate grazer biomass and inorganic sediment would be extremely low potentially allowing for benthic primary producers to have accelerated growth rates. During this period I would predict that macroconsumer nutrient excretion could become a particularly important bottom-up pathway allowing primary producer biomass to grow at a faster rate. Additionally, in the transition from dry to wet season, loose sediment is often washed into streams and may limit primary production. I predict that benthic grazing and excretion by macroconsumers could facilitate a longer “growing season” for primary producers by clearing sediments and reducing nutrient limitation (*sensu* Power 1990).

Interactions between abiotic disturbance and patterns of species abundance have long been acknowledged (Connell 1961), however investigating how abiotic factors

influence the direction and magnitude of macroconsumers top-down and bottom-up effects on lower trophic levels would be a novel path for future research.

REFERENCES

- Agrawal, A. A., M. T. J. Johnson, A. P. Hastings, and J. L. Maron. 2013. A Field Experiment Demonstrating Plant Life-History Evolution and Its Eco-Evolutionary Feedback to Seed Predator Populations. *American Naturalist* **181**:S35-S45.
- Allen, S. E. 1974. *Chemical Analysis of Ecological Materials*. Blackwell Scientific Publications, London, UK:565.
- Bassar, R. D., R. Ferriere, A. Lopez-Sepulcre, M. C. Marshall, J. Travis, C. M. Pringle, and D. N. Reznick. 2012. Direct and Indirect Ecosystem Effects of Evolutionary Adaptation in the Trinidadian Guppy (*Poecilia reticulata*). *American Naturalist* **180**:167-185.
- Bassar, R. D., M. C. Marshall, A. Lopez-Sepulcre, E. Zandonna, S. K. Auer, J. Travis, C. M. Pringle, A. S. Flecker, S. A. Thomas, D. F. Fraser, and D. N. Reznick. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. *Proceedings of the National Academy of Sciences of the United States of America* **107**:3616-3621.
- Becker, B., M. S. Moretti, and M. Callisto. 2009. Length-dry mass relationships for a typical shredder in Brazilian streams (Trichoptera: Calamoceratidae). *Aquatic Insects* **31**:227-234.
- Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society* **18**:308-343.
- Botosaneanu, L., and D. Sakal. 1992. Ecological observations on the caddisflies (Insecta: Trichoptera) from Trinidad and Tobago (W. Indies). *Revue d'hydrobiologie tropicale* **25**:197.
- Boyero, L., P. A. Rincon, and R. G. Pearson. 2008. Effects of a predatory fish on a tropical detritus-based food web. *Ecological Research* **23**:649-655.
- Bruno, J. F., and M. I. O'Connor. 2005. Cascading effects of predator diversity and omnivory in a marine food web. *Ecology Letters* **8**:1048-1056.
- Butman, D., and P. A. Raymond. 2011. Significant efflux of carbon dioxide from streams and rivers in the United States. *Nature Geoscience* **4**:839-842.

- Capps, K. A., A. Ulseth, and A. S. Flecker. 2014. Quantifying the top-down and bottom-up effects of a non-native grazer in freshwaters. *Biological Invasions*:1-14.
- Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. *Bioscience*:634-639.
- Carpenter, S. R., J. F. Kitchell, J. R. Hodgson, P. A. Cochran, J. J. Elser, M. M. Elser, D. M. Lodge, D. Kretchmer, X. He, and C. N. Vonende. 1987. Regulation of lake primary production by food web structure. *Ecology* **68**:1863-1876.
- Connell, J. H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology* **42**:710-723.
- Connelly, S., C. M. Pringle, R. J. Bixby, R. Brenes, M. R. Whiles, K. R. Lips, S. Kilham, and A. D. Huryn. 2008. Changes in Stream Primary Producer Communities Resulting from Large-Scale Catastrophic Amphibian Declines: Can Small-Scale Experiments Predict Effects of Tadpole Loss? *Ecosystems* **11**:1262-1276.
- Dorn, N. J., and J. M. Wojdak. 2004. The role of omnivorous crayfish in littoral communities. *Oecologia* **140**:150-159.
- Duffy, J. E. 2002. Biodiversity and ecosystem function: the consumer connection. *Oikos* **99**:201-219.
- El-Sabaawi, R. W., R. D. Bassar, C. Rakowski, M. C. Marshall, B. L. Bryan, S. N. Thomas, C. Pringle, D. N. Reznick, and A. S. Flecker. 2015. Intraspecific phenotypic differences in fish affect ecosystem processes as much as bottom-up factors. *Oikos*:n/a-n/a.
- Estes, J. A., J. Terborgh, J. S. Brashares, M. E. Power, J. Berger, W. J. Bond, S. R. Carpenter, T. E. Essington, R. D. Holt, J. B. C. Jackson, R. J. Marquis, L. Oksanen, T. Oksanen, R. T. Paine, E. K. Pikitch, W. J. Ripple, S. A. Sandin, M. Scheffer, T. W. Schoener, J. B. Shurin, A. R. E. Sinclair, M. E. Soule, R. Virtanen, and D. A. Wardle. 2011. Trophic Downgrading of Planet Earth. *Science* **333**:301-306.
- Feller, R. J., and R. M. Warwick. 1988. *Energetics*. in R. P. Higgins and H. Thiel, editors. *Introduction to the study of meiofauna*. Smithsonian Institution Press, Washington, DC.
- Finke, D. L., and R. F. Denno. 2002. Intraguild predation diminished in complex-structured vegetation: implications for prey suppression. *Ecology* **83**:643-652.
- Finke, D. L., and R. F. Denno. 2005. Predator diversity and the functioning of ecosystems: the role of intraguild predation in dampening trophic cascades. *Ecology Letters* **8**:1299-1306.

- Fitzpatrick, S. W., J. Torres-Dowdall, D. N. Reznick, C. K. Ghalambor, and W. C. Funk. 2014. Parallelism Isn't Perfect: Could Disease and Flooding Drive a Life-History Anomaly in Trinidadian Guppies? *The American Naturalist* **183**:290-300.
- Flecker, A. S. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical stream. *Ecology* **77**:1845-1854.
- Flint Jr, O. S. 1996. Studies of neotropical caddisflies LV: Trichoptera of Trinidad and Tobago. *Transactions of the American Entomological Society*:67-113.
- Fraser, D. F., J. F. Gilliam, M. P. MacGowan, C. M. Arcaro, and P. H. Guillozet. 1999. Habitat quality in a hostile river corridor. *Ecology* **80**:597-607.
- Fraser, D. F., and B. A. Lamphere. 2013. Experimental evaluation of predation as a facilitator of invasion success in a stream fish. *Ecology* **94**:640-649.
- Gelman, A. 2007. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press.
- Gilliam, J. F., D. F. Fraser, and M. Alkinkoo. 1993. Structure of a tropical stream fish community - a role for biotic interactions. *Ecology* **74**:1856-1870.
- Greathouse, E. A., C. M. Pringle, and W. H. McDowell. 2006. Do small-scale exclosure/enclosure experiments predict the effects of large-scale extirpation of freshwater migratory fauna? *Oecologia* **149**:709-717.
- Hedges, L. V., J. Gurevitch, and P. S. Curtis. 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* **80**:1150-1156.
- Huang, C., and A. Sih. 1991. Experimental studies on direct and indirect interactions in a three trophic-level stream system. *Oecologia* **85**:530-536.
- Hutchinson, G. E. 1965. The ecological theater and the evolutionary play. Yale University Press New Haven.
- Knoll, L. B., P. B. McIntyre, M. J. Vanni, and A. S. Flecker. 2009. Feedbacks of consumer nutrient recycling on producer biomass and stoichiometry: separating direct and indirect effects. *Oikos* **118**:1732-1742.
- Kohler, T. J., T. N. Heatherly, R. W. El-Sabaawi, E. Zandona, M. C. Marshall, A. S. Flecker, C. M. Pringle, D. N. Reznick, and S. A. Thomas. 2012. Flow, nutrients, and light availability influence Neotropical epilithon biomass and stoichiometry. *Freshwater Science* **31**:1019-1034.
- Konishi, M., S. Nakano, and T. Iwata. 2001. Trophic cascading effects of predatory fish on leaf litter processing in a Japanese stream. *Ecological Research* **16**:415-422.

- Liley, N., and B. Seghers. 1975. Factors affecting the morphology and behaviour of guppies in Trinidad. *Function and evolution in behaviour*:92-118.
- Loeb, S. L. 1981. An insitu method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems *Limnology and Oceanography* **26**:394-399.
- Magurran, A. E. 2005. *Evolutionary ecology: the Trinidadian guppy*. Oxford University Press.
- Maitland, D. P. 2003. Predation on snakes by the freshwater land crab *Eudaniela garmani*. *Journal of Crustacean Biology* **23**:241-246.
- Malmqvist, B. 1993. Interactions in stream leaf packs: effects of a stonefly predator on detritivores and organic matter processing. *Oikos*:454-462.
- Marshall, M. C., A. J. Binderup, E. Zandona, S. Goutte, R. D. Bassar, R. W. El-Sabaawi, S. A. Thomas, A. S. Flecker, S. S. Kilham, D. N. Reznick, and C. M. Pringle. 2012. Effects of Consumer Interactions on Benthic Resources and Ecosystem Processes in a Neotropical Stream. *Plos One* **7**.
- Merritt, R. W., K. W. Cummins, and M. B. Berg. 2008. *An Introduction to the Aquatic Insects of North America*. 4th edition. Kendall Hunt, Dubuque, IA.
- Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. de Ruitter, Q. Dong, A. Hastings, N. C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo, K. M. Scow, M. J. Vanni, and D. H. Wall. 2004. Detritus, trophic dynamics and biodiversity. *Ecology Letters* **7**:584-600.
- Moulton, T. P., M. L. Souza, E. F. Brito, M. R. A. Braga, and S. E. Bunn. 2012. Strong interactions of *Paratya australiensis* (Decapoda: Atyidae) on periphyton in an Australian subtropical stream. *Marine and Freshwater Research* **63**:834-844.
- Murray, K. *In Prep*. Evidence for inter-reach variation in the population size structure of a shredding caddisfly (*Phylloicus hansonii*) in headwater streams, Trinidad.
- Obernbörfer, R. Y., J. V. McArthur, J. R. Barnes, and J. Dixon. 1984. The effect of invertebrate predators on leaf litter processing in an alpine stream. *Ecology*:1325-1331.
- Palkovacs, E. P., M. C. Marshall, B. A. Lamphere, B. R. Lynch, D. J. Weese, D. F. Fraser, D. N. Reznick, C. M. Pringle, and M. T. Kinnison. 2009. Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philosophical Transactions of the Royal Society B-Biological Sciences* **364**:1617-1628.

- Palkovacs, E. P., and D. M. Post. 2009. Experimental evidence that phenotypic divergence in predators drives community divergence in prey. *Ecology* **90**:300-305.
- Pennisi, E. 2012. The Great Guppy Experiment. *Science* **337**:904-908.
- Perez, R. G. 1988. Guia para el estudio de los macroinvertebrados acuaticos del Departamento de Antioquia. Universidad de Antioquia, Medellin, Colombia.
- Petchey, O. L., A. L. Downing, G. G. Mittelbach, L. Persson, C. F. Steiner, P. H. Warren, and G. Woodward. 2004. Species loss and the structure and functioning of multitrophic aquatic systems. *Oikos* **104**:467-478.
- Petchey, O. L., P. T. McPhearson, T. M. Casey, and P. J. Morin. 1999. Environmental warming alters food-web structure and ecosystem function. *Nature* **402**:69-72.
- Plummer, M. 2003. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. Pages 20-22 in Proceedings of the 3rd International Workshop on Distributed Statistical Computing (DSC 2003). March.
- Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual Review of Ecology and Systematics*:297-330.
- Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. *American Naturalist* **147**:813-846.
- Post, D. M., E. P. Palkovacs, E. G. Schielke, and S. I. Dodson. 2008. Intraspecific variation in a predator affects community structure and cascading trophic interactions. *Ecology* **89**:2019-2032.
- Power, M. E. 1990. Resource enhancement by indirect effects of grazers: armored catfish, algae, and sediment. *Ecology*:897-904.
- Power, M. E., W. J. Matthews, and A. J. Stewart. 1985. Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. *Ecology* **66**:1448-1456.
- Pringle, C. M., and G. A. Blake. 1994. Quantitative effects of *Atyid* shrimp (Decapoda, *Atyidae*) on the depositional environment in a tropical stream - use of electricity for experimental exclusion. *Canadian Journal of Fisheries and Aquatic Sciences* **51**:1443-1450.
- Pringle, C. M., and T. Hamazaki. 1997. Effects of fishes on algal response to storms in a tropical stream. *Ecology* **78**:2432-2442.

- Pringle, C. M., and T. Hamazaki. 1998. The role of omnivory in a neotropical stream: Separating diurnal and nocturnal effects. *Ecology* **79**:269-280.
- Pringle, C. M., N. Hemphill, W. H. McDowell, A. Bednarek, and J. G. March. 1999. Linking species and ecosystems: Different biotic assemblages cause interstream differences in organic matter. *Ecology* **80**:1860-1872.
- Reznick, D., M. J. Butler, and H. Rodd. 2001. Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *American Naturalist* **157**:126-140.
- Reznick, D., and J. A. Endler. 1982. The impact of predation on the life-history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**:160-177.
- Reznick, D. A., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural-population. *Nature* **346**:357-359.
- Reznick, D. N., R. D. Bassar, J. Travis, and F. H. Rodd. 2012. Life-history evolution in guppies VII: The demographics of density regulation in guppies (*Poecilia reticulata*). *Evolution* **66**:2903-2915.
- Reznick, D. N., F. H. Shaw, F. H. Rodd, and R. G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* **275**:1934-1937.
- Rickson, F. R. 1977. Progressive loss of ant-related traits of *Cecropia peltata* on selected Caribbean islands. *American Journal of Botany*:585-592.
- Ripple, W. J., J. A. Estes, R. L. Beschta, C. C. Wilmers, E. G. Ritchie, M. Hebblewhite, J. Berger, B. Elmhagen, M. Letnic, M. P. Nelson, O. J. Schmitz, D. W. Smith, A. D. Wallach, and A. J. Wirsing. 2014. Status and Ecological Effects of the World's Largest Carnivores. *Science* **343**:151-+.
- Ripple, W. J., E. J. Larsen, R. A. Renkin, and D. W. Smith. 2001. Trophic cascades among wolves, elk and aspen on Yellowstone National Park's northern range. *Biological Conservation* **102**:227-234.
- Ruetz, C. R., R. M. Newman, and B. Vondracek. 2002. Top-down control in a detritus-based food web: fish, shredders, and leaf breakdown. *Oecologia* **132**:307-315.
- S Greig, H., and A. R McIntosh. 2006. Indirect effects of predatory trout on organic matter processing in detritus-based stream food webs. *Oikos* **112**:31-40.
- Schmitz, O. J., P. A. Raymond, J. A. Estes, W. A. Kurz, G. W. Holtgrieve, M. E. Ritchie, D. E. Schindler, A. C. Spivak, R. W. Wilson, and M. A. Bradford. 2014. Animating the carbon cycle. *Ecosystems* **17**:344-359.

- Schoener, T. W. 2011. The Newest Synthesis: Understanding the Interplay of Evolutionary and Ecological Dynamics. *Science* **331**:426-429.
- Schofield, K. A., C. M. Pringle, J. L. Meyer, and E. J. Rosi-Marshall. 2008. Functional redundancy of stream macroconsumers despite differences in catchment land use. *Freshwater Biology* **53**:2587-2599.
- Seber, G. A. F. 1982. The estimation of animal abundance. Griffin.
- Simon, T. N. *In Prep*. Isolating the effects of Trinidadian guppies from sympatric macroconsumers on the structure of the ecosystem using an *in situ* density manipulation.
- Sterner, R. W. 1986. Herbivores' direct and indirect effects on algal populations. *Science(Washington)* **231**:605-607.
- Strayer, D. L. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* **55**:152-174.
- Strong, D. R. 1992. Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. *Ecology* **73**:747-754.
- Sutherland, W. J., R. P. Freckleton, H. C. J. Godfray, S. R. Beissinger, T. Benton, D. D. Cameron, Y. Carmel, D. A. Coomes, T. Coulson, and M. C. Emmerson. 2013. Identification of 100 fundamental ecological questions. *Journal of Ecology* **101**:58-67.
- Tanabe, K., and T. Namba. 2005. Omnivory creates chaos in simple food web models. *Ecology* **86**:3411-3414.
- Taylor, B. W., A. S. Flecker, and R. O. Hall. 2006. Loss of a harvested fish species disrupts carbon flow in a diverse tropical river. *Science* **313**:833-836.
- Turcotte, M. M., D. N. Reznick, and J. D. Hare. 2011. The impact of rapid evolution on population dynamics in the wild: experimental test of eco-evolutionary dynamics. *Ecology Letters* **14**:1084-1092.
- Urban, M. C. 2013. Evolution mediates the effects of apex predation on aquatic food webs. *Proceedings of the Royal Society B-Biological Sciences* **280**.
- Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**:130-137.

- Wake, D. B., and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* **105**:11466-11473.
- Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science* **277**:102-104.
- Walsh, M. R., D. F. Fraser, R. D. Bassar, and D. N. Reznick. 2011. The direct and indirect effects of guppies: implications for life-history evolution in *Rivulus hartii*. *Functional Ecology* **25**:227-237.
- Walsh, M. R., and D. N. Reznick. 2009. Phenotypic diversification across an environmental gradient: a role for predators and resource availability on the evolution of life histories. *Evolution* **63**:3201-3213.
- Woodward, G., G. Papantoniou, F. Edwards, and R. B. Lauridsen. 2008. Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. *Oikos* **117**:683-692.
- Wu, X., J. E. Duffy, P. B. Reich, and S. Sun. 2011. A brown-world cascade in the dung decomposer food web of an alpine meadow: effects of predator interactions and warming. *Ecological Monographs* **81**:313-328.
- Yoshida, T., L. E. Jones, S. P. Ellner, G. F. Fussmann, and N. G. Hairston. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. *Nature* **424**:303-306.
- Zandona, E., S. K. Auer, S. S. Kilham, J. L. Howard, A. Lopez-Sepulcre, M. P. O'Connor, R. D. Bassar, A. Osorio, C. M. Pringle, and D. N. Reznick. 2011. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Functional Ecology* **25**:964-973.
- Zou, X., C. P. Zucca, R. B. Waide, and W. H. McDowell. 1995. Long-term influence of deforestation on tree species composition and litter dynamics of a tropical rain forest in Puerto Rico. *Forest Ecology and Management* **78**:147-157.

APPENDIX A

Table 1: Insect community composition on *Cecropia peltata* leaves in the enclosure experiment. Table showing functional feeding group (FFG) guild, order, and lowest taxonomic grouping for both KCG (killifish, crabs, and guppies) and KC (killifish and crabs) reaches. Biomass of each taxon were collected from control treatments (24 hour macroconsumer access) and summed across each sampling period (n=5) for the 4 week experiment. Percent (%) biomass indicate contributions of each taxon to the overall biomass and abundance of the entire community. *Phylloicus hansonii* is highlighted, because it was the only leaf shredding insect found in the community.

FFG	Order	Lowest Taxonomic Grouping	Total Biomass (mg)		% Biomass		
			<u>KCG</u>	<u>KC</u>	<u>KCG</u>	<u>KC</u>	
Collector Gatherer	Coleoptera	Elmidae	5.27	25.00	1.41	13.99	
		Diptera	Ceratopogonidae	2.93	0.27	0.78	0.15
	Ephemeroptera	Chironomidae	14.35	19.52	3.84	10.92	
		Tipulidae	0.00	0.03	0.00	0.02	
		<i>Baetis spp.</i>	0.92	3.25	0.24	1.82	
		<i>Baetodes spp.</i>	0.00	5.37	0.00	3.01	
		<i>Leptohyphes spp.</i>	2.97	7.10	0.79	3.97	
		<i>Tricorythodes spp.</i>	1.34	0.71	0.36	0.40	
		Trichoptera	Glossosomatidae	0.96	0.00	0.26	0.00
	Predator	Diptera	Hydropsychidae	0.18	0.00	0.05	0.00
			Nectopysche	0.12	0.00	0.03	0.00
		Odonata	Philopotamidae	14.63	0.00	3.91	0.00
			Tanypodinae	46.74	3.87	12.49	2.16
Scraper	Ephemeroptera	Argia	21.67	61.57	5.79	34.45	
		Hetaerina	9.07	21.76	2.42	12.17	
		Libellulidae	0.37	0.00	0.10	0.00	
Shredder	Trichoptera	<i>Thraulodes spp.</i>	1.08	8.39	0.29	4.69	
		<i>Phylloicus hansonii</i>	251.61	21.89	67.24	12.25	
Total:			374.20	178.73			

Table 2: *Phylloicus hansonii* normalized abundance [RSA (No. *Phylloicus* g⁻¹ *Cecropia* dry mass)], and length (mm), averaged over the 4 week landscape study. Landscape study conducted in streams consisting of a paired reach containing killifish, crabs, and guppies (KCG) downstream, and killifish and crabs (KC) upstream. Estimates of *Phylloicus* biomass, abundance, and length were replicated across streams (n=6 streams per reach type).

Reach	Mean (SE)	
	RSA	Length
KCG	3.64 (1.60)	8.25 (0.93)
KC	0.09 (0.06)	7.41 (0.98)